

2010

# Cytotoxicity Evaluation of EndoSequence Bioceramic and GuttaFlow sealers in vitro

Keivan Zoufan

*University of Connecticut Health Center*

Follow this and additional works at: [https://opencommons.uconn.edu/sodm\\_masters](https://opencommons.uconn.edu/sodm_masters)



Part of the [Dentistry Commons](#)

---

## Recommended Citation

Zoufan, Keivan, "Cytotoxicity Evaluation of EndoSequence Bioceramic and GuttaFlow sealers in vitro" (2010). *SoDM Masters Theses*. 179.

[https://opencommons.uconn.edu/sodm\\_masters/179](https://opencommons.uconn.edu/sodm_masters/179)

# **Cytotoxicity Evaluation of EndoSequence Bioceramic and GuttaFlow Sealers *in vitro***

**Keivan Zoufan, D.D.S.**

D.D.S., Tehran Azad University, Center of Dental Science, 1999  
D.D.S., University of Southern California, School of Dentistry, 2004

A Thesis  
Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Dental Science  
at the  
University of Connecticut  
2010


**APPROVAL PAGE**

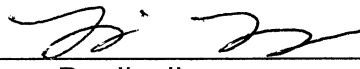
Master of Dental Science Thesis

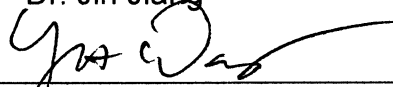
**Cytotoxicity Evaluation of EndoSequence Bioceramic  
and GuttaFlow Sealers *in vitro***

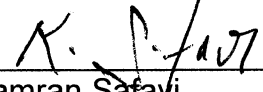
Presented by

Keivan Zoufan D.D.S.

Major Advisor  \_\_\_\_\_  
Dr. Qiang Zhu

Associate Advisor  \_\_\_\_\_  
Dr. Jin Jiang

Associate Advisor  \_\_\_\_\_  
Dr. Yu-Hsiung Wang

Associate Advisor  \_\_\_\_\_  
Dr. Kamran Safavi

University of Connecticut

2010

## **Acknowledgements**

I would like to thank all those who have helped me to carryout and complete my thesis. First and foremost, I would like to acknowledge the dedication and guidance of Dr. Qiang Zhu. His professionalism in the laboratory setting and his approach to research is stress-free and admirable.

I would like to thank Drs. Jiang, Wang for your assistance and guidance.

Dr. Safavi I can not thank you enough for these three wonderful and exciting years as an endodontic resident. You have provided me with the invaluable skills that not only apply to the clinical setting and my professional career but to life in general, and that is to be analytical and to question the things I do and to understand why.

To my parents who have provided me with the gift of education and exposed me to so many incredible experiences throughout my life, thank you.

To my wife, Setareh whom I admire dearly, having spent three years raising our two daughters, Nika and Nura, and working full-time. Thank you for your support, patience and understanding.

## Table of Contents

Title Page	i
Approval Page	ii
Acknowledgements	iii
Table of Contents	iv
Introduction and Background	1
Specific Aims	25
Materials & Methods	26
Results	28
Discussion	31
Conclusion	39
References	40
Figures	50

# **INTRODUCTION**

Sealing ability is one of the fundamental requirements of root filling materials. Numerous root fillings have been introduced to improve the seal of the root canal. However, even in the era of adhesive dentistry, microleakage between root canal dentin walls and root canal fillings still exist and may adversely affect endodontic treatment outcome (Zhang 2009a). Recently, EndoSequence BioCeramic and GuttaFlow sealers have been developed to overcome this issue.

Furthermore, biocompatibility of endodontic sealers is critical to clinical success of endodontic therapy (Orstavik 2005). Whereas the cytotoxicity of conventional endodontic sealers has been well documented, little is known about the toxicity of newer endodontic sealers such as BioCeramic sealer or GuttaFlow. Therefore, the purpose of the current study was to review the Endodontic etiology, various root fillings, biocompatibility tests and at last investigate the early biological response of these new endodontic sealers.

## **Endodontic Etiology**

Bacteria are the prime etiologic factor in development and progression of dental caries and pulp and periapical disease. Miller in 1890 was the first to describe the presence of several different types of bacteria in the necrotic pulp. Despite his findings, the role of microorganisms in the etiology of apical periodontitis eluded researchers and clinicians alike for a number of years.

Keyes(1960) demonstrated that dental caries did not develop in germ free animals fed a cariogenic diet. Kakehashi et al.(1965) elegantly demonstrated that germ-free rats were devoid of apical periodontitis when their pulps were kept exposed to the oral cavity. Soon thereafter, the crucial role of obligate anaerobes in endodontic infections was demonstrated (Möller, 1966). These findings were confirmed by others (Bergenholtz, 1974; Kantz and Henry, 1974; Wittgow and Sabiston, 1975). Sundqvist (1976) further demonstrated by sampling caries-free, human teeth requiring root canal treatment due to trauma. These teeth had necrotic pulps, but only the teeth harboring bacteria in their root canal systems developed periapical radiolucencies, which are the hallmark of apical periodontitis. Since then researchers have accepted the role bacteria and their byproducts play as the principal cause of apical periodontitis.

## **Endodontic Microflora**

The unique local environment of the root canal provides the necessary factors that select for the strict anaerobic genera found therein. Oxygen and its products play an important role in the determination of which species will thrive at the different levels of the root canal system. Traveling apically in the root canal system, oxygen is consumed with an increase in production of carbon dioxide and hydrogen. The developing low reduction-oxidation potential, together with the lack of oxygen selects for the growth and development of anaerobic species.

The nutrients available to the invading bacteria also play a role in determining which bacterial species will thrive in the root canal. When there is

carious exposure of the pulp space, the coronal third of the root canal contains exogenous nutrients such as fermentable carbohydrates which promote the growth and development of those species which derive their energy by carbohydrate fermentation. Facultative anaerobes such as streptococci derive their nutrients in this manner (Edvardsson 1967). However, further down into the middle and apical third of the root canal system, the availability of these carbohydrates decreases and the bacteria found there must derive their nutrients from different endogenous sources. Also, certain bacteria cause inflammation of the periapical tissues and this inflammation leads to an influx of protein-containing exudate. Anaerobic bacteria have the ability to hydrolyze proteins and glycoproteins into amino acids which they can use as nutrients for growth and development (Figdor 2007). Many bacteria also receive their nutrients from the by-products of other species found in the infected root canal system. It is known that mixed bacterial populations contain a food chain in which one species receives its sustenance from another bacterial species (Marsh 1989). For example, black-pigmented anaerobic rods such as *Porphyromonas* have a very specific requirement for Vitamin K and hemin in order to grow. Vitamin K is produced by the *Veillonella* genus and this in turn is used by the black-pigmented rods for growth and development (Gibbons 1964). Species belonging to the *Porphyromonas* and *Veillonella* genera are commonly found together in the infected root canal system.

The pathogenicity of endodontic microflora is determined by a number of factors. Their interactions with other microorganisms and their ability to develop



synergistic relationships with other bacteria are an integral part of establishing virulence and pathogenicity. The importance of the mixed bacterial infections has been well demonstrated in animal studies (Sundqvist *et al.*, 1979; Fabricius *et al.*, 1982). *Prevotella oralis* and 11 other species isolated from the infected, necrotic pulps of teeth with periapical lesions from experimental monkeys were inoculated in various combinations or as a single species into the root canals of other monkeys. Inoculation with individual species resulted in mild apical periodontitis. However, in combinations, the same bacterial species induced a more severe periapical reaction. Interestingly, *Prevotella oralis* did not establish in root canals as a mono-infection. In Contrast, it not only survived but dominated the endodontic flora when introduced with the other species involved (Fabricius *et al.*, 1982).

Bacteria usually do not live as a single microbial species but as a collection of “matrix embedded...multispecies organisms in microecosystems that may be immobilized on the dentinal wall”. Today these groups of bacteria living together, protected by a hydrated exopolysaccharide complex, are known as biofilms (Nair, 2004).

Bacterial lipopolysaccharide (LPS) is a powerful stimulus of the immune response. LPS, also known as endotoxin, forms an integral part of the cell walls of Gram negative bacteria. Endotoxin may be released by bacteria during division or after death. The profound effect of LPS is mediated through its induction of endothelial cells to express adhesion molecules and macrophages to secrete numerous proinflammatory molecular mediators (e.g. TNF-alpha)

(Metzger 2000). As the Gram negative bacteria multiply and/or die in the apical regions of the root canal, LPS leaches out in the periapical tissues (Yanagisawa, 1980) where it induces and sustains periapical inflammation (Dahlén, 1980; Dahlén *et al.*, 1981). The ability of LPS to trigger an immune response is also evidenced by mitogenic stimulation of B lymphocytes to produce non-specific antibodies which are taken up by soluble antigens released by the bacteria preventing the antibodies from acting against the microorganisms (Mims, 1988). This mechanism, like those of other pathogens, stimulates the immune response, but has a secondary mechanism to evade it.

The dynamic interaction between the microbes and their virulence factors and the host defenses determines the different stages of apical periodontitis. The causative agent in the vast majority of apical periodontitis lesions is bacteria (Takehashi *et al.* 1965; Bergenholtz 1975; Sjogren 1976), but iatrogenic trauma, irritation from chemicals or overfilled root fillings all can result in periapical inflammation. The resulting inflammation produces symptoms such as pain, hypereruption of the tooth, and tenderness to touch.

## **Endodontic Treatment**

The classical steps of Endodontic treatments are instrumentation, disinfection and obturation. Thorough cleaning and shaping of the canal system to render it as free as possible of bacteria, and bacterial products, followed by complete sealing of the canal system to prevent bacterial colonization and re-infection. Different materials are available to achieve this objective. Some have

been used for over one hundred years whereas others are relatively new to the endodontic armamentarium. Ongoing efforts to improve the outcome of endodontic therapy will continue to require the development of new materials.

Endodontic filling materials should not only be able to eliminate or minimize the ingress or egress of bacteria and their byproducts, but also have a favorable tissue response that promotes healing of the periapical tissues. Although endodontic sealers are designed to be used only within the root canal during endodontic therapy, they are frequently extruded through the apical constriction. Thus, they are often placed in intimate contact with the periapical tissues for extended periods of time. It is generally accepted that the biocompatibility of endodontic sealers is critical to clinical success of endodontic therapy. Whereas the cytotoxicity of conventional endodontic sealers has been well documented, little is known about the toxicity of newer endodontic sealers such as Bioceramic sealer, or GuttaFlow. Therefore, the purpose of the current study was to evaluate the early biological response of these new endodontic sealers.

## **Root Canal Filling Materials**

The first evidence of a “root filling” comes from a maxillary lateral incisor from the skeletal remains dating back to 200BCE (Hellenistic period) in the northern Negev Desert. It shows a 2.5mm bronze wire wedged in the root canal (Zias and Numeroff 1987). However, Edward Hudson in 1809 is generally given credit for placing the first root filling, which was made of gold foil (Taylor 1922).

The main objectives of root canal therapy are cleaning and shaping, obturating the root canal system in three dimensions and preventing reinfection. Complete sealing of the root canal system after cleaning and shaping is critical to successful endodontic therapy. The intimate contact between endodontic sealers and adjacent periapical tissues, coupled with inadvertent but common extrusion of sealers into the periapical region during treatment, make the biological and sealing properties of sealers paramount to clinical success.

Various techniques have been proposed for root canal obturation and the most common method uses semisolid materials such as gutta-percha in combination with a root canal sealer or paste. Several classes of endodontic sealers are currently used in clinical practice, but all have significant limitations. (Orstavik 2005).

## **Gutta Percha**

Gutta Percha is the most common root filling material in use today. This is interesting, because it is one of the oldest dental materials currently being used. The history of Gutta Percha goes back much earlier than its introduction into dentistry in 1860. The introduction of Gutta Percha into dentistry is credited to Dr. Asa Hill, a Connecticut dentist. Then Gutta Percha was introduced as a root filling material (Bowman 1867). Gutta-percha is a natural product made of the bark of the gutta-percha tree (*Isonandra percha*). Gutta Percha is chemically a polymer based on isoprene (Spangberg 1982). Gutta Percha endodontic points

are composed of approximately 66% zinc oxide, 20% gutta percha, 11% heavy metal sulfates, and 3% wax/resins (Friedman et al. 1975).

Animal studies have shown Gutta Percha to have an acceptable biocompatibility profile with a low degree of toxicity (Spangberg 1969a, Wolfson and Seltzer 1975). However, *in vitro* studies have shown gutta percha to be cytotoxic. This toxicity was believed to be attributed to toxic agents bound to water-insoluble substances (Spangberg 1969a). Later, it was believed that the cytotoxic nature of this material was due to the high content of zinc oxide in gutta percha (Pascon and Spangberg 1990). Finally, it was concluded by Sjogren et al. (1995), that the size and surface character of gutta percha determined the tissue reaction of the material.

It was also apparent that “gutta percha root fillings” in the absence of sealing cement were frequently linked to the manifestations of apical periodontitis manifested both clinically and radiographically (Orstavik 2005). To combat this problem, a cementing medium or sealer was used to accompany the solid gutta percha cone (Rickert 1925).

## **Coated Gutta Percha**

Gutta Percha is now available with the uniform resin layer that may achieve bonding between the solid core and a resin sealer. The manufacturer claims that this will inhibit leakage between the solid core and the sealer. The technique calls for use of EndoRez sealer (Ultradent, South Jordan, UT) with this new solid-core material. The material with a resin sealer was compared with

guttapercha for microleakage. The results indicated a bonding between core and sealer resulting in far less microleakage than gutta-percha (shipper 2004) .

## **Silver Cones**

Jasper (1933) introduced cones made of silver, which he claimed produced the same success rate as gutta-percha and were easier to use. However, when silver points contact tissue fluids or saliva, they corrode. The corrosion products have been found to be cytotoxic and produced pathosis. Therefore, silvercones are no longer used in endodontic practice (AAE position statement, Use of silver points, 2007).

## **Resilon**

Recently, the resin-based obturation systems Epiphany (Pentron Clinical Technologies), RealSeal (SybronEndo), and Resinate (Obtura Spartan, Earth City, MO) have been introduced as alternatives to gutta-percha that brings dentin bonding technology to root canal obturation. The principle of chemically bonding the root-fillings to the canal wall may have potential for improving both the apical and coronal seal of the canal. Resilon (Resilon Research, LLC, Madison, Connecticut) is a thermoplastic synthetic polymer based on polymers of polyester containing bioactive glass and radiopaque fillers. The filler content is approximately 65% by weight. Resilon handles like gutta percha, and is available in the same variety of master cones and accessory cones. The sealer used with Resilon is Epiphany dual cure resin cement (Pentron Clinical Technologies,

Wallingford, Connecticut). The resin sealer bonds to a Resilon core, and attaches to the etched root surface. The manufacturer claims that this forms a “monoblock”. With traditional techniques there is a gutta-percha–sealer interface and a tooth–sealer interface. With Resilon the resin sealer bonds to both the canal wall and the cone. Whether a monoblock is achievable remains controversial. An in-depth review article on the subject of monoblocks indicates that with current materials and techniques, the monoblock has yet to be achieved (Tay, 2007).

### **Activ GP**

Activ GP (Brasseler USA) consists of gutta-percha cones impregnated on the external surface with glass ionomer. Single cones are used with a glass ionomer sealer. The single cone technique is designed to provide a bond between the dentinal canal wall and the master cone. Like Resilon, this material is popular because of its ability to create a “monobloc” obturation. The Activ GP sealer is a glass ionomer sealer, which can adhere chemically and micro-mechanically to the Activ GP cones and bond to the dentin (Koch and Brave, 2006).

A bacterial leakage study comparing Activ GP/glass ionomer sealer, Resilon/Epiphany, and gutta-percha/AH Plus demonstrated no statistically significant differences in leakage at 65 days (Fransen 2008).

## **Endodontic Sealers**

An endodontic sealer should be biocompatible, antibacterial, radiopaque, hermetically seal the root canal system, be dimensionally stable and should have good adhesion to the core material and the root canal wall.

Endodontic sealers are necessary to seal the space between the dentinal wall and the obturating core interface. Sealers also fill voids and irregularities in the root canal, lateral and accessory canals, and spaces between core materials. According to the composition, Sealers can be classified in various classes. These include Silica based, Zinc-oxide-eugenol (ZOE), glass ionomer, resin based, calcium hydroxide based, and silicone based sealers (Orstavik, 2005).

## **Zinc-Oxide Eugenol**

ZOE sealers go by their commercial variants known as Kerr pulp Canal Sealer, Roth sealer, and TubliSeal. ZOE sealers possess some antibacterial activity, however, there have also been shown to exhibit some toxicity when placed directly on vital tissues (Orstavik 2005). This characteristic is partially attributed to the several chemical additives used in the various formulations. These include rosins used for greater dentin adhesion, paraformaldehyde for antimicrobial and mummifying effects, germicides for antiseptic action, and corticosteroids for anti-inflammatory action (Hauman and Love, 2003). However, eugenol itself has been shown to exhibit various properties depending on its concentration and duration of contact with cells. These properties include



inhibition of prostaglandin synthesis, inhibition of sensory nerve activity, and exhibition of antimicrobial activity (Hume 1988). Furthermore, analysis of the setting reaction of zinc-oxide eugenol cements, indicate that the eugenol oil becomes available as a result of surface hydrolysis of the zinc eugenolate chelate. The release rate of eugenol when placed in saline was found to be much greater than through intervening dentin (Hume 1988). Consequently, when in contact with wetter periapical tissues, the release is more rapid with a concentration sufficient to kill cells (Hauman and Love, 2003).

### **Glass Ionomer Sealers**

Glass ionomer sealer has the advantage of chemically bonding to dentin. Ketac-Endo glass ionomer sealer has been shown to be biocompatible and maintain the ability to adhere to dentin (Ray and Seltzer, 1991). Animal studies and *in vitro* cell culture studies have shown minor tissue irritation, low toxicity, and little cytotoxic effect on L-929 cells after a setting time of 24h and 1 week, respectively (Zetterqvist et al. 1987&1988, Pissiotis et al. 1991, Ersev et al. 1999). However, they have also been shown to leak and disintegrate (Schafer et al. 2003).

Active GP sealer is a glass ionomer sealer, which can adhere chemically and micro-mechanically to the Activ GP cones and bond to the dentin (Koch and Brave, 2006). Donadio et al. (2008) demonstrated that Active GP sealer showed moderate toxicity when fresh and less toxicity when set.

Glass ionomer sealers have been shown to be well tolerated by tissues

(Kolokuris et al., 1996). However, *in vitro* studies have also shown glass ionomer cements to be highly toxic to cells in culture (Hume and Mount 1988). This characteristic is reported to be due to the release of uncured acid from the material. However, dentin is believed to play a role in buffering the acid, which might explain its clinical usefulness in successful endodontic treatment outcomes (Friedman et al., 1995).

### **Resin Based Sealers**

Resin based sealers are widely used today. The most popular of these is the AH series developed over 50 years ago by Andre Schroeder in Switzerland. The AH 26 powder contains hexamethylenetetramine (HMT) which is made from formaldehyde and ammonia. HMT is a catalyst/disinfective agent which is hydrolyzed to ammonia and formaldehyde. The amount of formaldehyde release is determined by the amount of freshly mixed sealer which is in contact with water (Koch 1999). AH26 has been shown to release formaldehyde in the freshly mixed state for up to 2 days at which point levels drop off (Spangberg et al. 1993). Consequently, AH26 has been shown to exhibit some cytotoxicity. These findings are concurrent with *in vitro* cytotoxicity studies which show that cytotoxicity levels are greatest immediately after mixing and gradually decrease with time, ceasing after several days (Spangberg, 1969 and Spangberg. and Langeland, 1973). Other resin formulations include resorcin-formaldehyde type, also known as "Russian Red," Diaket, a polyvinyl chloride in polymer which sets by chelation, and EndoREZ, which is based on urethane dimethacrylate, and has

been shown to have hydrophilic properties (Orstavik 2005).

The hydrophilic properties of the urethane methacrylate based resin sealers along with resin coated gutta percha points, has sparked new interest in the development of a sealer based on BisGMA, UDMA, and hydrophilic methacrylates used in combination with a primer to coat the dentinal walls (Orstavik 2005). These materials have given rise to the concept of a homogeneous “monoblock” obturation.

### **Calcium hydroxide based sealers**

Calcium hydroxide based sealers were developed to taking advantage of the biocompatibility and possible bioactivity of calcium hydroxide in pulp capping and an interappointment dressing. However, to be effective in this respect, calcium hydroxide must dissociate into calcium and hydroxyl ions. For this to occur, it would require the sealer to break down or dissolve to some degree. If the dissociation occurred, the sealing ability would be compromised (Soares et al., 1990).

Calciobiotic root canal sealer (CRCS) is a zinc oxide–eugenol sealer with calcium hydroxide as one ingredient. Sealapex (SybronEndo) is a catalyst/base system. The base contains zinc oxide, calcium hydroxide, butyl benzene, sulfonamide, and zinc stearate. The catalyst contains barium sulfate and titanium dioxide as radiopacifiers. Apexit and Apexit Plus (Ivoclar Vivadent, Schaan, Liechtenstein) consist of an activator (disalicylate, bismuth hydroxide/bismuth carbonate, and fillers) and a base (calcium hydroxide, hydrated colophonium,

and fillers).

### **Silicone-based sealers**

Polydimethylsiloxanes are often referred to as Silicones. Silicones possess unique properties such as stability, low surface tension, lack of toxicity and have been utilized in all aspects of health care. Silicone-based sealers, based on silicones widely used as sealants in kitchens, bathrooms, and joining material in construction work, show sound biological performance and can be made to polymerize without shrinkage. The first of those materials were based on C-silicones (condensation cross-linking silicones); a newer material is based on A-silicones (addition cross-linking), such as RoekoSeal (Automix, Roeko Dental Products, Langenau, Germany).

The new root canal filling paste, GuttaFlow (GF; Coltene/Whaledent, Altstätten, Switzerland), is a modification of this sealer. It contains Gutta Percha particles as filler. According to the manufacturer, its ingredients include Gutta percha, zinc oxide, zircon dioxide, paraffin and silicone-based oils, hexachloroplatinic acid and silicic acid. The manufacturer is claiming an improved seal by slight (0.2%) expansion (Bouillaguet 2006). Roeko-Seal, Endo-Fill, and GuttaFlow are examples of silicone sealers (Ortsavik 2005).

## **Silica based sealers**

Recently, Silica based sealers have been introduced to the market and described by manufacturer as a bioceramic material. Bioceramic materials are “ceramic products or components employed in medical and dental applications, mainly as implants and replacements, which have osteoinductive properties.” Many materials used today in dentistry are considered to be bioceramics, such as zirconia, hydroxyapatite, tricalcium phosphate, tricalcium silicate, and dicalcium silicate (Zhang 2009a). According to this definition, both GMTA and WMTA are considered to be bioceramics.

EndoSequence BC sealer (Brasseler, Savannah, GA), also known as iRoot SP root canal sealer (Innovative Bioceramic Inc, Vancouver, Canada), has recently been introduced to the market. According to manufacturer’s description, BC sealer is premixed, injectable white hydraulic cement and composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and various filling and thickening agents which requires the presence of water to set and harden. To date there are few studies evaluating this new material.

## **Biocompatibility**

Biocompatibility describes the ability of a material to perform with an appropriate host response when applied as intended (Williams 1992). There are various culture methods utilized for the evaluation of the toxic characteristics of

dental materials. Organ culture, tissue culture, and cell culture comprise the range of culturing methods available. Of these methods, organ culture, due to its impracticality, was not developed for use. Consequently, tissue culture was the original technique employed for evaluating toxicity. While all methods assess the degree of toxicity, the techniques for evaluating cell injury are many. Depending on the type of study, cell injury measures included the degree of proliferation from the explant periphery, attachment to or detachment from a substrate, colony inhibition or growth inhibition in assay, cell counting and mitotic index, cell membrane permeability as measured by dye exclusion, vital staining,  $^{51}\text{Cr}$  release, and finally, evaluation of macromolecular biosynthesis and analysis of differentiated functions (Spangberg 1981).

The MTT assay is also developed for evaluating toxicity of a test material at the cellular level. This method was first described by (Mosmann 1983). This assay is widely used for biocompatibility evaluation in *in vitro* studies (Huang et al. 2002b, Camps and About 2003, Huang et al., 2004). MTT stands for [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide]. It measures the ability of a mitochondrial dehydrogenase enzyme found in viable cells to cleave the tetrazolium rings of MTT (pale yellow). This cleavage results in the formation of formazan crystals (dark blue). These crystals are largely impermeable to cell membranes and accumulate within healthy cells. The addition of a detergent results in solubilization of the cells and the release of the crystals. The number of surviving cells is directly proportional to the level of the formazan crystals produced. The color can be quantified using a simple colorimetric assay. The

results can then be read on a multiwell scanning spectrophotometer (ELISA reader). The advantages of this assay method are that it is simple, rapid, and precise and does not require the use of radioisotopes (Eldeniz et al., 2007).

The majority of *in vitro* techniques are based upon material-cell contact by diffusion and evaluation of the effects by calculating the degree of destruction of the monolayer. Monolayer disruption, target cell lysis, cell migration inhibition, and colony formation represent the array of methods for calculating these effects. However, the major flaw in all these methods is the lack of direct cell-material contact and the reactions proceed slowly. Moreover, the criteria for cell death are not clearly defined. Finally, the evaluation criterion is a subjective measurement (Spangberg 1973).

In response to these drawbacks, Spangberg (1973) adopted a technique utilized in immunologic research that incorporates the use of radioactive markers. In this method, DNA-synthesizing cells are incubated with  $^{51}\text{Cr}$  and the radiolabel is incorporated into the DNA. Once the cells are exposed to the toxic material, cell damage occurs and the labeled DNA is released from the cell. Consequently, this technique provides a rapid and highly sensitive method for objective quantification of cell damage with good accuracy while allowing for adequate cell-material contact for both soluble and non-soluble materials (Spangberg 1973). Spangberg and Langeland (1973) used this technique to evaluate several root canal filling materials. Amongst these were various zinc-oxide eugenol formulations, N2, Tubli-Seal, AH26, gutta-percha, and chloropercha. In the fresh state, AH26 demonstrated a high degree of toxicity though less than the

remaining materials at 1h cell material contact but significantly increased at 4h as measured by  $^{51}\text{Cr}$  release. Cell lysis occurred for all materials except Moyco chloropercha at 24h. Gutta-percha points only slightly increased release of  $^{51}\text{Cr}$  after 24h of cell material contact. Lastly, analysis of the medium extracts showed AH26 to exhibit a significant increase in radiochromium release though less than Tubli-Seal. N2 and Proco-Sol caused complete cell lysis (Spangberg and Langeland, 1973).

In conclusion, studies have shown gutta percha to be relatively inert. Based on these early cytotoxicity studies, Langeland (1974) outlined the properties of root canal sealants and pastes relative to toxicity and resorbability and evaluated via tissue culture experiments, implant studies, and usage tests. These criteria still hold true today. He concluded that 1) in their freshly mixed state, all sealers cause irritation, 2) once set or cured, some sealers lose their irritant properties and become relatively inert, 3) all sealers are resorbable, 4) the possibility of sealer components to travel and settle in internal organs is a risk, 5) paste only root fillings are not acceptable, 6) the root canal should be filled as much as possible with a solid or semi-solid nonirritating material, and 7) no amount of sealer should be in contact with remaining pulp or the periapical tissue.

The ISO (1984) in technical report 7405 established a structured approach for evaluating the biocompatibility of dental materials. It includes three levels. 1) initial tests (cytotoxicity, mutagenicity); 2) secondary tests (sensitization, implantation tests, mucosal irritation); and 3) usage tests.



Biocompatibility of endodontic materials can be evaluated by various tests. These include genotoxicity, mutagenicity, carcinogenicity, cytotoxicity, histocompatibility or microbial effects. Therefore, it is impossible to determine the relative biological characteristics of a material using one test alone. Several structured *in vitro* and *in vivo* tests are necessary for this type of assessment (Hauman and Love 2003).

### **Cell Culture Studies**

Cell culture tests have been employed to evaluate the cytotoxic reactions induced by endodontic materials (Rappaport et al. 1964, Keresztesi and Kellner 1966). Permanent cell lines such as HeLa, 3T3 or L929 cells and oral fibroblasts are commonly used for these studies. Despite this, primary cell lines are thought to be more relevant for biocompatibility studies because the materials tested are more likely to come into contact with cells such as human fibroblasts. On the contrary, the advantage that is offered by permanent cell lines is that they continue to grow as long as there is nutrient present, whereas primary cell lines have a predetermined life span which means that growth will plateau regardless of the growth conditions (Key et al., 2006). In addition, L929 cells are commonly used because they have been shown to be more prone to toxic products than human gingival fibroblasts (Pissiotis and Spangberg 1991).

Various biological endpoints are used to measure the degree of toxicity elicited by a given material. These include growth inhibition, determination of the effective dose 50 (ED<sub>50</sub>), membrane integrity, DNA, RNA, or protein synthesis,

and alteration in cell morphology (Hauman and Love 2003).

## **Implantation Studies**

Implantation studies represent an *in vivo* method for evaluating nonspecific tissue reactions caused by endodontic materials and are subsequently evaluated histologically after the material has been implanted into various animal tissues. Test materials may be directly injected or implanted either directly or placed within teflon, silicone or polyethylene tubes. Implantation sites can vary from subcutaneous connective tissue, muscle or bone of rats, rabbits, guinea pigs, hamsters and ferrets (Hauman and Love 2003).

## **Usage Tests**

Usage tests are categorized as specific *in vivo* toxicity tests which involve the use of the test material in animals. Materials are usually deliberately overfilled to determine their effects on the periapical tissues. It is important to note, that due to ethical considerations, usage tests in humans are rarely performed. Moreover, they are time consuming, expensive and difficult to control (Hauman and Love 2003). Furthermore, it is often difficult to control for the influence of other factors both technical and biological such as infection or dentin chips between the filling material and vital, reacting tissue (Orstavik, 1988).

In evaluating all three levels of biocompatibility testing, it should be noted that usage tests, in addition to their inherent pitfalls, have a tendency to demonstrate less tissue-irritating characteristics of sealers than one would expect

from cell culture or implantation experiments. On the other hand, while cell culture studies are valuable for identifying individual toxic components of a material and are the most sensitive test, they provide little relevant clinical information. Therefore, ranking of toxicity will differ depending on the technique (Orstavik 1988, Mjor 1988).

## **New Root Filling Materials**

### **GuttaFlow**

GuttaFlow (Coltene Whaledent, Switzerland) is a silicone based sealer which consists of a mixture of gutta-percha powder, poly-dimethylsiloxane and silver particles. According to the manufacturer, its ingredients include Gutta-Percha, zinc oxide, zircon dioxide, paraffin and silicone-based oils, hexachloroplatinic acid and silicic acid. It comes in a unidose capsule and is injected after mixing. The silicone is mixed with gutta-percha powder to form what the company calls a “two-in-one” cold filling system. GuttaFlow is flowable, and sets within 10 min. It is supposed to be easily applied using lentulo spiral or application syringe. The manufacturer claims that GuttaFlow slightly expands (0.2%) during setting, which results in an improved apical seal of the root end fill. The major advantages of this material are that it flows at room temperature and that a primer can be used to increase its wettability to radicular dentin.

Polydimethylsiloxane has been used widely in prosthodontics because of its low dimensional change (0.6–0.15%) and low water absorption. Silicon soft liners have been proposed for use in patients with irritation of the denture bearing mucosa. Room temperature silicon is popular as a maxillofacial material for correcting facial defects because of its ease of processing and good physical properties. Also silicon-low temperature isotropic in a subperiosteal implant is found to be very biocompatible (Philipps, 1981). Also, Guttaflow was ineffective in killing the bacteria *in vitro*, (Orstavik 2004).

## **EndoSequence BC sealer**

EndoSequence BC sealer has been recently developed to improve the seal of root canal filling. The sealer consists of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents. The manufacturer claims that superior biocompatibility and sealing ability are due to its high hydrophilic properties unlike traditional hydrophobic materials. As a hydrophilic sealer it utilizes moisture within the canal to complete the setting reaction and it does not shrink on setting. It is biocompatible and exhibits antimicrobial properties during the setting reaction (Zhang 2009b).

## **SPECIFIC AIMS**

Even though GuttaFlow and BC sealer have been on the market, there are few studies to investigate their biocompatibility. Therefore, the purpose of this experiment was to test the null hypothesis that there was no difference in cytotoxicity between these two contemporary systems, GuttaFlow and BC sealer and the traditional root canal sealers, AH plus and TubliSeal.

The specific aim of this study is to evaluate the cytotoxicity of EndoSequence BC sealer and GuttaFlow sealer in an *in vitro* cell culture system, and compare them with traditional AH plus sealer and TubliSeal sealer.

# **MATERIALS AND METHODS**

## **Cell Culture**

L929 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in Eagle's minimum essential medium (EMEM) (ATCC), supplemented with 10% fetal bovine serum (Hyclone Laboratories Inc. Logan UT), and 1% antibiotic/antimycotic cocktail (300 units/ml penicillin, 300 µg/ml streptomycin, 5 µg/ml amphotericin B; Gibco BRL, Gaithersburg, MD) under standard cell culture conditions (37 °C, 100% humidity, 95% air and 5% CO<sub>2</sub>).

## **Root Canal Sealers**

The root canal sealers used in this study were:

1. EndoSequence BC Sealer (Brasseler USA, Savannah, Georgia).
2. GuttaFlow (Coltene/Whaledent, Langenau, Germany).
3. AH Plus Jet (Dentsply DeTrey GmbH, Konstanz, Germany).
4. Tubliseal Xpress (SybronEndo, Orange, California).

## **Cell Cytotoxicity Assay**

The cytotoxicity of the different sealers was evaluated in two ways:

In one set of experiments, set sealer was used. Sealers were mixed according to manufacturer's instruction, placed into the 24-well plates at 0.5

mg/well and incubated for 72 hours in a cell culture incubator to allow the sealer to become set.

In another set of experiments, fresh mixed sealers were placed into the 24-well plates at 0.5 mg/well.

The fresh and set sealers were incubated with three different amounts of cell culture medium: 300  $\mu$ L, 600  $\mu$ L, and 1000  $\mu$ L for 24 and 72 hours. Each sealer had a total of 12 eluate groups to be evaluated. There were a total of 48 eluate groups for the four sealers.

For the cell cytotoxicity assay, L929 cells were seeded into 96-well plates at  $3 \times 10^4$  cells/well and cultured for 24 hours to allow adhesion. Then 100  $\mu$ L of the sealer eluate was placed into the culture wells. Cells cultured with 100  $\mu$ L medium served as a control group. After 24h incubation, the cytotoxicity was evaluated by MTT assay according to manufacturer's instructions (ATCC, Manassas, VA).

## **Statistical Analysis**

Cell viability was calculated as the ratio of the number of live cells in the experimental group to that in the control group, and the results were analyzed with one-way ANOVA. Post hoc tests were done with Scheffe's test. Each experiment was repeated three times.



## **RESULTS**

### **Sealer Setting:**

When the tested sealers were left on the bench top at room temperature, EndoSequence BC sealer did not set even after 2 months (Figure 1). The other three sealers had become set when they were checked after 24 hours (Figure 1). No indentation or penetration with number 15 blade could be made in the sealer pellet after setting.

When the tested sealers were placed in a cell culture incubator for 24 hours, all sealers became set (Figure 2). EndoSequence BC sealer released a clear liquid (Figure 2).

### **Cytotoxicity of Set Sealers**

When L929 cells were cultured with one-day eluate of set sealers, AH Plus and Tubliseal were more cytotoxic than GuttaFlow and EndoSequence BC sealer, and Tubliseal exhibited the most cytotoxicity in the 300  $\mu$ L eluate group (Fig. 3). In the 600  $\mu$ L eluate group, Tubliseal was more cytotoxic than the other three sealers and no cytotoxicity difference was observed among AH Plus, BC sealer and Tubliseal (Fig. 3). In the 1000  $\mu$ L eluate group Tubliseal was more cytotoxic than AH Plus and BC sealer. No cytotoxicity difference was observed between Tubliseal and GuttaFlow (Fig. 3).

When cells were cultured with three-day eluate of set sealers, AH Plus and Tubliseal were more cytotoxic than GuttaFlow and EndoSequence BC sealer

in the 300  $\mu$ L and 600  $\mu$ L eluate groups (Fig. 4). AH Plus was more cytotoxic than Tubliseal in the 600  $\mu$ L eluate groups (Fig. 4). Tubliseal was more cytotoxic than AH Plus in the 1000  $\mu$ L eluate group (Fig. 4). Also no cytotoxicity difference was observed among AH Plus, BC sealer and GuttaFlow, and among Tubliseal, BC sealer and GuttaFlow (Fig. 4). There was no cytotoxicity difference between GuttaFlow and EndoSequence BC sealer in all the eluate groups (Fig. 4).

### **Cytotoxicity of Fresh Sealers:**

When cells were cultured with one-day eluate of fresh sealers, AH Plus exhibited the most cytotoxicity, and Tubliseal was more cytotoxic than BC sealer and GuttaFlow in the 300 and 600  $\mu$ L eluate groups (Fig. 5). In the 1000  $\mu$ L eluate groups, AH Plus exhibited the most cytotoxicity, and there was no cytotoxicity difference among BC sealer, Tubliseal, and GuttaFlow (Fig. 5).

When cells were cultured with three-day eluate of fresh sealers, AH Plus exhibited the most cytotoxicity, and Tubliseal was more cytotoxic than BC sealer and GuttaFlow in all the 300, 600, and 1000  $\mu$ L eluate groups (Fig. 6). No cytotoxicity difference was observed between GuttaFlow and EndoSequence BC sealer in all the eluate groups (Fig. 4).

## **Summary**

No cytotoxicity difference was observed between EndoSequence BC sealer and GuttaFlow, and they were less cytotoxic than AH Plus and Tubliseal.

## **DISCUSSION**

Biomaterials science and technology have been expanding tremendously in recent years. The results of this evolution are obvious in dental applications especially with the contemporary development of Nanotechnology. Among biomaterials, bioceramics possess a specific field due to various interactions with the biological tissues.

Interaction between biomaterials and natural tissues is a significant subject for biomaterials science. Information originating from this interaction is essential to aid the design and fabrication of new biocompatible and bioactive materials. In last few decades, 'biomaterials' is a great developing research area in the interdisciplinary field of materials-related sciences with various applications in medical and dental treatment. The development and manufacture of biomaterials demand high standards of production process, due to the combination of both high-level mechanical and biological properties.

Bioceramics related to their interactions with the biological tissues are classified as inert and bioactive.

### **Inert Bioceramics**

Inert bioceramics are biocompatible materials, exhibiting a morphological fixation with the surrounding tissues without any biochemical bonding. In dentistry, the most significant representatives are alumina ( $\text{Al}_2\text{O}_3$ ), zirconia ( $\text{ZrO}_2$ ) and carbons (C), which have also been widely used in medical fields, e.g., orthopedics, such as joint or tissue replacements and for coating metal implants

to improve their biocompatibility. During the last decades, Ti and its alloys have replaced these inert bioceramics to a great extent. However nowadays, with the advance of nanotechnology these inert bioceramics have gained active roles.

## **Bioactive bioceramics**

The bioactive materials elicit a specific biological response at the interface, which results in the formation of a biological bond between the adjacent tissues and the material itself. They include Calcium Phosphate Ceramics (CPC), bioactive glasses, bioactive glass ceramics and Mineral Trioxide Aggregate (MTA). The rapid healing, the thickness and the strength of the bonding zone depend upon the various materials. The common characteristic of all the known bioactive implant materials is that in order to have a bond with tissues, a layer of biologically active hydroxylcarbonate apatite must form at the interface (Adamopoulos 2007). The formation of this apatite, which resembles bone apatite, is mostly due to the calcium and phosphorous ions coming out from the biomaterial surface. The apatite layer is the bridge connecting the ionically bonded bioceramic to the organically bonded bone.

Bioceramic (BC) sealer is composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and various filling and thickening agents. As a hydrophilic sealer it utilizes moisture within the canal to complete the setting reaction. Moisture from dentin is supposed to facilitate the hydration reactions of calcium silicates to produce calcium silicate hydrogel and calcium hydroxide. Calcium hydroxide partially reacts with the phosphate to form

hydroxyapatite and water (Zhang 2009b). The water is supposed to start again the reaction cycle and react with calcium silicates to produce calcium silicate hydrogel and calcium hydroxide.

BC sealer possesses antibacterial effect due to the combination of high pH, hydrophilicity, and active calcium hydroxide diffusion. However, the antimicrobial effect was greatly diminished at 7 days after mixing. The antibacterial effect might be a combination of high pH, hydrophilicity, and active calcium hydroxide diffusion (Zhang 2009a). The material also has excellent radiopacity and is available in a preloaded syringe with intracanal tips that can be bent to facilitate placement in clinical situations. Also Zhang (2009b), in the *in vitro* study, showed equal sealing ability with BC and AH plus sealers.

GuttaFlow is a recently introduced alternative root filling material. It consists of a polydimethylsiloxane matrix highly filled with gutta percha powder (<30 micron) and nano-silver particles (GuttaFlow instruction Inc. 2008). The finely ground gutta percha powder of the sealer is claimed to create bonds with the master gutta percha cone providing better seal. In addition, there is nano-silver in the material to prevent the re-growth of bacteria (GuttaFlow instruction Inc. 2008). GuttaFlow showed similar leakage results when used with lateral compaction or System B techniques, but it demonstrated expanding capacity over time compared to compacted gutta-percha and AH26 over a 12-month period (Kontakiotis 2007). When GuttaFlow was compared to two single-cone obturation systems, it was found that the apical seal was as effective as AH Plus

used with vertical compaction (Monticelli, 2007). However, Vasiliadis (2010) demonstrated neither AH Plus nor GuttaFlow completely sealed the root canal.

## **Biocompatibility of Sealers**

This study compared the cytotoxicity of BC and GuttaFlow sealers with the conventional root canal sealers, AHplus and Tubliseal. In the present study, L929 mouse fibroblasts were placed in the prepared elute of the tested materials. L929 mouse fibroblasts represent cells of a permanent cell line that are commercially available. L929 is easy to prepare and culture, provides more reproducible results, and is routinely used for cytotoxicity studies (Groth et al., 1995).

The MTT assay is a standard assay to evaluate the cytotoxicity of endodontic materials. The MTT assay was used in this study to measure the cytotoxicity elicited by the given test materials. This assay measures cytotoxicity, proliferation or activation of cells. By measuring the metabolic activity of cells, as measured by the ability of mitochondrial dehydrogenase enzymes to cleave [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] and form dark blue formazan crystals, the assay detects living, but not dead cells. The level of formazan product created is proportional to the number of surviving cells and quantified in a multiwall scanning spectrophotometer. The main advantages of this assay are that it is simple, rapid, precise, and does not require the use of radioisotopes (Mossman 1983).

The elution of the sealers mainly tests whether the material will leach cytotoxic substances which inhibit cell activity and growth. The advantage of this

method is not only quantitative and reproducible, but also to test fresh and set material at the various stages. A series of elutions with various times could provide the cytotoxic degree of the tested material. However, different materials may have different elution processes, so the elution volume and elution time should be carefully considered.

In this study, the cytotoxicities of four sealers were evaluated. AH Plus was found to be the most cytotoxic when fresh and less cytotoxic when set. These findings are consistent with other studies which have demonstrated that AH26 is most cytotoxic initially after mixing and the cytotoxicity gradually decreases with time (Spangberg, 1969 and Spangberg and Langeland, 1973). This has been shown to be related to the release of formaldehyde (Spångberg et al., 1993).

AH26 and AH Plus demonstrated both cytotoxicity and genotoxicity in vitro, when tested with rat cerebral astrocyte cell culture (Huang TH, 2002). When used with human cervical carcinoma (HeLa) cells and mouse skin fibroblasts (L929), AH Plus was found to be more toxic than AH26 (Miletic, 2000). Pinna et al., found AH Plus to be severely toxic to cells during the first 72 hours, but toxicity decreased over 5 weeks ( Pinna, 2008). The results of the present experiment are in agreement with these earlier studies.

Although zinc-oxide eugenol (ZOE) containing sealers are the most widely used sealers in endodontics, non-specific biocompatibility tests showed eugenol elicited a pronounced tissue irritation (Langeland,1978). Araki et al.,(1993) demonstrated that the increased cytotoxicity of ZOE sealer was caused by its



eugenol content. Hume (1986) demonstrated that when ZOE was in contact with the soft tissue, release of eugenol caused local cell death. Kolokouriset al (1998) also demonstrated that eugenol liberation from ZOE containing compounds was initially high immediately after mixing, but decreased overtime with a progressive decrease in connective tissue reaction.

The TubliSeal sealer, a eugenol based sealer, was shown to be cytotoxic when fresh and set, but its toxicity gradually declined and was less than AH Plus. Eugenol has been identified as one of the major ingredients responsible for the material's cytotoxicity (Serene et al., 1988, Guigand et al., 1999, Hauman and Love, 2003). The results of our study are in agreement with these earlier studies.

The first silicone-based endodontic sealer, RoekoSeal has been reported to be non-cytotoxic (Miletic et al. 2005). GuttaFlow is the most recent silicone based sealer which is manufactured by adding gutta-percha powder to the silicone matrix. Silicone sealers, based on their composition, are expected to be biocompatible.

However, there are few reported studies on the cytotoxicity of GuttaFlow. Gerosaetal (2003) compared the cytotoxicity of GuttaFlow with Pulp Canal Sealer and resin-based Acroseal (Septodont, France) and found GuttaFlow is less cytotoxic than Pulp Canal Sealer and Acroseal. Miletic et al., (2005) found no cytotoxic effects of the silicon-based sealer RoekoSeal on HeLa cells and mouse skin fibroblasts (L929). Gencoglu et al., (2003) investigated the connective tissue response of RoekoSeal and found new granulation tissue with fibrous tissue adjacent to RoekoSeal on day 30 after treatment. In the animal study, Gencoglu

et al (2009) investigated the remote organ toxicity and connective tissue reaction to GuttaFlow. Slight cytotoxic effects were observed in all of the investigated organs such as lung, liver, kidney and skin of albino Wistar rats within a period of 24 h and this effect decreased in time and was found acceptable.

In contrast, GuttaFlow was shown to exhibit a comparatively low cytotoxicity and less cell damage than with resin-based systems or AH26 (Bouillaguet et al., 2006) and it was only slightly more cytotoxic than the pure silicone sealer (Eldeniz, 2005). Guttaflow and RoekoSeal demonstrated comparatively low cytotoxicity, especially when tested fresh (Bouillaguet 2006, Eldeniz 2007). The results of our study demonstrated that GuttaFlow was comparatively non-cytotoxic.

Bioceramics currently in use in both dentistry and medicine have been tested for biocompatibility. Alumina and zirconia are among the bioinert ceramics used for prosthetic devices. Based on their composition, no toxicity would be expected and in this study relative biocompatibility of BC sealer was observed. BC sealer showed cell viability similar to the control group in freshly mixed and set conditions. During setting, BC sealer released a clear fluid. It would be expected to be water for the reason that hydration reactions of calcium silicates produce calcium silicate hydrogel and calcium hydroxide. Calcium hydroxide partially reacts with the phosphate to form hydroxyapatite and water (Zhang 2009b).

Setting time, antimicrobial activity, dimensional stability, solubility, and effective root canal sealing should be investigated further

## CONCLUSION

It is generally accepted that the biocompatibility of endodontic sealers is critical to clinical success of endodontic therapy. Biocompatibility is only one of the several properties which define the ideal root filling material. While the majority of biocompatibility studies are done *in vitro*, understanding the relevance to the clinical setting is often difficult to impossible. Furthermore, various biological endpoints are used to measure a material's toxicity which does not allow for uniformity and a direct comparison of materials from one study to another. Therefore, a standardized approach to all *in vitro* material testing that is as clinically relevant as possible needs to be implemented. In conclusion, the findings of this study demonstrated that BC and GuttaFlow sealers showed comparable biocompatibility. Other properties of BC and GuttaFlow, such as solubility, dimensional stability, *in vivo* endodontic usage, etc., need to be investigated in the future. The clinical implications of this study may suggest that caution should be taken not to overfill when using the AH Plus or TubliSeal Sealers.

## REFERENCES

- Adamopoulos O, Papadopoulos T. Nanostructured bioceramics for maxillofacial applications. *J Mater Sci Mater Med* 2007;18:1587-97.
- Anthony LP, Grossman LI. A brief history of root-canal therapy in the United States. *J Am Dent Assoc* 1945;32:43-50.
- Bergenholtz G. Retreatment of endodontic fillings. *Scand J Dent Res* 1979;87:217-24.
- Beyer-Olsen EM, Orstavik D, Eriksen HM. Radiographic voids and leakage along root fillings in vitro. *Int Endod J* 1983;16:51-8.
- Bianchi F, Vassalle C, Simonetti M, Vozzi G, Domenici C, Ahluwalia A. Endothelial cell function on 2D and 3D micro-fabricated polymer scaffolds: application in cardiovascular tissue engineering. *J Biomater Sci Polym Ed* 2006;17:37-51.
- Biggs SG, Knowles KI, Ibarrola JL, Pashley DH. An in vitro assessment of the sealing ability of Resilon/Epiphany using fluid filtration. *J Endod* 2006;32:759-61.
- Bouillaguet S, Wataha JC, Tay FR, Brackett MG, Lockwood PE. Initial in vitro biological response to contemporary endodontic sealers. *J Endod* 2006;32:989-92.
- Bowman GA. A history of dentistry in Missouri. Ovid Bell Press, Fulton, MO, 1938, p.423.
- Browne RM, Friend LA. An investigation into the irritant properties of some root filling materials. *Arch Oral Biol* 1968;13:1355-70.
- Camps J, About I. Cytotoxicity testing of endodontic sealers: a new method. *J Endod* 2003;29:583-6.
- Chogle S, Mickel AK, Huffaker SK, Neibaur B. An in vitro assessment of iodoform gutta-percha. *J Endod* 2005;31:814-6.
- Curson I. History and Endodontics. *Dent Pract Dent Rec* 1965;15:435-439.
- Das S. Effect of certain dental materials on human pulp in tissue culture. *Oral Surg Oral Med Oral Pathol* 1981;52:76-84.
- Donadio M, Jiang J, He J, Wang YH, Safavi KE, Zhu Q. Cytotoxicity evaluation of Activ GP and Resilon sealers in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009 ;107:e74-8.

Economides N, Koulaouzidou EA, Beltes P, Kortsaris AH. In vitro release of hydroxyl ions from calcium hydroxide gutta-percha points. *J Endod* 1999;25:481-2.

Edwardsson S, Krasse B. Human streptococci and caries in hamsters fed diets with sucrose or glucose. *Arch Oral Biol* 1967;12:1015-6.

Eldeniz AU, Mustafa K, Ørstavik D, Dahl JE. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. *Int Endod J* 2007;40:329-37.

Engstrom, B., The correlation between positive culture and the prognosis of root canal therapy. *Odont Revy* 1964;15:257.

Ersev H, Schmalz G, Bayirli G, Schweikl H. Cytotoxic and mutagenic potencies of various root canal filling materials in eukaryotic and prokaryotic cells in vitro. *J Endod* 1999;25:359-63.

Fabricius L (1982). Oral bacteria and apical periodontitis. An experimental study in monkeys (dissertation). Göteborg, Sweden: University of Göteborg.

Fabricius L, Dahlén G, Öhman AE, Möller ÅJR. Predominant indigenous oral bacteria isolated from infected root canal after varied times of closure. *Scand J Dent Res* 1982a;90:134–144.

Fabricius L, Dahlén G, Holm SC, Möller ÅJR. Influence of combinations of oral bacteria on periapical tissues of monkeys. *Scand J Dent Res* 1982b;90:200–206.

Figdor D, Sundqvist G. A big role for the very small – understanding the endodontic microbial flora. *Aust Dent J* 2007;52(1Suppl):S38-51.

Fransen JN, He J, Glickman GN, Rios A, Shulman JD, Honeyman A: Comparative assessment of Activ GP/glass ionomer sealer, Resilon/Epiphany, and gutta-percha/AH Plus obturation: a bacterial leakage study. *J Endod* 2008;34:725.

Friedman S, Lost C, Zarrabian M, Trope M. Evaluation of success and failure after endodontic therapy using a glass ionomer cement sealer. *J Endod* 1995;21:384-90.

Friedman CM, Sandrik JL, Heuer MA, Rapp GW. Composition and mechanical properties of gutta-percha endodontic points. *J Dent Res* 1975;54:921-5.

Gencoglu N, Sener G, Omurtag GZ, Tozan A, Uslu B, Arbak S, Helvacioğlu D. Comparison of biocompatibility and cytotoxicity of two new root canal sealers. *Acta Histochem*. 2009. [Epub ahead of print]

Geurtsen W, Leinenbach F, Krage T, Leyhausen G. Cytotoxicity of four root canal sealers in permanent 3T3 cells and primary human periodontal ligament fibroblast cultures. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:592-7.

Gibbons RJ, Engle LP. Vitamin K compounds in bacteria that are obligate anaerobes. *Science* 1964;146:1307-9

Grossman LI. Physical properties of root canal cements. *J Endod* 1976;2:166-175.

Groth T, Falck P, Miethke RR. Cytotoxicity of biomaterials—basic mechanisms and in vitro test methods: a review. *ATLA* 23 (1995), pp. 790–799.

Guigand M, Pellen-Mussi P, Le Goff A, Vulcain JM, Bonnaure-Mallet M. Evaluation of the cytocompatibility of three endodontic materials. *J Endod* 1999;25:419-23.

Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. *Int Endod J* 2003;36:75-85.

Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 2. Root Canal Filling Materials. *Int Endod J* 2003;36:147-160.

Heitman EP, Joyce AP, McPherson JC 3rd, Roberts S, Chuang A. An in vitro evaluation of the growth of human periodontal ligament fibroblasts after exposure to a methacrylate-based endodontic sealer. *J Endod* 2008;34:186-9.

Hiraishi N, Papacchini F, Loushine RJ, Weller RN, Ferrari M, Pashley DH, Tay FR. Shear bond strength of Resilon to a methacrylate-based root canal sealer. *Int Endod J* 2005;38:753-63.

Hommeze GM, Coppens CR, De Moor RJ. Periapical health related to the quality of coronal restorations and root fillings. *Int Endod J* 2002;35:680-9.

Hunter, H.A. The effect of gutta percha, silver points and Rickert's root sealer on bone healing, *J Can Dent Assoc* 23 (1957), p. 385

Huang FM, Tai KW, Chou MY, Chang YC. Cytotoxicity of resin-, zinc oxide-eugenol-, and calcium hydroxide-based root canal sealers on human periodontal ligament cells and permanent V79 cells. *Int Endod J* 2002;35:153-8.

Huang TH, Ding SJ, Hsu TZ, Lee ZD, Kao CT. Root canal sealers induce

cytotoxicity and necrosis. J Mater Sci Mater Med 2004;15:767-71.

Hume WR. In vitro studies on the local pharmacodynamics, pharmacology and toxicology of eugenol and zinc oxide-eugenol. Int Endod J 1988;21:130-4.

Hume WR, Mount GJ. In vitro studies on the potential for pulpal cytotoxicity of glass-ionomer cements. J Dent Res 1988;67:915-8.

Jasper EA. Root Canal therapy in modern dentistry. D Cosmos 1933.

Takehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-349.

Key JE, Rahemtulla FG, Eleazer PD. Cytotoxicity of a new root canal filling material on human gingival fibroblasts. J Endod 2006;32:756-8.

Kirkevang, L.L., et al., Periapical status and quality of root fillings and coronal restorations in a Danish population. Int Endod J 2000;33:509-15.

Koch MJ. Formaldehyde release from root-canal sealers: influence of method. Int Endod J 1999;32:10-6.

Koch K, Brave DG. Activ GP: a single-cone obturation technique. Inside Dentistry 2006;Jan/Feb:76-7.

Kontakiotis EG, Tzanetakis GN, Loizides AL: A 12 month longitudinal in vitro leakage study on a new silicon-based root canal filling material (Gutta-Flow). Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:854.

Larsen MJ, Horsted-Bindslev P. A laboratory study evaluating the release of hydroxyl ions from various calcium hydroxide products in narrow root canal-like tubes. Int Endod J 2000;33:238-42.

Leonardo MR, Utrilla LS, Rothier A, Lenonardo RT, Consolaro A. Comparison of subcutaneous connective tissue responses among three different formulations of gutta-percha used in thermatic techniques. Int Endod J 1990;23:211-7.

Lodiene G, Morisbak E, Bruzell E, Orstavik D. Toxicity evaluation of root canal sealers in vitro. Int Endod J 2008;41:72-7.

Lohbauer U, Gambarini G, Ebert J, Dasch W, Petschelt A. Calcium release and pH-characteristics of calcium hydroxide plus points. Int Endod J 2005;38:683-9.

Lui JN, Sae-Lim V, Song KP, Chen NN. In vitro antimicrobial effect of chlorhexidine-impregnated gutta percha points on *Enterococcus faecalis*. Int



Endod J 2004;37:105-13.

Marsh PD. Host defenses and microbial homeostasis: role of microbial interactions. J Dent Res 1989;68:1567-75.

Merdad K, Pascon AE, Kulkarni G, Santerre P, Friedman S. Short-term cytotoxicity assessment of components of the epiphany resin-percha obturating system by indirect and direct contact millipore filter assays. J Endod 2007;33:24-7.

Miller WD. The micro-organisms of the human mouth. 1890. Philadelphia: White Dental. Mjör IA. Endodontic materials: reactor response. Adv Dent Res 1988;2:25-8.

Möller ÅJR (1966). Microbiological examination of root canals and periapical tissues of human teeth (dissertation). Göteborg, Sweden: University of Göteborg. Mfg. CO

Moorer WR, Genet JM. Evidence for antibacterial activity of endodontic gutta-percha cones. Oral Surg Oral Med Oral Pathol 1982;53:503-7.

Moorer WR, Genet JM. Antibacterial activity of gutta-percha cones attributed to the zinc oxide component. Oral Surg Oral Med Oral Pathol 1982;53:508-17.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.

Monticelli F, Sword J, Martin RL, et al: Sealing properties of two contemporary single-cone obturation systems. Int Endod J 2007;40:374.

Munaco FS, Miller WA, Everett MM. A study of long-term toxicity of endodontic materials with use of an in vitro model. J Endod 1978;4:151-7.

Murphy WM. An in vitro model for testing biocompatibility of endodontic materials to bone. Biomaterials 1985;6:427-30.

Nair, P.N., Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004;15:348-81.

Nielsen, Benjamin A. et al. Setting times of Resilon and other Sealers in Aerobic and Anaerobic Environments. J Endod 2006;32:130-132.

Onay EO, Ungor M, Orucoglu H. An in vitro evaluation of the apical sealing ability of a new resin-based root canal obturation system. J Endod 2006;32:976-8.

Orstavik, D. Materials used for root canal obturation: technical, biological, and

clinical testing. Endodontic Topics 2005;12:25-38.

Pascon EA, Spangberg LSW. In vitro cytotoxicity of root canal filling materials: 1. gutta-percha. J Endod 1990;16:429-33.

Pissiotis E, Sapounas G, Spångberg LS. Silver glass ionomer cement as a retrograde filling material: a study in vitro. J Endod 1991;17:225-9.

Pitout E, Oberholzer TG, Blignaut E, Molepo J. Coronal leakage of teeth root-filled with gutta-percha or Resilon root canal filling material. J Endod 2006;32:879-81.

Podbielski A, Boeckh C, Haller B. Growth inhibitory activity of gutta-percha points containing root canal medications on common endodontic bacterial pathogens as determined by an optimized quantitative in vitro assay. J Endod 2000;26:398-403.

Ray H, Seltzer S. A new glass ionomer root canal sealer. J Endod 1991;17:598-603.

Ray, H.A. and M. Trope, Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. Int Endod J 1995;28:12-8.

Rappaport, Lilly GE, Kapsimalis P. Toxicity Of Endodontic Filling Materials. Oral Surg Oral Med Oral Pathol 1964;18:785-802.

Rickert UG. Dental Cosmos 1925;67:635.

Rickert, U.G. and Dixon, C.M. Tissue Tolerance to Foreign Materials. JADA 1933;20:1458-1472.

Schäfer E, Zandbiglari T. Solubility of root-canal sealers in water and artificial saliva. Int Endod J 2003;36:660-9.

Seregin VV, Coffey JL. Biomineralization of calcium disilicide in porous polycaprolactone scaffolds. Biomaterials 2006;27:4745-54.

Schwarze T, Leyhausen G, Geurtsen W. Long-Term cytocompatibility of various endodontic sealers using a new root canal model. J Endod 2002;28:749-53.

Serene TP, Vesely J, Boackle RJ. Complement activation as a possible in vitro indication of the inflammatory potential of endodontic materials. Oral Surg Oral Med Oral Pathol 1988;65:354-7.

Shipper G, Orstavik D, Teixeira FB, Trope M. An evaluation of microbial leakage in roots filled with a thermoplastic synthetic polymer-based root canal filling material (Resilon). *J Endod* 2004;30:342-7.

Shipper G, Teixeira FB, Arnold RR, Trope M. Periapical inflammation after coronal microbial inoculation of dog roots filled with gutta-percha or Resilon. *J Endod* 2005;31:91-6.

Siqueira, J.F., Jr., et al., *Periradicular status related to the quality of coronal restorations and root canal fillings in a Brazilian population*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;100:369-74.

Sjogren, U., et al., *Factors affecting the long-term results of endodontic treatment*. *J Endod* 1990;16:498-504.

Sjögren U, Sundqvist G, Nair PN. Tissue reaction to gutta-percha particles of various sizes when implanted subcutaneously in guinea pigs. *Eur J Oral Sci* 1995;103:313-21.

Soares I, Goldberg F, Massone EJ, Soares IM. Periapical tissue response to two calcium hydroxide-containing endodontic sealers. *J Endod* 1990;16:1669.

Sousa CJ, Montes CR, Pascon EA, Loyola AM, Versiani MA. Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ, and Epiphany root canal sealers. *J Endod* 2006;32:656-62.

Spångberg L. Biological effects of root canal filling materials. 6. The inhibitory effect of solubilised root canal filling materials on respiration of HeLa cells. *Odontol Tidskr* 1969a;77:121-31.

Spångberg L. Biological effects of root canal filling materials. Experimental investigation of the toxicity of root canal filling materials in vitro and in vivo. *Odontol Revy* 1969b;20(suppl 16):1-32.

Spångberg L. Biological effects of root canal filling materials. 4. Effect in vitro of solubilised root canal filling materials on HeLa cells. *Odontol Revy*. 1969c;20:289-99.

Spångberg L. Biological effects of root canal filling materials. 5. Toxic effect in vitro of root canal filling materials on HeLa cells and human skin fibroblasts. *Odontol Revy* 1969d;20:427-36.

Spangberg L. Kinetic and quantitative evaluation of material cytotoxicity in vitro. *Oral Surg Oral Med Oral Pathol* 1973;35:389-401.

Spangberg L, Langeland K. Biologic effects of dental materials. 1. Toxicity of root canal filling materials on HeLa cells in vitro. *Oral Surg Oral Med Oral Pathol* 1973 ;35:402-14.

Spångberg LS. In vitro assessment of the toxicity of endodontic materials. *Int Endod J* 1981;14:27-33.

Spangberg LSW. Endodontic Filling Materials. In: Smith DC, Williams DF, eds. *Biocompatibility of Dental Materials*. Boca Raton: CRC Press 1982:223-257.

Spångberg LS, Barbosa SV, Lavigne GD. AH 26 releases formaldehyde. *J Endod* 1993;19:596-8.

Spangberg L, Pascon EA. The importance of material preparation for the expression of cytotoxicity during in vitro evaluation of biomaterials. *J Endod* 1988;14:247-50.

Stratton RK, Apicella MJ, Mines P. A fluid filtration comparison of gutta-percha versus Resilon, a new soft resin endodontic obturation system. *J Endod* 2006;32:642-5.

Sundqvist G (1976). Bacteriological studies of necrotic dental pulps (dissertation). Umeå, Sweden: University of Umeå.

Susini G, About I, Tran-Hung L, Camps J. Cytotoxicity of Epiphany and Resilon with a root model. *Int Endod J* 2006;39:940-4.

Szep S, Grumann L, Ronge K, Schriever A, Schultz M, Heidemann D. In vitro cytotoxicity of medicated and nonmedicated gutta-percha points in cultures of gingival fibroblasts. *J Endod* 2003;29:36-40.

Tay FR, Pashley DH, Yiu CK, Yau JY, Yiu-fai M, Loushine RJ, Weller RN, Kimbrough WF, King NM. Susceptibility of a polycaprolactone-based root canal filling material to degradation. II. Gravimetric evaluation of enzymatic hydrolysis. *J Endod* 2005;31:737-41.

Tay FR, Pashley DH: Monoblocks in root canals: a hypothetical or a tangible goal. *J Endod* 2007;33:391.

Taylor JA. History of Dentistry. Lea & Ferber. Philadelphia, 1922, p. 78

Teixeira FB, Teixeira EC, Thompson J, Leinfelder KF, Trope M. Dentinal bonding reaches the root canal system. *J Esthet Restor Dent* 2004;16:348-54.

Tronstad L, Barnett F, Flax M. Solubility and biocompatibility of calcium hydroxide-containing root canal sealers. *Endod Dent Traumatol* 1988;4:152-9.

Tronstad, L., et al., Influence of coronal restorations on the periapical health of endodontically treated teeth. *Endod Dent Traumatol* 2000;16:218-21.

Tunga U, Bodrumlu E. Assessment of the sealing ability of a new root canal obturation material. *J Endod* 2006;32:876-8.

Van Lieshout M, Peters G, Rutten M, Baaijens F. A knitted, fibrin-covered polycaprolactone scaffold for tissue engineering of the aortic valve. *Tissue Eng* 2006;12:481-7.

Vasiliadis L, Kodonas K, Economides N, Gogos C, Stavrianos C. Short- and long-term sealing ability of Gutta-flow and AH-Plus using an ex vivo fluid transport model. *Int Endod J* 2010;43:377-81.

Verma D, Katti K, Katti D. Bioactivity in in situ hydroxyapatite-polycaprolactone composites. *J Biomed Mater Res A* 2006;78:772-80.

Weiger R, Heuchert T, Hahn R, Löst C. Adhesion of a glass ionomer cement to human radicular dentine. *Endod Dent Traumatol* 1995;11:214-9.

Wennberg, A. (1978), Evaluation of methods for biological screening of dental materials. Thesis, University of Lund.

Willershausen B, Marroquin BB, Schafer D, Schulze R. Cytotoxicity of root canal filling materials to three different human cell lines. *J Endod* 2000;26:703-7.

Willershausen B, Hagedorn B, Tekyatan H, Marroquin BB. Effect of calcium hydroxide and chlorhexidine based gutta-percha points on gingival fibroblasts and epithelial tumor cells. *Euro J Med Res* 2004;9:345-50.

Williams DF (1987) Definitions in Biomaterials. Oxford: Elsevier.

Williams C, Loushine RJ, Weller RN, Pashley DH, Tay FR. A comparison of cohesive strength and stiffness of Resilon and gutta-percha. *J Endod* 2006;32:553-5.

Wilson AD, Batchelor RF. Zinc oxide-eugenol cements: II. Study of erosion and disintegration. *J Dent Res* 1970;49:593-8.

Wolfson EM, Seltzer S. Reaction of rat connective tissue to some gutta-percha formulations. *J Endod* 1975;1:395-402.

Zias J, Numeroff K. Operative dentistry in the second century BCE. *J Am Dent Assoc* 1987;114:665-6.

Zeldow BI, Ingle JI. Correlation of the positive culture to the prognosis of endodontically treated teeth: a clinical study. J Am Dent Assoc 1963;66:9-13.

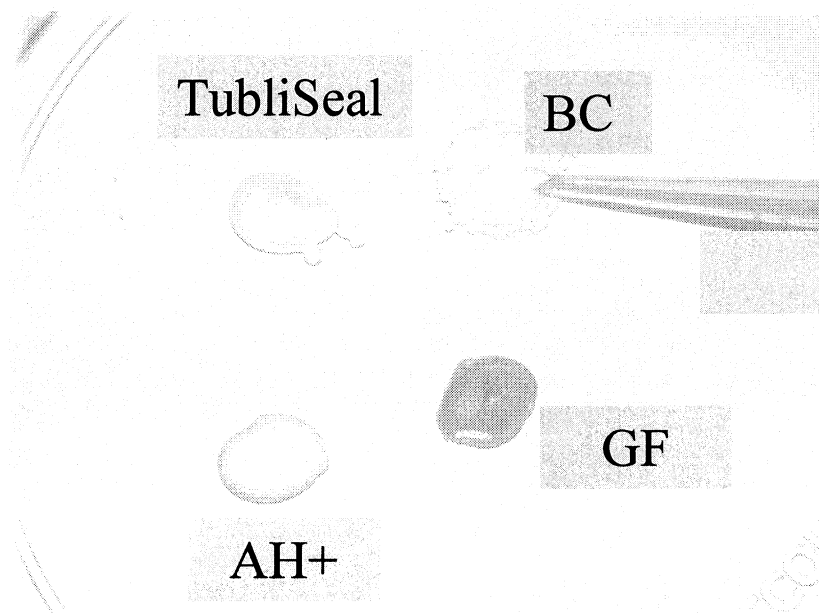
Zetterqvist L, Anneroth G, Nordenram A. Glass-ionomer cement as retrograde filling material. An experimental investigation in monkeys. Int J Oral Maxillofac Surg 1987;16:459-64.

Zetterqvist L, Anneroth G, Danin J, Röding K. Microleakage of retrograde fillings- a comparative investigation between amalgam and glass ionomer cement in vitro. Int Endod J 1988 ;21:1-8.

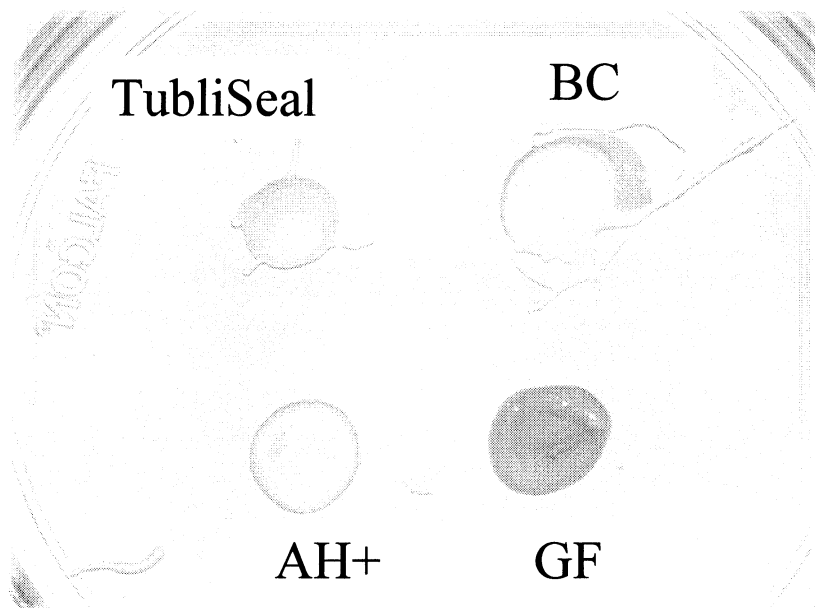
Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. J Endod 2009;35:1051-5.

Zhang W, Li Z, Peng B. Assessment of a new root canal sealer's apical sealing ability. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:e79-82.

## FIGURES

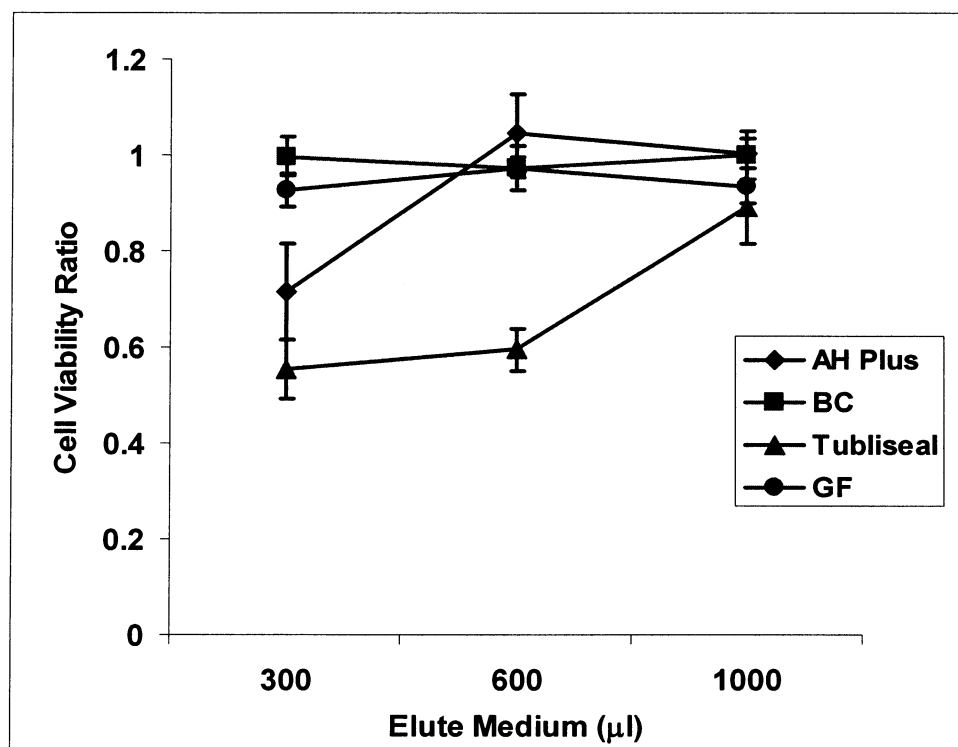


**Figure 1.** Sealers were left in the laboratory bench top for 2 months.

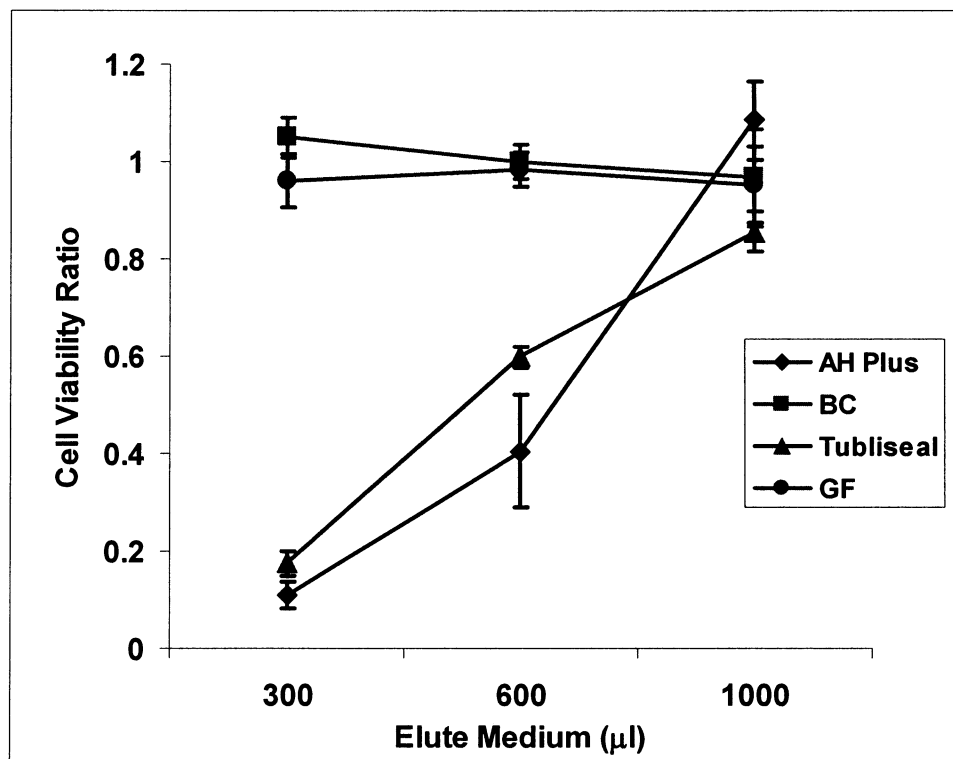


**Figure 2.** Sealers were placed in cell culture incubator for 24 hours.

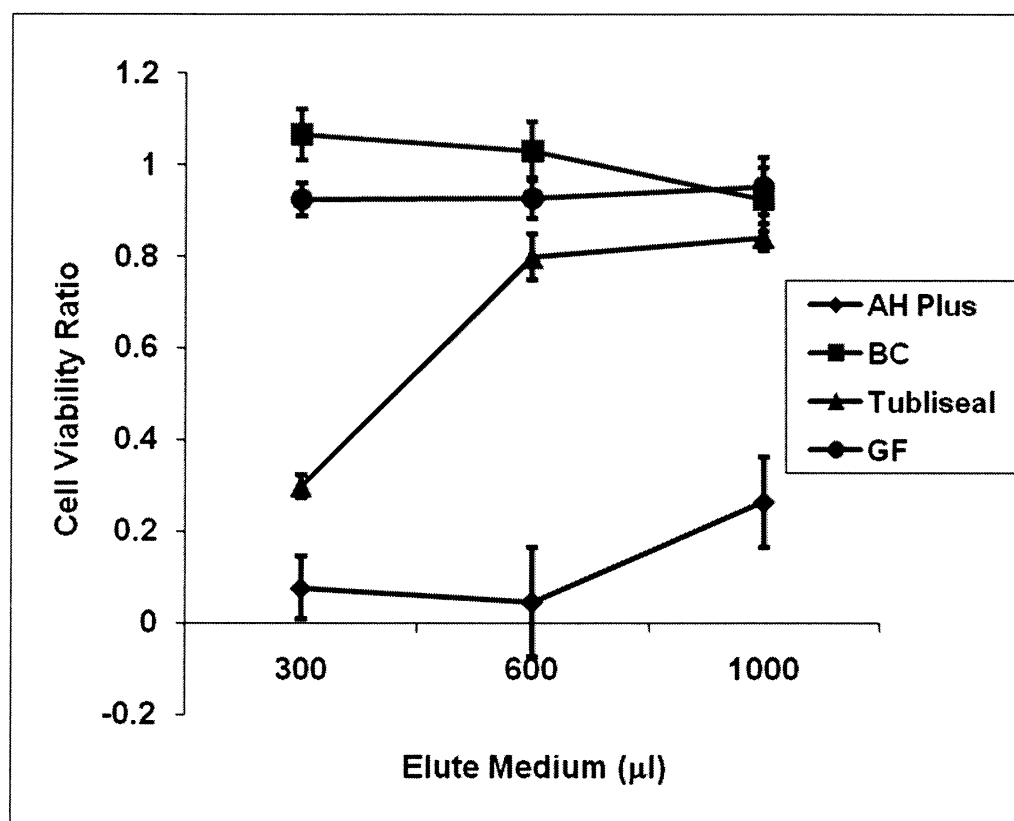




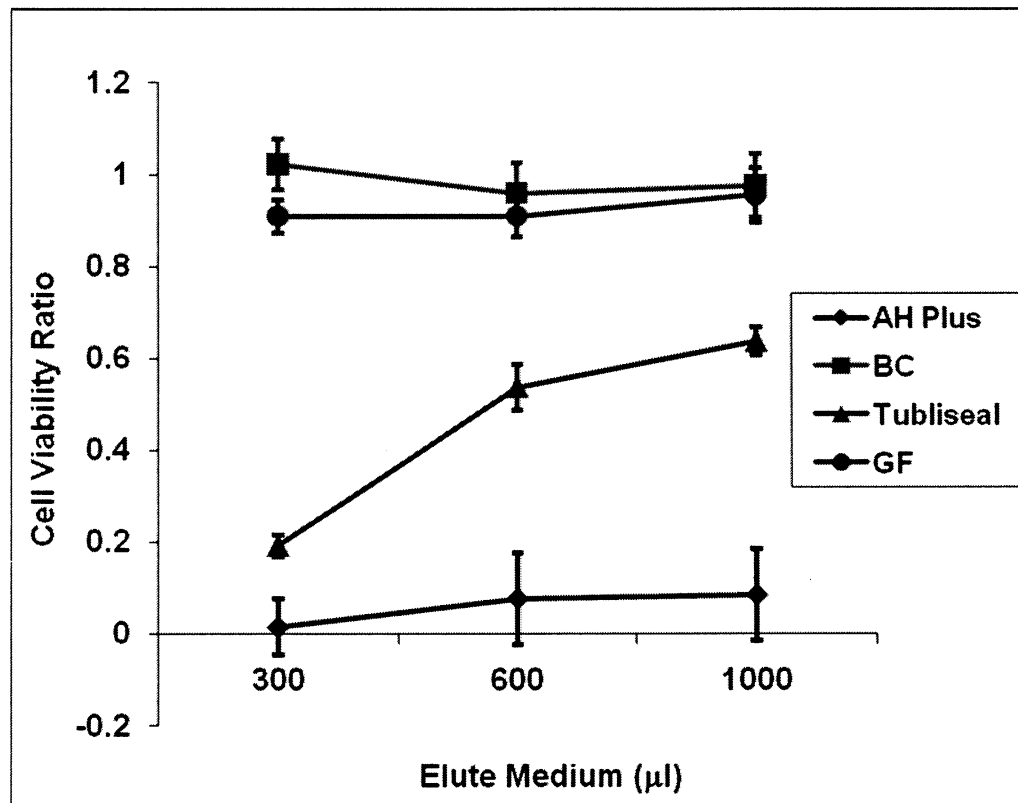
**Figure 3.** Cell viability of L929 cells after culture with 1 day eluate of set sealers.



**Figure 4.** Cell viability of L929 cells after culture with 3 day eluate of set sealers.



**Figure 5.** Cell viability of L929 cells after culture with 1 day eluate of fresh mixed sealers.



**Figure 6.** Cell viability of L929 cells after culture with 3 day eluate of fresh mixed sealers.