CHAPTER II

LITERATURE REVIEW

2.1 Irrigants

Cleaning and shaping root canals are essential steps in root canal treatment. Unfortunately, the mechanical action of instruments is unable to reach areas of the root canal system due to anatomical complexities. As a result, irrigating solutions have an important role in chemomechanical preparation.

The major function of an irrigant is flushing debris from the canal. The irrigant may have additional properties that aid in cleaning and shaping. Below are outlined the characteristics of an ideal irrigant.

Properties of ideal irrigant (Walton *et al.*, 2002)

1. Tissue or debris solvent: In regions inaccessible to instruments, the irrigant could dissolve or disrupt soft tissue or hard tissue remnants to permit their removal.

2. No toxicity: The irrigant should be noninjurious to periradicular tissues.

3. Low surface tension: This property promotes flow into dentinal tubules and into inaccessible areas. Alcohol added to an irrigant decreases surface tension and increases penetrability, whether this enhances is unknown.

4. Lubricant: Lubrication helps instruments to slide down the canal.

5. Sterilization (or at least disinfection).

6. Removal of smear layer: The smear layer is a layer of microcrystalline and organic particle debris spread on the walls after canal penetration. There are solutions that chelate and decalcify remove the smear layer.

7. Availability

8. User friendliness

9. Cheap

10. Convenience

11. Adequate shelf life

12. Ease of storage
13. No stain

Function of an irrigant

1. Gross debridement
2. Elimination of microbes
3. Dissolution of remnant pulp tissue
4. Lubricant
5. Remove smear layer

2.1.1 Sodium hypochlorite (NaOCl)

Sodium hypochlorite has been demonstrated to be an effective agent against a broad spectrum of bacteria and dissolve vital as well as necrotic tissue. However, it has been shown also that sodium hypochlorite has toxic effects on vital tissue, resulting in haemolysis, skin ulceration and necrosis. It has a pH approximately 11-12 that cause injury primarily by oxidation of proteins.

Sodium hypochlorite solution is the most commonly employed root canal irrigant, but no general agreement exists regarding its optimal concentration, which ranges from 0.5% to 5.25%. Its antimicrobial activity is proportional to the drug concentration, as shown in the present work. To obtain acceptable cytotoxic levels, 0.5% sodium hypochlorite is recommended, but this concentration needs at least 30 minutes to inhibit the growth of facultative microorganisms. On the other hand, study of Vianna *et al.* (2004) found that 5.25% sodium hypochlorite kills microorganisms in seconds.

It seems that the antimicrobial activity of sodium hypochlorite depends on the concentration of undissociated hypochlorous acid (HOCl) in solution. HOCl exerts its germicidal effect by an oxidative action on sulfydryl groups of bacterial enzymes. As essential enzymes are inhibited, important metabolic reactions are disrupted, result in the death of bacterial cells.

2.1.2 Chlorhexidine (CHX)

Properties of chlorhexidine

1. Bacterostatic or bacteriocidal
2. Biocompatibility
3. Low toxicity

Action of chlorhexidine

Chlorhexidine is active against a wide range of gram-positive and gram-negative organisms, yeast, fungi, facultative anaerobes, and aerobes. Its action is the result of the absorption of the chlorhexidine onto the cell wall of the microorganism, resulting in the leakage of intracellular components.

- At low chlorhexidine concentrations, small molecular weight substances, such as potassium and phosphorus, will leach out, exerting a bacteriostatic effect.

- At higher concentrations, chlorhexidine is bacteriocidal because of precipitation or coagulation of the cytoplasm, probably caused by protein cross-linking.

The bacteriocidal effect is thought to be less important than the bacteriostatic effect provided by a slow release of chlorhexidine.

Because of chlorhexidine's cationic properties, it binds to the hydroxyapatite of tooth enamel, the pellicle on the tooth surface, salivary proteins, bacteria, and extracellular polysaccharides of the bacterial origin. Between third to a half of the chlorhexidine retained in the mouth is bound to phosphate group. The current view is that much of the chlorhexidine binding in the mouth occurs on coatings of mucous membrane surfaces. The adsorbed chlorhexidine gradually is released for up to 24 hours, as the concentration in the mouth decreases. Thus, chlorhexidine is thought to reduce bacterial colonization of the tooth surfaces (Fardal *et al.*, 1986).

Chlorhexidine liquid, in all concentrations (0.2%, 1% or 2%), killed all microorganism in 30 seconds or less, whereas chlorhexidine gel took from 22 seconds (2% chlorhexidine gel) to 2 hours (0.2% chlorhexidine gel) (Vianna *et al.*, 2004). This could be explained that chlorhexidine liquid mixed very well with the bacterial suspension, immediately exerting its antimicrobial action. Whereas the gel formulation,

which is more difficult to mix, prevented direct contact between bacterial cells and chlorhexidine. Thus requiring a longer time to act against the microorganism.

The time required to eliminate microorganism depended on the concentration and type of irrigant used. Oncag *et al.* (2003) compared the antibacterial properties and toxicity of various root canal irrigants that showed 0.2%, 1% and 2% chlorhexidine gluconate were more effective and had more residual antibacterial effects and lower toxicity than 5.25% sodium hypochlorite.

Chlorhexidine gluconate has the possible clinical advantage of being relatively compatible to vital tissue. This could influence a decision to use chlorhexidine gluconate in perforations, open apices, or cases with difficulty in isolation. Another advantage of using chlorhexidine gluconate is that it could be used in patients who are allergic to sodium hypochlorite. The major disadvantage of using chlorhexidine gluconate as the primary endodontic irrigant is that it lacks the ability to dissolve necrotic pulp tissue.

Side effect

Chlorhexidine can cause brownish tooth discoloration (interaction between the anionic groups of the dye molecules and the cationic groups of the chlorhexidine molecules that may be related to the staining of teeth).

The disadvantage of using chlorhexidine gluconate is that it is more expensive.

Combination of sodium hypochlorite and chlorhexidine gluconate

It has been postulated that the use of sodium hypochlorite and chlorhexidine gluconate, combined as root canal irrigants:

- an additive antimicrobial action
- a tissue dissolution property better than using chlorhexidine alone
- a solution less toxic than sodium hypochlorite.

Kuruvilla and Kamath (1998) compared that the effect of antimicrobial of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate combined within the root canal. The results showed that in the greatest percentage reduction of post-irrigant positive cultures. This reduction was significant compared to used of sodium hypochlorite alone

but not significant compared to used of chlorhexidine gluconate alone. The speculated reason for this could be due to the following reaction.

- Chlorhexidine is a base, itself capable of forming salts with a number of organic acids.
- Sodium hypochlorite is an oxidizing agent that may be capable of oxidizing the gluconate part of chlorhexidine gluconate to gluconic acid. The chloro groups might get added on to the guanidine component of the chlorhexidine molecule, there by forming " chlorhexidine chloride ".

If these were to happen, it would increase the ionizing capacity of the chlorhexidine molecule, and the solution would incline toward an alkaline pH. This was evident when the pH of sodium hypochlorite solution, chlorhexidine gluconate solution, and their combination were recorded using a pH meter.

The pH was recorded as follows:

- 2.5% sodium hypochlorite: pH 9
- 0.2% chlorhexidine gluconate: pH 6.5
- Combination: pH 10

2.1.3 Ethylene-diaminetetraacetic acid (EDTA)

Properties of EDTA

EDTA acts upon the inorganic components of the smear layer, causes the decalcification of peri- and intertubular dentine, and leaves the collagen exposed.

Combination of sodium hypochlorite and EDTA

The study of Teixeira *et al.* (2005) reported that the association of 15% EDTA (3 ml) and 1% sodium hypochlorite solutions (3 ml) proved effective in removing the smear layer for 1, 3 and 5 min (Fig. 1).

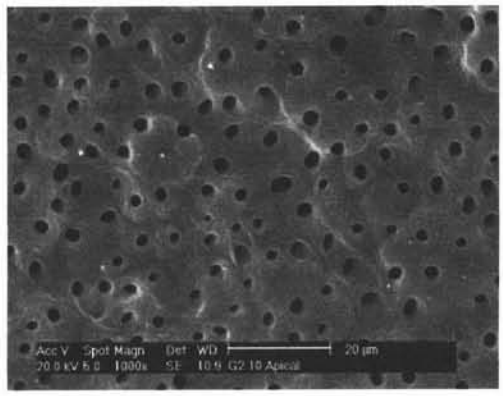

Fig. 1 Effect of 15% EDTA for 1 min, followed by 1% sodium hypochlorite for 1 min on the root canal (Modified from Teixeira *et al.*, 2005)

2.2 Root canal sealers

Methacrylate resin sealer

Recently, a new thermoplastic synthetic polymer base root filling material was introduced (Resilon®; Pentron Clinical Technologies, Wallingford, CT, USA). This material resembles gutta-percha in appearance; It has similar handling properties and is available both in cone format and in pellets for warm injection. The corresponding sealer (Pentron Clinical Technologies) is a dual curable dental resin composite. This so-called 'Epiphany' system (Resilon® and sealer combined with self-etching of the canal wall) is claimed to form a 'monoblock' which adheres to the dentine walls, prevents leakage and increases resistance to fracture (Shipper *et al.*, 2004; Teixeira *et al.*, 2004).

Resilon® is a thermoplastic synthetic polymer-based root canal core filling-material that contains bioactive glass and radiopaque fillers. It performs like gutta-percha, has the same handling properties, and for re-treatment purposes it may be softened with heat or dissolved with solvents such as chloroform. Because it is a synthetic, polymer-based, the resin sealer attaches to it, as well as to the bonding agent used penetrate into the dentinal tubules, forming a "monoblock" composed of filling material, resin sealant, bonding agent, and dentin (Fig. 2) (Teixeira *et al.*, 2004).

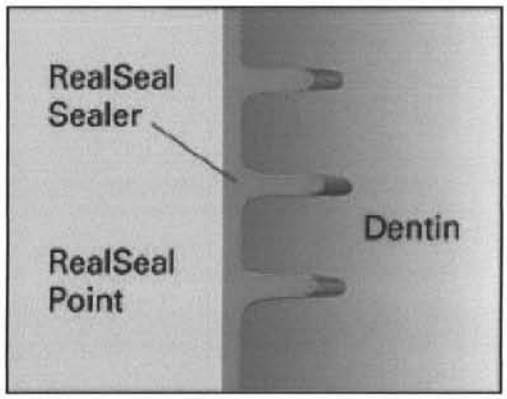

Fig. 2 Monoblock: filling material, resin sealant, bomding agent, and dentin (Modified from Teixeira *et al.*, 2004)

Methacrylate resin sealer composed of (Gomes *et al.*, 2002)

1. Self-etch primer (Epiphany® primer, Pentron Clinical Technologies)
2. Dual-curable, polymer, composite sealer (Epiphany® sealant, Pentron Clinical Technologies)
   - The polymer matrix is a mixture of dimethacrylate, ethoxylated dimethacrylate, urethane dimethacrylate, and hydrophilic difunctional methcrylates.
   - The total filler content in the sealer is about 70% by weight. (Calcium hydroxide, barium sulfate, barium glass, and silica)

2.2.1 Chemical composition (Resilon®; Pentron Clinical Technologies, Wallingford, CT, USA)

2.2.1.1 Chemical composition of Resilon® cone (Fig. 3)

- Polycaprolactone or Tone (57.6 ± 0.2 vt%)
- Bifunctional methacylates resin
- Bioactive glass
- Bismuth oxychloride Fillers 42 ± 0.2 vt%
- Barium sulfate 65 wt%

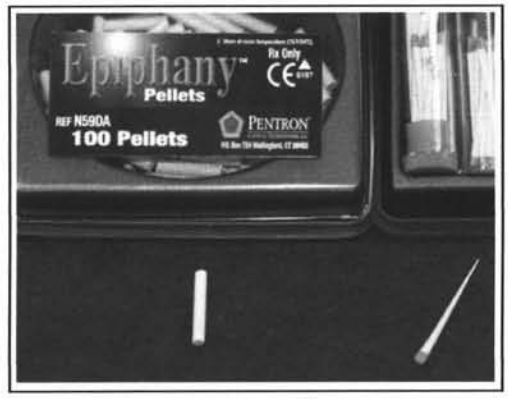

Fig. 3 Resilon® cone

(Modified from Pentron Clinical Technologies, Wallingford, CT, USA)

2.2.1.2 Chemical composition of Resilon® sealer (Fig. 4)

The filler: content of approximately 70% WT

- Calcium hydroxide (41.46 mg/L) (Versiani *et al.*, 2006)
- Barium sulfate
- Barium glass
- Bismuth oxychloride
- Silica

2.2.1.3 Chemical composition of Resilon® primer (Fig. 4)

"Aqueous solution of acidic monomer"

- Sulfonic acid-terminated functional monomer
- Hydroxyethyl-methacrylate (HEMA)
- Water
- Polymerization initiator

Fig. 4 Resilon® primer and Resilon® sealer

(Modified from Pentron Clinical Technologies, Wallingford, CT, USA)

2.2.2 Physical properties

2.2.2.1 Thermal properties

Miner *et al.* (2006) compared melting point, specific heat, enthalpy change with melting and heat transfer between gutta-percha and Resilon®. (Endodontic sealer was not used during the experiment.)

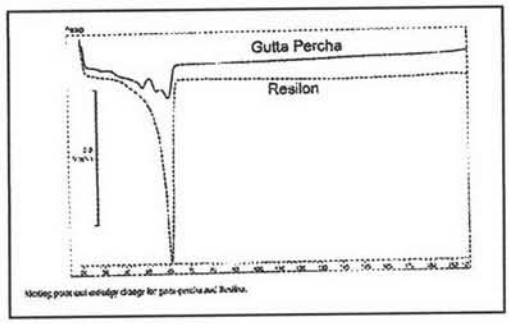

Fig. 5 Melting points between gutta-percha and Resilon®.
(Modified from Miner *et al.*, 2006)

Results showed no significant difference between gutta-percha and Resilon® for the melting point temperature (Gutta-percha = 60.01°C, Resilon® = 60.57°C) (Fig.5).

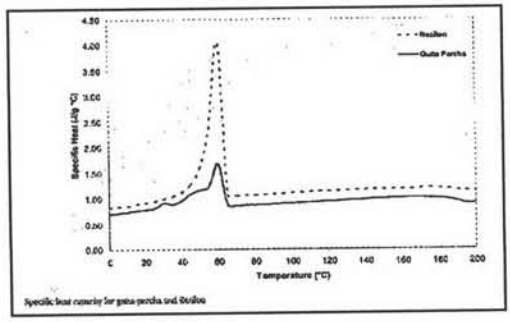

Fig. 6 Specific heat capacities between gutta-percha and Resilon®.
(Modified from Miner *et al.*, 2006)

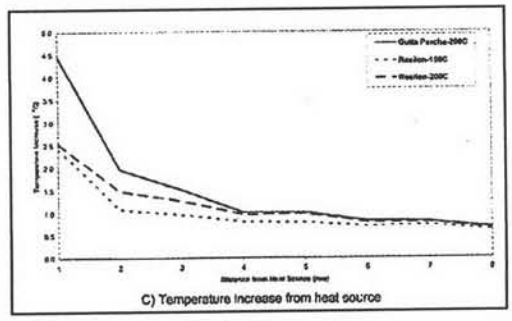

Fig. 7 Heat transfer between gutta-percha and Resilon®.

(Modified from Miner *et al.*, 2006)

There was significant difference in specific heat capacity and endothermic enthalpy change between the two materials (Fig. 6). The heat transfer test showed a significant difference in temperature increase between gutta-percha and Resilon® within 3 mm of the heat source (Fig. 7).

Although the melting temperatures are similar, the results of the study suggest that Resilon® may not thermoplasticized similar to gutta-percha because there is a higher specific heat, higher enthalpy change with melting and less heat transfer. It is not known at this time whether the lack of heat transfer within 3 mm from the heat source is clinically relevant.

2.2.2.2 Setting time

The ANSI/ADA (2000) requirements require that a sealer shall be within 10% of that stated by the manufacturer. According to the guidelines for AH Plus and Epiphany®, the cements have 8 hours (480 min) and 25 min of setting time respectively.

Resilon® sealer set in 30 minutes in anaerobic environments. In the presence of air, Resilon® took a week to set and when placed in PBS (phosphate buffered saline), an uncured layer remained on the surface (Nielsen *et al.*, 2006).

2.2.2.3 Solubility

The ANSI/ADA specification 57 states that the root canal cement should not exceed 3% by mass when the solubility of the set material is tested. In contrast to the

AH Plus mean result (0.21%), the solubility of Epiphany® sealer did not conform to ANSI/ADA standardization (3.14%) (Versiani *et al.*, 2006).

2.2.2.4 Flow test

The ANSI/ADA (2000) requires that a sealer shall have a diameter of no less than 20 mm. Both cements conformed to ANSI/ADA standard as the results were 38.57 (± 3.85) and 35.74 (± 0.47) mm to AH Plus and Epiphany® respectively (Versiani *et al.*, 2006).

2.2.2.5 Film thickness

The ANSI/ADA (2000) requires that a sealer shall have a film thickness of no more than 50 μm. Both cements conformed to ANSI/ADA standardization as the results were 10.6 (± 0.54) and 20.1 (± 8.12) μm to AH Plus and Epiphany® respectively (Versiani *et al.*, 2006).

2.2.2.6 Dimensional alteration

The ANSI/ADA (2000) requirements for this test state that the mean linear shrinkage of the sealer shall not exceed 1% or 0.1% in expansion. Neither cement conformed to the ANSI/ADA standardization. The results showed expansions of 1.3% and 8.1% for AH Plus and Epiphany® respectively (Versiani *et al.*, 2006).

Table 1 Physical properties of Epiphany® and AH Plus.

(Modified from Versiani *et al.*, 2006)

Physical properties	Epiphany®	AH Plus
Setting time	25.03 ± 1.93 (min)	8 h (480 min)
Solubility*	3.41% (Fe 0.56, Ni 0.06, Ca 41.46, Mg 0.80, Zn 0.05, Na 4.11 and K 0.50 mg/L)	0.21%
Flow	35.74 ± 0.47 mm	38.57 (± 3.85) mm
Film thickness	20.1 ± 8.12 μm	10.6 (± 0.54) μm
Dimensional change*	Expasion 8.1%	Expasion 1.3%

In conclusion, setting time, flow, and film thickness tests of both cements conformed to American National Standards specification for endodontic filling materials ANSI/ADA (2000). However, the solubility and dimensional alteration values of Epiphany® sealer, and dimensional alteration values of AH Plus were higher than those considered acceptable for the ANSI/ADA specifications (ANSI/ADA 2000) (Table 1) (Versiani *et al.*, 2006).

2.2.2.7 Biodegradation

Polycaprolactone (PCL) can be degraded by microorganisms as well as by a hydrolytic mechanism under physiological conditions. It is reported by Tay *et al.* (2005) that PCL is susceptible to both alkaline and enzymatic hydrolyzes. They examined the susceptibility of Resilon®, a polycaprolactone-based root filling composite, to alkaline hydrolysis using field-emission acanning electron microscopy and energy dispersive X-ray analysis. The surface resinous component of Resilon® was hydrolyzed after 20 min of sodium ethoxide immersion, exposing the spherulitic polymer structure of PCL and subsurface glss and bismuth oxychloride filler. More severe erosion occurred after 60 min of sodium ethoxide treatment (Fig. 8-12) (Tay *et al.* 2005b; Tay *et al.* 2005c).

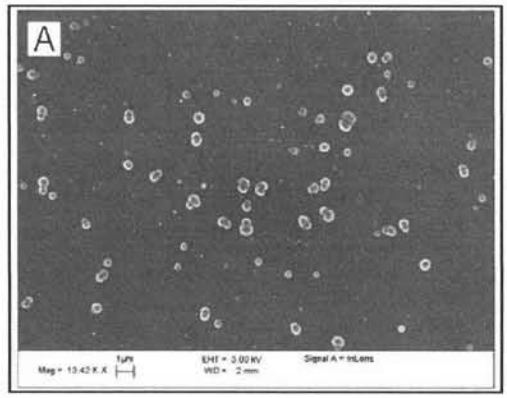

Fig. 8 Resilon®: without alkaline treatment

(Modified from Tay *et al.*, 2005b)

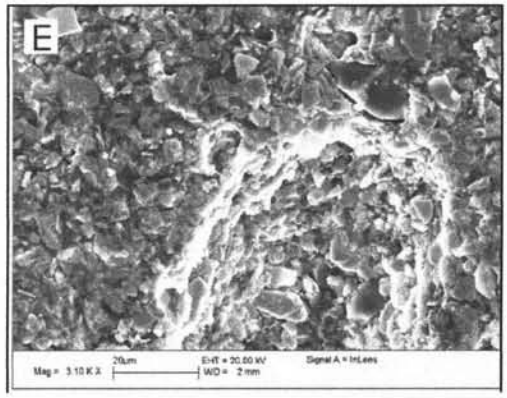

Fig. 9 Resilon®: treated with 20% sodium ethoxide 60 min.
(Modified from Tay *et al.*, 2005c)

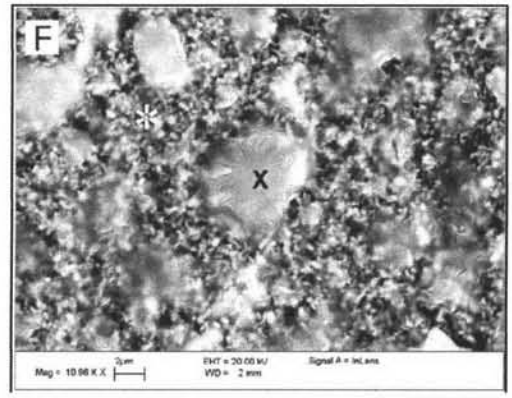

Fig. 10 Gutta-percha: treated with 20% sodium ethoxide 60 min.
(Modified from Tay *et al.*, 2005c)

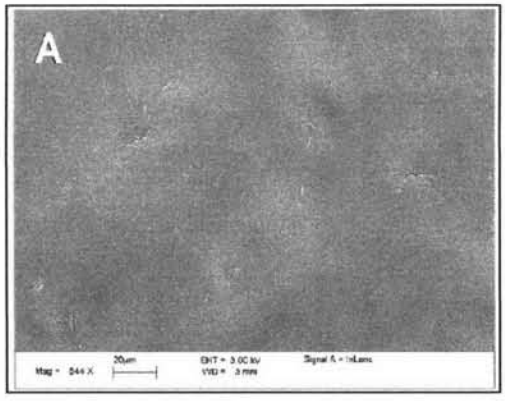

Fig. 11 Resilon®: before enzyme immersion (lipase PS).
(Modified from Tay *et al.*, 2005c)

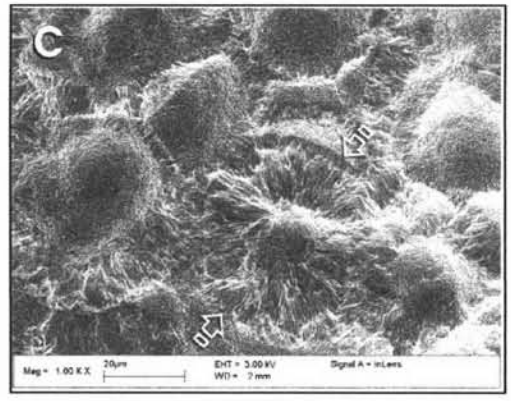

Fig. 12 Resilon®: after enzyme immersion (lipase PS).
(Modified from Tay *et al.*, 2005c)

2.2.3 Mechanical properties

2.2.3.1 Bond strength between sealer/dentine " push-out test "

Skidmore *et al.* (2006) compared the micropush-out bond strength of Resilon® to that the gutta-percha (Kerr pulp root canal sealer). The results showed that the mean micropush-out bond strength of the Resilon® group (1.51 ± 1.22 MPa) was significantly higher ($p < 0.05$) than that of the gutta-percha group (0.66 ± 0.39 MPa). Mode of failure

of the samples revealed the bond failure to be predominantly adhesive between the sealer and dentin interface for both groups.

Gesi *et al.* (2005) compared the interfacial strengths and failure mode of Resilon®/Epiphany® and gutta-percha/AH Plus. The results showed that the mean interfacial strengths of Resilon®/Epiphany® (0.05 ± 0.41 MPa) was significantly lower (p = 0.025) than that of the gutta-percha/AH Plus (0.94 ± 0.77 MPa).

Urgor *et al.* (2006) showed Epiphany® sealer and gutta-percha core combination had the highest bond strength, whereas the AH Plus sealer and Resilon® core combination had the lowest values. Interestingly, the Epiphany® sealer and Resilon® core combination showed lower bond strength values than expected. One possible explanation is that gutta-percha is more compactable than Resilon®, and thus helps resist dislodgment.

The bond strength of the AH Plus/gutta-percha showed higher bond strength than the Epiphany®/Resilon® combination. The result is similar to the findings of Gesi *et al.* (2005) who used the same methodology.

Sly *et al.* (2007) evaluated the push-out bond strength to intraradicular dentin of two polymeric endodontic obturation systems, Epiphany®/Resilon® and gutta-percha/AH26. It was concluded that the push-out bond strength achieved with Epiphany®/Resilon® (mean 0.51 ± 0.30) to intraradicular dentin is not superior to that of gutta-percha/AH26 (mean 1.7 ± 0.71).

2.2.3.2 Shear bond strengths

Hiraishi *et al.* (2005) evaluated the adhesion strength of Resilon® to Next® root canal sealer, a methacrylate-based root canal sealer, using a modified microshear bond testing design. The results showed the low shear bond strength of Resilon® to a methacrylate-based sealer compared with composite control. Increasing the surface roughness of the Resilon® surface did not contribute to further improvement in shear bond strength for this methacrylate-based sealer.

Tay *et al.* (2006) evaluated the contribution of chemical coupling by bonding to smooth surfaces, and the contribution of micromechanical retention by bonding to

surfaces with a different roughness, to the overall adhesion of Resilon® to Methacrylate-based sealer (RealSeal).

However, polycaprolactone-based thermoplastic composite may not yet be optimized for effective chemical coupling to methacrylate resins.

2.2.3.3 Cohesive strength (that is the tensile stress when they begin to flow or break) and modulus of elasticity (or stiffness)

Williams *et al.* (2006) compared the cohesive strength and stiffness of Resilon® and gutta-percha. The results of this study showed that the cohesive strength and modulus of elasticity of gutta-percha and Resilon® were relatively low. In conclusion, the stiffness of Resilon® and gutta-percha were too low to reinforce roots after root canal therapy.

2.2.4 Clinical properties

2.2.4.1 Biocompatibility

Key *et al.* (2006) evaluated the cytotoxicity of root canal sealing materials Resilon® and Epiphany® versus gutta-percha, Grossman's sealer, Thermaseal, and sealapex. Using human gingival fibroblasts were stained with trypan blue, to determine number of dead cells. The results showed that Resilon® had a lower cytotoxicity and that Epiphany® was more cytotoxicity than conventional materials.

Susini *et al.* (2006) evaluated the cytotoxicity of Resilon® and Epiphany® using root model and used to measure the cytotoxicity on mouse fibroblasts L929 with MTT assay that recorded the mitochondrial activity of the target cells and ISO 10993-5 standards. Epiphany® and Resilon® were the most cytotoxic materials at 1 and 2 days, due mainly to Epiphany®, decrease after 2 days to reach a level comparable with commonly used root canal sealer.

Merdad *et al.* (2007), this study was to evaluate the cytotoxicity of the Epiphany® system's component using the indirect contact Millipore filter assay, and direct contact assay. Results of both assays showed both freshly mixed sealers elicited a moderate cytotoxic response, with significantly larger unstained zones around AH Plus specimens

than around Epiphany® specimens. The 24- and 48- hour set sealers, Resilon®, and gutta-percha were all characterized by noncytotoxic response.

2.2.4.2 Effective retreatment

Ezzic *et al.* (2006), this study was to evaluate the effectiveness of retreatment technique for a Resilon®/Epiphany® and gutta-percha/AH Plus. With the same technique, it took less time to remove Resilon®/Epiphany® when compared to gutta-percha/AH Plus.

De Oliveira *et al.* (2006), showed results supporting the study of Ezzic *et al.* (2006) that with the same technique, it took less time to remove Resilon®/Epiphany® when compared to gutta-percha/AH26.

Teixeira *et al.* (2002) showed that Epiphany® primer conditions the root canal dentinal surface and Epiphany® sealer bonds both to the root canal walls and Resilon® cones forming a monoblock. They also stated that this monoblock provides good adaptation to root canal wall and reduces leakage.

2.2.4.3 Fracture resistance

Sagsen *et al.* (2007) compared the fracture resistance of roots filled with different materials (Table 2).

Table 2 The fracture resistance of roots filled with different materials.

(Modified from Sagsen *et al.*, 2007)

Groups	Mean force of fracture values
Resilon® + Epiphany® sealer	967.0
AH26 + gutta-percha	859.0
MCS + gutta-percha	1043.0
Control	517.5

In conclusion, all the materials used in the present study reinforced the prepared root canal.

In the study of Teixeira *et al.* (2002), it was reported that the group filled with Resilon® cones and Epiphany® sealer were more resistant to fracture than the groups filled with AH26 and gutta-percha. The authors attributed the reinforcing effect of Resilon® groups to the monoblock that forms within the root canal: they also found no difference between the experimental groups and the unfilled control group.

Schafer *et al.* (2007) compared the fracture resistance of roots filled with different materials. The results showed that the roots obturated with RealSeal were significantly stronger than those obturated with gutta-percha and AH Plus.

2.3 Sealing ability

2.3.1 Methodologies

Methodologies *in vitro* are used to estimate sealing quality, generally by measuring microleakage that allows the tracer agent to penetrate the filled canal. Commonly used tracers are dyes, radioisotopes, bacteria and their products, such as endotoxin. Bacteria used as tracers most closely approximate what happens clinically in terms of leakage. Other methodologies, such as fluid filtration and dye extraction methods have also been used, their main advantage being high reproducibility.

Although there are many leakage studies, there is no consensus about the endodontic sealer and core material sealing capacities. One of the reasons is that investigations 'do not use a standardized methodology and this frequently leads to contradictions.

2.3.1.1 Methodology using dyes

The methodology using tooth immersion in various types of dyes (eosin, methylene blue, black India ink, and others), reported for the first time by Grossman in 1939, is perhaps most widely used, mainly because it is easy to perform. The phenomenon of capillarity is importance in this passive method used mainly for assessing apical leakage, as the tooth apex is submerged in the dye that penetrates through any space between the canal wall and filling material (Camps *et al.*, 2003). Next, the teeth are sectioned longitudinally, transversely, or cleared and the linear penetration of the dye is recorded.

The longitudinal sectioning method enables examination on of the exposed filling material and any dry penetration into the material and at the interface of the dentinal wall on one side. Ahlberg *et al.* (1995) suggested a variation of this technique; whereby the roots are worm down to visualize the leakage through a thin layer of dentin, thus reducing the risk of dye dissolution during sectioning. They also affirmed that this technique provides more reliable information about the leakage pattern that transverse section or clearing. The disadvantages of longitudinal dentinal sectioning seem to be the random choice of the cut axis and the very low probability of the section being made through the deepest dye penetration point, with consequent underestimation of leakage and recording of unreliable data.

According to Ahlberg *et al.* (1995) transverse root sectioning results in loss of part of the dentinal tissue and dye to the technique itself (saw thickness), and only allows one to determine whether or not there is penetration in each section.

The clearing technique recommended by Okumura in 1927, in which the teeth become transparent after a process of demineralization, dehydration and immersion in methyl salicylate, provides a three-dimensional view of the internal anatomy of root canal without the loss of dental substance, making it easier to view the leakage area. It is simple, fast, performed with substances low in toxins and does not require complex equipment. Martin *et al.* (2002) also affirmed that technique makes it easier to observe the lateral and accessory canals, and clearly reflects the relation between the sealing material and apical foramen. Furthermore, the demineralization times differs, as greater the weight of the dentinal part, the greater the mineral content present and the longer it would take to complete the process. According to Tagger *et al.* (1983), the endpoint of this step could be easily assessed by inserting a thin needle in an unimportant area of the crown or by radiography. Another potential problem is that incomplete dehydration will leave opaque areas in the teeth, but this can be corrected by additional dehydration in 100% ethyl alcohol (Robertson *et al.*, 1980). Ahlberg *et al.* (1995) pointed out that immersion in acids such as nitric and alcohol for a long period may cause dye dissolution in this technique. Martin *et al.* (2002) showed that the clearing technique was

more precise than the transverse section for detecting apical leakage, as it allows the leakage to be visualized in tenths of millimeters, while transverse sectioning only determines whether or not leakage has occurred in each section. The clearing system could not, however, be used to measure the volume of tracer ingress (Youngson *et al.*, 1999).

With regard to dyes, particle molecule size, pH and chemical reactivity are expected to affect the degree of penetration (Martin *et al.*, 2002). A larger number of studies used methylene blue as dye because it is inexpensive, easy to manipulate, has a high degree of staining and a molecular weight even lower than that of bacterial toxins. It has been suggested that methylene blue presents the same leakage as butyric acid (Kersten *et al.*, 1989), a microbial product that has greater penetration than Indian ink. This present a few disadvantages such as dissolution during the demineralization and clearing process, in addition to being difficult to observe its maximum penetration point in some cases.

Indian ink particles with diameter smaller than or equal to 3 μm are also widely used, as is unlikely that bacterial invasion would occur in spaces inside the canal where this dye is unable to penetrate (Schafer *et al.*, 2002). However, it has been reported that the weight and of Indian ink molecules are smaller than those of the bacterial molecules found in the root canal. Therefore, this substance may also not faithfully represent the molecules of fluids coming from periradicular tissues, giving false positive result during analysis of the leakage.

One of the major considerations with respect to dye penetration studies is air entrapped in voids along the root canal filling may hinder fluid movement. It has been recommended that dye penetration should be performed under reduced pressure, incorrectly referred to as vacuum (Wu *et al.*, 1994). However, it is much more difficult to remove the trapped air by applying reduced pressure to small empty spaces, such as those of 2 μm in diameter which, in principle, are permeable to bacteria (Wu *et al.*, 1994). Kontakiotis *et al.* (2001) investigated the influence of hydration on voids along root filling through a fluid transport made and dye penetration in which transport air was

applied to remove water from gaps in one group, and found that methylene blue penetrates more easily in dry gaps than in water filled gaps. Methylene blue passes along air-filled gaps by capillary action, whereas in water-fill gaps, it passes by diffusion. Wimonchit *et al.* (2002) comparing different coronal dye leakage test techniques, observed that the vacuum method resulted in significantly more dye penetration than fluid filtration and passive dye penetration. The result of this study emphasized the importance of the use of reduced air pressure in dye penetration. Spångberg *et al.* (1989) found that passive dye penetration resulted in incomplete void filling, regardless of void size, whereas vacuum dye delivery resulted in complete void filling. Katz *et al.* (1998) found no significant differences between a horizontally positioned experimental group under reduced pressure and groups in passive immersion, but when the apices were in an upright position, the mean leakage was significantly higher under reduce pressure. Thus, the authors showed that tooth positioning had a significant effect on linear dye penetration under reduced pressure and emphasized the need to standardize factors that may influence penetration when assessing the methodology of leakage studies.

2.3.1.2 Fluid filtration or transportation methodology

The fluid filtration method, in which the sealing capacity is measured by means of air bubble movement inside a capillary tube, was developed by Pashley ' s group in 1987 and modified by Wu *et al.* in 1993 for use in root canal. It consists of a filled canal that has its coronal portion connected to a tube filled with water under atmospheric pressure, and its apex to a 20 µl glass capillary tube 170 mm long and of uniform caliber filled with water. Finally, a pressure of 0.1 atm is applied through the coronal part, which forces the water through the empty spaces along the root canal (Wu *et al.*, 1994). The results are generally expressed in µl/min (Pommel *et al.*, 2001a).

The above–mentioned method present many advantages in comparison with dye penetration methods, as the samples are not destroyed, therefore it allows both the apical and coronal sealing to be assessed after a long period.

Furthermore, the results are recorded automatically, thus providing quantitative measurements and avoiding operator errors; the results are precise, as small volume can be recorded and it would be more sensitive then dye penetration in detecting empty spaces along the canal. System sensitivity can be adjusted by altering the pressure used or the diameter of the micropipette (Cobankara *et al.*, 2002). However, according to Pommel and Camps (2001) the materials and methods used in this technique are not standardized, as the pressure used may rang from 10 to 20 psi, and the measuring time from 1 min to 3 hrs. This would alter the result obtained, sine lower filtration values have been found associated with longer recording time, and the values recorded were higher when high pressure was used in comparison with low pressure (Pommel and Camps, 2001a). According to the authors, 20 psi pressure would appear to be far too high because it corresponds to 1406 cm H_2O pressure. Therefore, to be as close as possible as possible to physiological pressures, 15 cm by H_2O would appear to be sufficient when highly sensitive equipment is used. The pressures should include in the results and should be expressed as µl/min cm H_2O instead of µl/min.

Thus, various parameters that could change the test results such as diameter of the capillary that contains the bubble. Bubble length, measuring time and pressures applied (Pommel and Camps, 2001a), must be mentioned in the materials and methods section.

Orucoglu *et al.* (2005) developed a new computerized fluid filtration meter based on light refraction at starting and ending positions of air bubble movement inside micropipette. It has some advantages over the conventional ones with the computer computer control and digital air pressure arrangement. Additionally, the movement of air bubbles can be observed by laser diodes which are computer controlled rather than visual finding. Additionally, the computerized fluid filtration method has the advantage that the movement of air bubble can be observed by laser diodes computer controlled rather than visual following.

2.3.1.3 Dye Extraction Method

In the extraction or dissolution method, the teeth are dissolved in acids that release all the dye from the interface and the optical density of the solution is measured by a spectrophotometer. It is fast and can be carried out with equipment available at most universities (Camps and Pashley, 2003).

According to Camps and Pashley (2003), there was no correlation between dye penetration and the fluid filtration and dye extraction techniques which determine microleakage. The fluid filtration technique gave similar results to those of dye extraction, because both take into consideration the porosity of the interface between the filling material and the root. Both techniques are based on quantitative measurements of liquids passing through these interfaces. The dye extraction method presents an advantage over the fluid filtration method, because the filtration values tend to diminish over time, as the water penetrates all the irregularities until a plateau is reached.

Dye-penetration studies are commonly used because they are easy to accomplish and do not require sophisticated materials. Pitt Ford (1983) who compared the dye leakage of several sealers found the differences seen did not produce noticeably different tissue responses *in vivo*.

Methylene blue dye was used in this study because it easily allows quantitative measurement of the extent of dye penetration by linear measurement techniques. Its molecular size is similar to bacterial by-products such as butyric acid which can leak out of infected root canals to irritate periapical tissues (Kersten and Moorer, 1989). Matloff *et al.* (1982) did not find any correlation between a dye-penetration study and a radioisotopic technique. Additionally, Barthel *et al.* (1999) found no correlation between a dye-penetration study and a bacterial leakage study, whereas Wu *et al.* (1993a) and Pommel *et al.* (2001b) found no correlation between a dye penetration study and a fluid filtration technique.

A study by Clamps and Pashley (2003) demonstrated the poor correlation of dye penetration studies compared to the fluid filtration technique for evaluation leakage.

2.3.1.4 Bacteria and toxin infiltration method

According to Timpawat *et al.* (2001) the use of bacteria to assess leakage (mainly coronal) is considered to be of greater clinical and biological relevance then the dye penetration method. Many different strains of bacteria have been used to assess marginal leakage and this has lead to contradictory results, because methods depend on the type of bacteria used. Moreover, if the sealer has antimicrobial activity, it is unfeasible to employ the bacteria method (Schafer and Olthoff, 2002). The system generally comprise two chambers and enable the apical and and coronal extremities of each specimen to be completely separated. The turbidity of the broth in that in apical chamber is the first indication of contamination by microorganisms (Carratu *et al.*, 2002).

If the pulp chamber becomes contaminated, it may serve as a reservoir of microorganisms and toxins. This could cause a problem in either of two ways. First, the apical seal may be affected adversely and cause the root canal treatment to fail. Second, movement of microorganisms and toxins though accessory canals in the floor of the pulp chamber may result in periodontal furcation involvement (Chailertvanitkul *et al.*, 1997).

These bacterial studies have been qualitative rather than quantitative. If only one bacteria passes through the obturated root canal, it may multiply in the enriched broth and cause turbidity (Chailertvanitkul *et al.*, 1997).

According to Barthel *et al.* (1999), bacteria or bacterial product penetration may start or reactivate the inflammatory process, and saliva leakage may stimulate the growth of the bacteria that persist inside the root canal. The size of the test agent molecule must be representative of the bacteria and/or components of the bacterial cell wall and/or nutrient fluid.

The differences in behavior between bacteria and endotoxins must be related to their chemical activities. Endotoxins are lipopolysaccharides of the external membrane of gram-negative bacteria and consist of a lipid portion, Lipid A, and a polysaccharide portion, which is the external part of the membrane. The possibility of the bacteria exerting enzymatic action on the gutta-percha, sealer and dentin and creating a

passage through the seal, has not yet been demonstrated (Carratu *et al.*, 2002). It has been reported that endotoxin preceded bacterial penetration of canal system (Williamson *et al.*, 2005).

Xu *et al.* (2005) introduced a new method for analysis of endodontic microleakage based on the filtration rate of glucose along the root canal filling. The model consists of a tube containing concentrated glucose solution that is connected to the coronal aspect of the tooth, whilst the apical region in water. Glucose that accumulates in apical chamber is measure with a spectrophotometer, following an enzymatic reaction. The amount of leakage was quantified with spectrophotometer. Glucose was selected as the tracer because of its small molecular size (MW = 180 Da) and as it is a nutrient for bacteria. So, if glucose could enter the canal from the oral cavity, bacteria that survive root canal preparation and could multiply and lead to periapical inflammation.

2.3.2 Sealing ability of Epiphany root canal sealer

A study of Tay *et al.* (2005a) using scanning electron microscopy (SEM) for gaps along canal walls, and using a transmission electron microscopy (TEM) for apical leakage. Single-rooted extracted human teeth were prepared using crown-down technique, debrided with sodium hypochlorite and EDTA, obturated with either Resilon®/Epiphany® or guta-percha/AH-Plus and using the continuous wave condensation technique. The transmission electron microscopy has shown the presence of silver deposites along the sealer–hybrid layer interface in Epiphany®/Resilon® combination, and between the AH-Plus gutta-percha combinations. SEM revealed both gap-free regions, and gap-containing regions in canals filled with both materials. It is concluded that a complete hermatic apical seal cannot be achieved with either root filling materials.

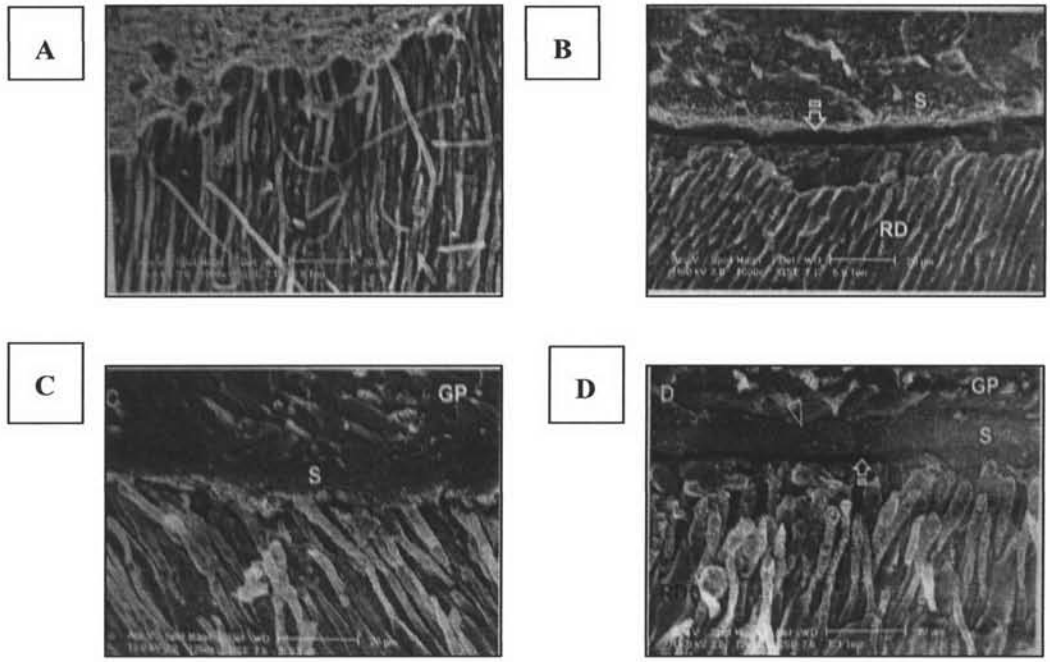

Fig. 13 FE-ESEM revealed excellent coupling of Resilon® to Epiphany® sealer. (Modified from Tay *et al.*, 2005a)

FE-ESEM revealed excellent coupling of Resilon® to Epiphany® sealer, deposite the presence of both gap-free (A) and gap-containing (B) regions along the sealer-dentin interface within the same tooth. Profuse resin tag formation was evident in gap-free regions (A), but resin tags were either sparse or completely absent in the gap-containing regions (B). Likewise, both gap-free (C) and gap-containing regions (D) could be identified in control gutta-percha specimens (Fig. 13).

Undermineralized, unstained TEM sections from-exhibiting regions of the Resilon®-filled root canal revealed heavy silver deposits between the methacrylate resin sealer and root dentin.

Trope (2006) discussed the study of Tay *et al.* (2005)

1. Maybe the silver tracer is so small that it can penetrate root filling and thus is not able to help assess the superiority of one over the other. This would the result clinically irrelevant.

2. More likely the silver tracer molecules penetrated not through the sealer-dentin junction but from the outside in through exposed dentinal tubules in the apical 2 mm of root. The reported depth of penetration of 4 mm corresponds to the depth of dentinal tubules at the apical root.

A study by Bodrumlu and Tunga (2006) using a dye penetration method to measure apical leakage. Single-rooted human anterior teeth were fully instrumented by using the "step-back" technique. The specimens were obturated by the lateral condensation technique, with gutta-percha and AH26 or AH Plus sealers, or Epiphany® sealer and Resilon® core material. The teeth were sectioned longitudinally in a bucco-lingual direction through the center of root. Linear apical leakage was measured from the apex to the coronal extent of the methylene blue dye penetration. The linear break through of the dye was estimated using a stereomicroscope (Olympus BX50, Japan). This study showed Resilon® core material and Epiphany® sealer had the least apical dye penetration than the other groups.

A study by Dultra *et al.* (2006) using a dye penetration method, that measured apical leakage. Single-rooted human anterior teeth (maxillary canine) were fully instrumented by using the "crown-down" technique. The specimens were obturated by the lateral condensation technique, with gutta-percha and Endofil (Grossman's sealer), EndoREZ (resin-based sealer) and AH Plus sealers, except Epiphany® sealer, in which Resilon® core material. The teeth were immersed in India ink for seven days and clarified using methyl salicylate. The extent of apical dye penetration was measured with a measuroscope in all aspects of the canal.

Table 3 Mean values and standard deviation of apical dye penetration for each root canal sealer (Modified from Dultra *et al.*, 2006)

Groups	Means and SD
EndoFill	0.83 ± 0.73
EndoREZ	0.32 ± 0.62
AH Plus	0.02 ± 0.07
Epiphany®	0.00 ± 0.00

AH Plus, Epiphany® and EndoREZ did not differ statistically to each other ($p>0.01$). The resin based root canal sealer presented lesser apical microleakage than the zinc-oxide eugenol based sealer. No statistical differences were observed among resin based sealers.

A study by Tunga and Bodrumlu (2006) using a fluid filtration method (Fluid transport device, Wu *et al.*, 1993), that was measured leakage quantity. Single-rooted human anterior teeth were fully instrumented by using the "step-back" technique. The specimens were obturated by the lateral condensation technique, with gutta-percha and AH26 or AH Plus sealers, or Epiphany® sealer and Resilon® core material. It was concluded that of the materials tested under the conditions of this study, Epiphany® allowed the least leakage.

A study by Biggs *et al.* (2006) using a fluid filtration method, that was measured leakage. The purpose of this study was to compare the sealing ability of the Resilon®/Epiphany® Obturation System to that of conventional gutta-percha obturations using lateral condensation, but not removed the smear layers before obturated root canal. The results of this study indicate that Resilon®/Epiphany® was equivalent, but not superior to gutta-percha/conventional endodontic sealers (AH Plus, Roth).

A study by Stratton *et al.* (2006) using a fluid filtration method, was to compare the sealing ability of gutta-percha and AH Plus sealer versus Resilon® and Epiphany® resin root canal sealer using three different final irrigants. Obturation was performed using the continuous wave of condensation. Under the conditions of this study, the Resilon® groups with self-etch primer and Epiphany® resin root canal sealer were significantly more resistant to fluid movement than the gutta-percha and AH Plus sealer groups and no statistically significant difference in leakage between irrigants. Although there was no statistically significant difference between the irrigants used with either obturation material, there was a trend towards more leakage with the Resilon® material when the final irrigant used was sodium hypochlorite.

A study by Sagsen *et al.* (2006) using the computerized fluid filtration meter, that was measured apical leakage quantity. Obturation was performed using the single cone

technique. In the present study, no significant difference was found between the apical leakage amounts of the groups filled with AH Plus/gutta-percha and sealapex/gutta-percha, but Resilon®/Epiphany® allowed least leakage.

A study by Onay *et al.* (2006) using the computerized fluid filtration meter, that was measured apical leakage quantity. The purpose of this study was to assess the apical sealing ability of the new resin-based Epiphany®/Resilon® root canal filling system, and to compare this with the sealing abilities of different pairings of AH Plus, gutta-percha, Epiphany® and Resilon®. The canal spaces were filled with different combinations of core and sealer using lateral condensation (Table 4).

Table 4 Mean values and standard deviation of different combinations of core and sealer (Modified from Onay *et al.*, 2006)

Material	N	Mean microleakage ± SD ($\mu l/cmH_2O/min^{-1}$ 1.2 atm)
Group I AH Plus + gutta-percha	14	0.000147 ± 0.000069 [a, b]
Group II AH Plus + Resilon®	13	0.000250 ± 0.000149 [b]
Group III Epiphany® + Resilon®	14	0.000153 ± 0.000085 [a, b]
Group IV Epiphany® + gutta-percha	15	0.000067 ± 0.000028 [a]

[a, b] Differences between groups identified with the same superscript symbol were not statistically significant. ($p > 0.05$)

The results for the present study indicating that the Epiphany® sealer and the gutta-percha core combination had the least amount of microleakage than all the other groups. One possible explanation is that the gutta-percha is more compactable than Resilon®, and thus may partically compensate for interfacial stresses by lateral compaction and also help resist dislodgement. However, the result indicated that the Epiphany® sealer and Resilon® core combination was not superior to that of the AH Plus sealer and gutta-percha core combination.

A study by Shipper *et al.* (2004) using the microbial leakage evaluation (split chamber microbial leakage model) has shown significantly higher leakage with AH26 gutta-percha and also Epiphany®-gutta-percha combinations than Epiphany®-Resilon® combination.

The authors tested resistance to bacterial penetration of Resilon®/Epiphany® in extrated single-rooted teeth, and compared it to gutta-percha with sealer filled roots. *Streptococcus mutans* and *Enterococcus faecalis* penetration were tested over a 30 days period through gutta-percha with sealer and Resilon® and Epiphany® using filling technique, namely lateral and warm vertical condensation or a continuous wave of condensation (system B). Percentage of specimens in each group showed leakage during 30 days. Root filled with Resilon® and Epiphany® sealer leaked significantly less than all other roots.

The excellent sealing capacity of Resilon® may be attributed to "Monoblock" which is created by the Resilon® filling closely adapting to the Epiphany® sealer and in turn the Epiphany® sealer adhering to the dentin walls. In contrast, the high-power SEM micrograph showed how the gutta-percha filling pulled away from the AH26 sealer, where as the sealer remained against the dentin wall with its resin tags penetrating the dentin tubes. This gap between gutta-percha and sealer may create an avenue for microleakage, which may explain the rapid rate of leakage in the gutta-percha specimens.

In follow up study by Shipper *et al.* (2005), a dog model was used to compare, in vivo, the efficacy of gutta-percha and AH26 sealer versus Resilon® with Epiphany® primer and sealer in preventing apical periodontitis subsequent to coronal inoculation with oral microorganisms over a period of 14 weeks. The authors concluded that the Resilon® based system was associated with less apical periodontitis than gutta-percha and AH26 sealer (Fig. 14-15).

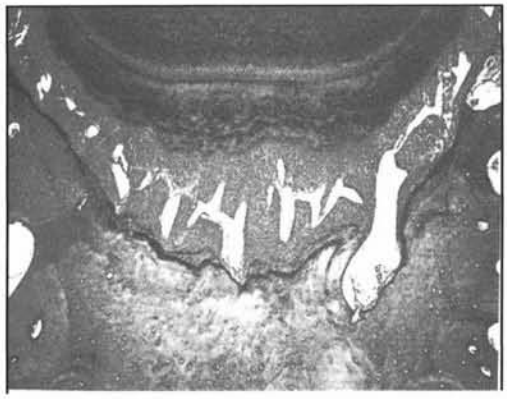

Fig. 14 Healthy periodontium
(Modified from Shipper *et al.*, 2005)

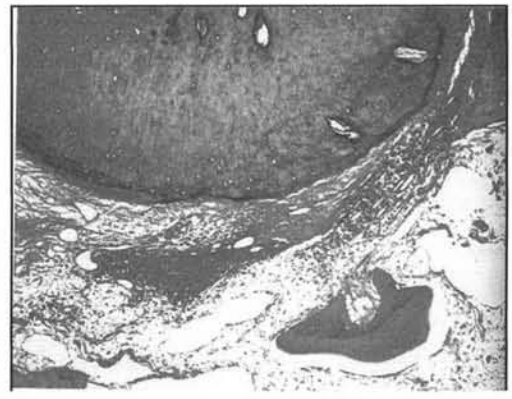

Fig. 15 Inflamed PDL, bone resorpion, abundant inflamed cell (82% of roots filled with gutta-percha/AH26, 19% of roots filled with Resilon®).
(Modified from Shipper *et al.*, 2005)

A study by Wang *et al.* (2006) using a split chamber microbial leakage model using *Streptococcus mutans*, was to investigate the effects of the use of calcium hydroxide as an intracanal dressing on the sealing ability of a thermoplastic synthetic polymer-based root filling (Resilon®) and the leakage was evaluated daily for a period of 30 days. The results showed there was no statistically significant difference in leakage between the groups with calcium hydroxide dressing and the group without calcium hydroxide. Under the condition of this study, using calcium hydroxide as an intracanal medication for 1 week did not adversely affect the apical seal of the root-canal system filled with Resilon®.

A study by Piout *et al.* (2006), compared the micro-leakage of a root canal filled with Resilon® and Epiphany® sealer or gutta-percha (GP) and Roth root canal cement (Zinc oxide eugenal sealer), utilizing either cold lateral condensation or System B. The bacterial micro-leakage test showed no significant difference between results obtained when using gutta-percha or Resilon®, whether using the cold lateral condensation technique or the system B technique, which may explain Roth root canal cement is

known to have an anti-bacterial effect and polymerization shringkage of the Epiphany® sealer was suggest as the possible cause of gap formation leading to apical leakage when using Resilon®. However, the results of the dye penetration test support those of the bacterial micro-leakage test and the antibacterial effect of Roth root canal cement could not have affected the dye penetration.

2.3.3 Sealing ability associated the irrigant

Effect of root canal irrgants on sealing ability

The smear layer, as it relates to the root canal system, is the layer debris on the root canal wall and has been shown to be packed into some of the dentinal tubule. This layer is a direct result of canal instrumentation and is not present uninstrumented canals. The thickness of this layer varies. However, it will generally be about 1 to 2 μm. The depth of tubular packing also varies and has been shown to be as much as 40 μm. The make-up of the smear layer is primarily inorganic particles of calcified tissue. It is also believed to contain some organic material, including necrotic and/or viable pulp tissue, odontoblastic process, bacterial, and blood cells. This layer can not be seen with the naked eye, but under a scanning electron microscope it appears to be amorphous with an irregular and granular surface (Mader *et al.*, 1984).

Goldman *et al.* (1989), using sodium hypochlorite and EDTA, developed what has become recognized as the most effective way to remove smear layer. Some consider that it is desirable to remove the smear layer, as it covers prepared areas and prevents medicaments and filling material from penetrating the dentinal tubules or even contacting the canal wall.

Gettleman *et al.* (1991) the influence of a smear layer on the adhesion of sealer cements to dentin was assessed in recently extracted human anterior teeth. The smear layer removed by washing for 3 min with 17% EDTA followed by 5.25% sodium hypochlorite. Evidence of the ability to remove the smear layer was verified by scanning electron microscopy. The sealer (AH26, Sultan, Sealapex) placed into a mounting jig which was designed for the Intron Universal Testing Machine so that only a tensile load

was applied without shearing. The result show significant difference ($p < 0.001$) among AH26, Sultan, Sealapex, with AH26 being the strongest. The only significant difference with regard to the presence or absence of the smear layer was found with AH26, which had a stronger bond when the smear layer was removed.

The study of Marley *et al.* (2001) demonstrated no significant differences in apical leakage using three irrigants (sterile saline, 5.25% sodium hypochlorite, Peridex (0.12% chlorhexidine)) and three different sealers (Roth'811, Sealapex, and AH26) at both 90- , 180-, 270- and 360-day observation periods. The results from this study demonstrate that there was no sealer leakage differences found using 0.12% chlorhexidine gluconate, therefore chlorhexidine gluconate should be considered useful as an alternative endodontic irrigant with no adverse effect on the apical seal.

Many articles have been written on the physical properties of root canal sealers, including their adhesive strength to dentin and gutta-percha. Adhesion strength measurements may be important to clinical usage, because higher adhesion strengths may reduce leakage in clinical situations. Removal of the smear layer prior to filling the root canal system may enhance the ability of filling materials to enter dentinal tubules. This may actually increase the adhesive strength of sealers to dentin and improve the sealing ability of the filling (White *et al.*, 1984).