BIODEGRADATION OF POLYACID MODIFIED COMPOSITE RESINS BY HUMAN SALIVARY ESTARASES

By

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For the degree of Masters of Applied Science
Biomaterials Department
Faculty of Dentistry
University of Toronto

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ABSTRACT

Polyacid modified composite resins (PMCR) are designed to combine the aesthetics of composites-resins with the fluoride release of glass-ionomers. Objectives: to compare the relative biostability and fluoride release of PMCR (F2000 [3M]; Dyract eXtra [DENTSPLY]) and a composite-resin (Z250 [3M]). Standardized samples were incubated in either buffer or human saliva derived esterases (HSDE) for up to 14 days. High-performance-liquid-chromatography revealed higher amounts of degradation products for all HSDE incubated groups, as compared with the buffer. Z250 samples released higher amounts of bishydroxypropoxyphenylpropane (Bis-HPPP) and triethylene-glycol-dimethacrylate (TEGDMA) than both PMCR. Dyract eXtra and F2000 samples released unique degradation products, respectively di-ester of 2-hydroxyethyl di-methacrylate with butane tetracarboxylic acid (TCB) and glyceryl dimethacrylate (GDMA). F2000 samples released more fluoride for both incubation periods in the presence of HSDE as compared with Dyract eXtra samples. Scanning electron microscopy analysis confirmed the greater degradation of both PMCR, as compared with Z250.
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# TABLE OF CONTENTS

ABSTRACT

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

LIST OF FIGURES

CHAPTER 1 – INTRODUCTION

1.1 HYPOTHESES

1.2 OBJECTIVES

CHAPTER 2 – LITERATURE REVIEW

2.1 COMPOSITE RESINS

2.2 GLASS IONOMER CEMENTS (GIC)

2.3 POLYACID MODIFIED COMPOSITE RESINS (PMCR)

2.4 RESIN MODIFIED GLASS IONOMER CEMENTS (RMGIC)

2.5 HUMAN SALIVA

2.6 BIODEGRADATION OF RESIN BASED RESTORATIVE MATERIALS

CHAPTER 3 – MATERIALS AND METHODS

3.1 SAMPLE PREPARATION
CHAPTER 8 – APPENDICES

APPENDIX 1: REPRESENTATIVE CHROMATOGRAMS AND MASS SPECTRA

APPENDIX 2: PREINCUBATION TCB AND GDMA RELEASE

APPENDIX 3: CALIBRATION CURVES

APPENDIX 4: CALCULATION OF MA, BIS-HPPP AND TEGDMA RELEASE (μg) FROM Z250, F2000 AND DYRACT EXTRA USING CALIBRATION CURVES
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>Bis-EMA</td>
<td>Ethoxylated bisphenol A dimethacrylate</td>
</tr>
<tr>
<td>Bis-GMA</td>
<td>Bisphenol glycidyl dimethacrylate</td>
</tr>
<tr>
<td>Bis-HPPP</td>
<td>Bis-hydroxypropoxyphenyl propane</td>
</tr>
<tr>
<td>BPA</td>
<td>Bisphenol-A</td>
</tr>
<tr>
<td>CDMA</td>
<td>Dimethacrylate functional oligomer derived from citric acid</td>
</tr>
<tr>
<td>CE</td>
<td>Cholesterol esterase</td>
</tr>
<tr>
<td>ChE</td>
<td>Cholinesterases</td>
</tr>
<tr>
<td>CQ</td>
<td>Camphoroquinone</td>
</tr>
<tr>
<td>DPICI</td>
<td>Diphenylliodoniumchlotide</td>
</tr>
<tr>
<td>FRP</td>
<td>Fiber reinforced polymers</td>
</tr>
<tr>
<td>GDMA</td>
<td>Glyceryl dimethacrylate</td>
</tr>
<tr>
<td>GIC</td>
<td>Glass ionomer cements</td>
</tr>
<tr>
<td>HEMA</td>
<td>Hydroxylethylmethacrylate</td>
</tr>
<tr>
<td>HMBP</td>
<td>2-hydroxy-4-methoxybenzophenone</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>MA</td>
<td>Methacrylic acid</td>
</tr>
<tr>
<td>MMA</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer solution</td>
</tr>
<tr>
<td>PCE</td>
<td>Psuedo-choline esterase</td>
</tr>
<tr>
<td>PMCR</td>
<td>Polyacid-modified composite resins</td>
</tr>
<tr>
<td>RMGIC</td>
<td>Resin modified glass ionomer cements</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TCB</td>
<td>Di-ester of 2-hydroxyethyl di-methacrylate with butane tetracarboxylic acid</td>
</tr>
<tr>
<td>TEG</td>
<td>Triethylene glycol</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>Triethylene glycol di-methacrylate</td>
</tr>
<tr>
<td>TEGMA</td>
<td>Triethylene glycol methacrylate</td>
</tr>
<tr>
<td>TMPTMA</td>
<td>Trimethylopropane trimethacrylate</td>
</tr>
<tr>
<td>UDMA</td>
<td>Urethane dimethacrylate</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

CHAPTER 2 – LITERATURE REVIEW

FIG. 2.1: STRUCTURE OF BIS-GMA MONOMER ........................................ 4
FIG. 2.2: STRUCTURE OF UDMA MONOMER ......................................... 4
FIG. 2.3: STRUCTURE OF TEGDMA MONOMER ....................................... 4
FIG. 2.4: SETTING REACTION OF CONVENTIONAL GIC ......................... 11
FIG. 2.5: STRUCTURE OF TCB RESIN ................................................... 17
FIG. 2.6: STRUCTURE OF GDMA .......................................................... 18

CHAPTER 3 – BIODEGRADATION OF POLYACID MODIFIED COMPOSITE
RESINS BY HUMAN SALIVARY ESTARASES

FIG. 3.1: CHEMICAL STRUCTURE OF P-NBP ......................................... 36
FIG. 3.2: CHEMICAL STRUCTURE OF BTC ............................................ 38
FIG. 3.3: CHEMICAL STRUCTURE OF DTNB ......................................... 38
FIG. 3.4: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY [HPLC] SYSTEM ................................................................. 40
FIG. 3.5: THE ORION 930 IONALYZER SYSTEM ..................................... 42

CHAPTER 4 – RESULTS

FIG. 4.1: RELATIVE ACTIVITY OF PCE/CE WHEN INCUBATED IN THE PRESENCE AND ABSENCE OF DYRACT AP, DYRACT EXTRA, F2000 AND Z250. ................................................................. 45
FIG. 4.2: RELATIVE ACTIVITY OF HSDE WHEN INCUBATED IN THE PRESENCE AND ABSENCE OF DYRACT AP, DYRACT EXTRA, F2000 AND Z250. ................................................................. 46
FIG. 4.3: INCREMENTAL RELEASE OF MA (A), BIS-HPPP (B) AND TEGDMA (C) FOR Z250, DYRACT EXTRA AND F2000 AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS.

FIG. 4.4: INCREMENTAL RELEASE OF THE TWO TCB RELATED PRODUCTS (15.8 MINUTES AND 16.4 MINUTES RETENTION TIME) (A) AND TOTAL INCREMENTAL TCB RELEASE FOR DYRACT EXTRA (B) AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS.

FIG. 4.5: INCREMENTAL GDMA RELEASE AT 14 AND 16 MINUTES FOR F2000 (A) AND TOTAL INCREMENTAL GDMA RELEASE (B) AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN PBS AND HSDE.

FIG. 4.6: INCREMENTAL FLUORIDE RELEASE FROM DYRACT EXTRA, F2000 AND Z250 FOLLOWING INCUBATION IN HSDE AND PBS FOR 2, 4, 7 AND 14 DAYS (WITH FILTRATION OF INCUBATION SOLUTIONS).

FIG. 4.7: FLUORIDE MEASURED FOLLOWING INCUBATION OF 1, 2.5 AND 5 PPM FLUORIDE IN HSDE AND PBS FOR 3 DAYS.

FIG. 4.8: FLUORIDE RELEASE FROM DYRACT EXTRA, DYRACT AP, F2000 AND Z250 SAMPLES FOLLOWING INCUBATION IN HSDE AND PBS FOR 1ST AND 2ND WEEKS.

FIG. 4.9: SEM IMAGES FOR DYRACT EXTRA, F2000 AND Z250 SAMPLES. NON-INCUBATED (A); FOLLOWING 14 DAYS INCUBATION WITH PBS (B); FOLLOWING 14 DAYS INCUBATION WITH HSDE (C).
CHAPTER 8 – APPENDICES

APPENDIX 1:

FIG. 8.1.1: REPRESENTATIVE HPLC CHROMATOGRAM OF MA STANDARD. 75

FIG. 8.1.2: REPRESENTATIVE HPLC CHROMATOGRAM OF BIS-HPPP STANDARD. 75

FIG. 8.1.3: REPRESENTATIVE HPLC CHROMATOGRAM OF TEGDMA STANDARD 75

FIG. 8.1.4: REPRESENTATIVE HPLC CHROMATOGRAM OF DYRACT EXTRA FOLLOWING INCUBATION IN PBS. 76

FIG. 8.1.5: REPRESENTATIVE HPLC CHROMATOGRAM OF DYRACT EXTRA FOLLOWING INCUBATION IN HSDE. 76

FIG. 8.1.6: REPRESENTATIVE HPLC CHROMATOGRAM OF DYRACT EXTRA FOLLOWING 48 HOURS OF PRE-INCUBATION IN PBS. 76

FIG. 8.1.7: REPRESENTATIVE HPLC CHROMATOGRAM OF F2000 FOLLOWING INCUBATION IN PBS. 77

FIG. 8.1.8: REPRESENTATIVE HPLC CHROMATOGRAM OF F2000 FOLLOWING INCUBATION IN GDMA. 77

FIG. 8.1.9: REPRESENTATIVE HPLC CHROMATOGRAM OF F2000 FOLLOWING 48 HOURS OF PRE-INCUBATION IN PBS. 77

FIG 8.1.10: MASS SPECTRUM OF GDMA MONOMER ISOLATED AT RETENTION TIMES OF 14 AND 16 MINUTES IN F2000 SAMPLES INCUBATED IN PBS AND HSDE. 78

FIG 8.1.11: MASS SPECTRUM OF TCB RESIN ISOLATED AT RETENTION TIMES OF 15.8 AND 16.4 MINUTES IN DYRACT EXTRA SAMPLES INCUBATED IN PBS AND HSDE. 78

APPENDIX 2:

FIG. 8.2.1: TOTAL INCREMENTAL TCB RELEASE FOR DYRACT EXTRA AT
EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS AND FOLLOWING 48 HR. PREINCUBATION IN PBS AT 37°C.

FIG. 8.2.2: TOTAL INCREMENTAL TCB RELEASE FOR GDMA AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS AND FOLLOWING 48 HR. PREINCUBATION IN PBS AT 37°C.

APPENDIX 3:

FIG. 8.3.1: CALIBRATION CURVE FOR MA.

FIG. 8.3.2: CALIBRATION CURVE FOR TEGDMA.

FIG. 8.3.3: CALIBRATION CURVE FOR BIS-HPPP.

APPENDIX 4:

FIG. 8.4.1: INCREMENTAL RELEASE (μG) OF MA FOR Z250, DYRACT EXTRA AND F2000 AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS.

FIG. 8.4.2: INCREMENTAL RELEASE (μG) OF BIS-HPPP FOR Z250, DYRACT EXTRA AND F2000 AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS.

FIG. 8.4.3: INCREMENTAL RELEASE (μG) OF TEGDMA FOR Z250, DYRACT EXTRA AND F2000 AT EACH TIME POINT (2, 4, 7 AND 14 DAYS) FOLLOWING INCUBATION IN HSDE AND PBS.
CHAPTER 1 - INTRODUCTION

Composite resins are the material of choice for most direct restorations in the anterior teeth. Posterior composite restorations are gaining popularity due to increasing demand for aesthetic restorations and concerns regarding amalgam toxicity. Previous studies have shown that composite resins’ organic matrix contains monomers that have ester bonds that are prone to enzymatic hydrolysis by salivary esterase enzymes (Santerre, 1999; Finer, 2004; Jaffer, 2002; Munksgaard, 1990).

Polyacid modified composite resins (PMCR or compomers) were introduced as a new class of dental materials, designed to combine the aesthetics of traditional composites with the fluoride release and adhesion of glass-ionomer cements (GIC), and have replaced the use of dental amalgam in a number of clinical applications, mainly in paediatric dentistry (Hse, 1999; Nicholson, 2007).

Similarly to resin composites, PMCR contain methacrylate based monomers; and therefore have the potential to undergo enzymatic degradation. In addition they contain reactive fluoro-alumino-silicate glass particles that are susceptible to acid attack and provide the source of fluoride ions and unique matrix monomers, which react simultaneously with the dimethacrylate monomers and with the cations liberated from the glass particles (Hse, 1999; Nicholson, 2007).

Most previous degradation studies on PMCR were in vitro elution analyses in which PMCR samples were incubated in water, ethanol and/or acids and analysed for leached unreacted monomer components (Michelsen, 2003; Michelsen, 2007; Guertsen, 1998; Becher, 2006; Lygre, 1999). A previous study demonstrated the ability of a model salivary esterase, cholesterol esterase (CE), to degrade two PMCR, Dyract AP (DENTSPLY) and F2000 (3M ESPE) and a traditional resin composite Z250 (3M ESPE). High performance liquid chromatography (HPLC) analysis provided qualitative and quantitative differences between the two PMCR and the composite. The findings of this study suggested that the two PMCR products degraded more extensively than the composite material. Dyract AP samples released significantly higher amounts of Bis-HPPP, TEGDMA and MA than the F2000 and Z250 samples. Fluoride was
released only from the PMCR materials; with Dyract AP releasing more fluoride, at each time point in the presence of CE, as compared with F2000 (Revuelta, 2006).

While the above studies provided some insight to the hydrolysis of PMCR materials, their interactions with human salivary esterases is yet to be fully investigated. Since biodegradation products could influence the restoration and the oral micro-environment, the present study is aimed to provide a better understanding of the widely used PMCR and can contribute to future \textit{in vivo} investigations. Since the formulation of Dyract has been changed, the study is expected to provide an updated view of the biostability of the latest formulation of Dyract (Dyract eXtra) as compared with its previous formulation (Dyract AP).

1.1 HYPOTHESES

1. The hydrolytic degradation of PMCR (Dyract eXtra and F2000) is enhanced by human saliva derived esterases as compared with buffer.

2. The level of fluoride release from the PMCR is proportional to the amount of the polymer degradation products.

1.2 OBJECTIVES

- To measure and compare the relative biostability in human saliva of two commonly used PMCR, Dyract eXtra and F2000 and a composite resin, Z250.
- To isolate and identify the biodegradation products by high performance liquid chromatography (HPLC) in combination with UV spectroscopy.
- To confirm the identity of the released products by means of mass spectrometry (MS).
- To analyze the biodegradation effect on the surface morphology of the specimens by scanning electron microscopy (SEM).
- To measure and compare levels of fluoride release from the Dyract AP, Dyract eXtra, F2000 and Z250 in the presence and absence of human salivary esterases.
CHAPTER 2 – LITERATURE REVIEW

As the search for the ideal aesthetic direct restoration dental material continues, it is apparent that no one material is universal. Composite resins and compomers were introduced as alternative restoration materials due to public concern over the possible toxicity of amalgam and an increasing demand for aesthetic restorations, and have become widely used. Compomers have gained great acceptance and have replaced the use of dental amalgam in a number of clinical applications, mainly in paediatric dentistry, due to their good handling characteristics and fluoride release, potentially reducing recurrent caries rate.

2.1 Composite Resins

The evolution of aesthetic restorative materials began about 150 years ago with the discovery of acrylic acid. The first particulate filled ceramic-polymer composite was patented by Knock and Glenn in 1951 (Eliades, 2003). Bowen conducted further work and in 1962 patented a new resin, bisphenol glycidyl dimethacrylate (Bis-GMA) that formed a strongly cross-linked polymer (Bowen, 1992). "Bowen's composite resin" has become the basis for all composite resins used in dentistry today.

2.1.1 Composition of composite resins

A composite is a mixture of two or more components; each of these materials contributes to the overall properties of the composite and may be clearly distinguished from one another (Van Noort, 2002).

The four major components of composite resins are:

2.1.1.1 Organic resin matrix – The resin is the chemically active component of the composite. Originally the predominant resin monomers were composed of methacrylate resins and later Bis-GMA was introduced. Bis-GMA, along with urethane dimethacrylate (UDMA), is the most commonly used oligomer in dental composites today. Both compounds contain reactive carbon double bonds at each end that can undergo addition polymerization. A few products use both Bis-GMA and UDMA oligomers (Craig, 2002).
Because of their high viscosity, diluents must be added to Bis-GMA and UDMA in order to reduce the viscosity to a useful clinical level. The most commonly used viscosity controller is triethylene glycol dimethacrylate (TEGDMA) (Van Noort, 2002).

2.1.1.2 *Inorganic Fillers* – The inclusion of fillers contributes body and strength and adds dimensional stability. In the past, fillers most commonly used were composed of quartz particles. Today, most composites contain a variety of glass fillers, including fine colloidal silica particles, lithium-aluminum silicate glass and silica glasses containing barium, strontium or zinc which are used to add radio-opacity, thus improving diagnostics (Van Noort, 2002; Ferracane, 1995). Composites are classified based on their filler particles. Currently, this classification can be divided into two general categories: microhybrid and microfilled products. Microhybrid composites are composed mainly of fine particles (0.04-0.2 µm) with some microfine particles (0.01-0.05 µm). The filler concentration of microhybrids is approximately 77-84% by weight (60-70% filler by volume). Today, most of the composite resins used are microhybrids.
Microfilled composites contain silica microfine particles (0.01-0.05µm) with the filler concentration being less than in microhybrids, at approximately 38% by weight (25% by volume). Because of the greater percentage of resin, this material exhibits increased water sorption and a higher coefficient of thermal expansion when compared to microhybrids. However, microfilled composites provide better esthetics (Craig, 2002; Van Noort, 2002). Nanotechnology has become a reality in different areas of engineering with the development of materials and functional structures containing particles within a size interval of 0.1–100 nm. It is also one of the most noticeable advances in composite filler technology, involving the incorporation of silica fillers of nanometer size. Nanofillers are found in microfill and some hybrid composites that can be considered predecessors of the newer nanoparticulate composites. A study evaluating the mechanical properties of experimental composites with or without nanofillers found a positive effect of the presence of nanofiller particles, observed as an improvement in flexural strength, surface hardness and fracture toughness (Ferracane, 1995).

2.1.1.3 *Coupling agents*- The bond between the organic (matrix) and inorganic (filler) components has to be strong and durable for the composite to have successful properties. The bond is achieved by treating the surface of the filler with a coupling agent that has characteristics of both the filler and the matrix, before mixing it with the oligomer. The most common coupling agents are organic silicon compounds called silanes. Resins are hydrophobic, whereas silica-based glasses are hydrophilic due to a surface layer of hydroxyl groups bound to the silica. Therefore, the resin and the glass surface have no natural affinity to form a bond. The silane coupling agent has hydroxyl groups on one end, which are attracted to the hydroxyl groups on the glass surface. On its other end the silane has a methacrylate group that is able to bond to the resin via a carbon double bond. This coupling reaction has tremendous influence on the mechanical properties of the composite (Van Noort, 2002; Ferracane, 1995).

2.1.1.4 *Initiator-accelerator*- that allows for curing of the resin. Composite systems are designed to polymerize from a viscous resin into a rigid solid via a free radical polymerization process by chemical (self-curing) or photochemical (light curing) means. Light-activated systems possess several advantages, including increased working time, decreased curing time, greater wear resistance and color stability, and therefore, are most commonly used. The initiator in light
activated dental composites is camphoroquinone (CQ), which is sensitive to blue light (Ferracane, 1995).

*Other components* include inorganic oxides to enhance tooth color matching, additives to improve color stability and inhibitors to retard premature polymerization (Craig, 2002).

### 2.1.2 Resin adhesives

Composite resins are chemically bonded in place with polymer bonding agents. The steps when restoring a tooth are: removing decay, etching and priming of the involved tooth structure, a liquid adhesive resin is placed over the etched and primed area and bonded using a curing light. Finally, the composite resin is placed into the prepared tooth and polymerized (Vanable, 2004).

### 2.1.3 Properties of composite resins

The physical properties of composite resins are related to the amount and type of filler content.

#### 2.1.3.1 Polymerization shrinkage/contraction

Polymerization contraction and its accompanying stresses are among the biggest problems facing the expanded use of composites. The polymerization shrinkage of a composite resin is dependent upon the type of resin employed and the amount of resin present in its unpolymerized form, since it is the polymerization of the resin monomers that causes the contraction. In general, a higher proportion of glass filler results in a lower final shrinkage. However, polymerization shrinkage remains a concern since it can create marginal gaps that may predispose the dental restoration to recurrent decay, post-operative sensitivity and marginal staining. The effect of polymerization shrinkage can be reduced by placing the restoration in increments or layers, polymerizing each increment separately (Van Noort, 2002; Ferracane, 1995).

#### 2.1.3.2 Coefficient of thermal expansion

Is the rate of change in the material when it is exposed to changes in temperature. Composite resins’ coefficient of thermal expansion is greater than that of tooth structure; therefore it may decrease adhesion and increase microleakage around the
restoration. The higher the ratio of resin to filler particles, the greater the coefficient of thermal expansion and the lower the strength of the restoration (Craig, 2002).

2.1.3.3 Thermal conductivity- composite resin is not a good conductor of heat, which allows it to be placed without insulating bases and liners to protect the pulp (Craig, 2002).

2.1.3.4 Aesthetics- The microfilled composites have the most ideal esthetic qualities, due to their excellent polishability and capacity to retain surface smoothness over time.

2.1.3.5 Solubility- Inadequate polymerization results in greater water sorption and solubility, which in turn affect the color stability and wear resistance (Van Noort, 2002).

2.1.3.6 Water sorption- composite resins absorb water from the intraoral environment, which softens the resin matrix, leading eventually to degradation of the material and has a detrimental effect on the color stability. The greater the resin content, the more water absorption, the higher expansion when exposed to water. Water sorption by the polymer network contributes to a reduction of its strength, stiffness and wear resistance by softening the polymer network. Water sorption can be reduced by the use of more hydrophobic monomers, such as the ethoxylated version of Bis-GMA (Bis-EMA), which does not contain unreacted hydroxyl groups on the main polymer chain (Ferracane, 1995).

2.1.3.7 Wear and abrasion- wear in the mouth can occur when there is direct contact between two teeth or restorations or during mastication with food acting as an abrasive agent. Wear can also result after an acid attack on a composite restoration. The rate of wear is related to the filler particle size and spacing between the filler particles. The resin matrix is abraded first, leaving filler particles exposed, which are then plucked from the resin during later abrasions. Reducing the size of filler particles reduces the filler plucking, thus reducing abrasive wear (Ferracane, 1995).

2.1.3.8 Fluoride release- composite resins may contain fluoride in a variety of forms, such as inorganic salts, leachable glass or organic fluoride. Three different approaches for development
of fluoride releasing composites have been reported: addition of water-soluble salts such as NaF or SnF$_2$, Fluoride-releasing filler systems and matrix bound fluoride. Incorporation of inorganic fluoride resulted in increased fluoride release but it left voids in the matrix. Fluoride levels leached from composites are usually much lower compared to levels released from conventional or resin-modified GIC and compomers (Wiegand, 2007).

2.1.4 Clinical applications of composite resins

Composite is currently used in dentistry for more than 95% of direct restorations in anterior teeth. Demands for improved esthetics and concerns regarding mercury in the amalgam have led to a dramatic increase of posterior composite resin direct restorations (Craig, 2002). Flowable composites, containing fewer filler particles, thus flow more readily and are used in preventive dentistry in place of the traditional unfilled resin sealant materials. They are also used as liners under large restorations or as restorative material in conservative preparations (Stein, 2005).

2.1.5 Biocompatibility of composite resins

Nearly all the major components of composite resins have been found to be released following curing. These materials include uncured resins and diluents, UV stabilizers, plasticizers and initiators that may have detrimental effects not only on pulp tissue but also on adjacent and remote tissues. The amount of release depends on the type of composite and the method and degree of cure that has been achieved. General usage of these materials over more than 20 years indicates a high benefit to risk ratio and they are believed to be relatively trouble-free (Van Noort, 2002; Yap, 2000; Bayne, 1992).

2.1.5.1 Estrogenity- Bisphenol-A (BPA) is a precursor of Bis-GMA and has been shown to be an estrogenic material (Olea, 1996). This study tested a sealant based on Bis-GMA and it was suggested that BPA could be released from the Bis-GMA and cause proliferation of breast cancer cells in a culture essay. Bis-GMA alone was unable to do so. However, other studies did not detect BPA as an eluted compound of resin composites (Ortengren, 2002; Spahl, 1996) therefore
the clinical relevance of the exposure to estrogenic compounds is still controversial (Schweikl, 2006).

2.1.5.2 *Pulpal irritation* - pulpal irritation following placement of a composite restoration is attributed to bacterial leakage and chemical byproducts. A thick layer of dentin, together with the smear layer, contributes to the reduction of irritation following a composite restoration. It is important to obtain as complete polymerization as possible through the entire restoration to minimize pulpal responses, especially in deep cavity preparations when an incomplete curing of the resin permits higher concentrations of residual unpolymerized monomer in close proximity to the pulp (Stanley, 1992). TEGDMA, the diluent that is added to Bis-GMA to decrease viscosity, has been reported to stimulate the growth rate of cariogenic micro-organisms (*Streptococcus mutans*, *S. salivarius*, *S. sobrinus* and *Lactobacillus acidophilus*) (Hansel, 1998; Khalichi, 2004). Pulpal reactions associated with resin composite restorations are correlated with the amount of bacteria; therefore comonomers released from resin materials may contribute to pulpal irritation under resin composite restorations, as well as to increase plaque formation on the surface or marginal gaps of restorative materials (Stanley, 1992).

2.1.5.3 *Toxicity and allergic reactions* - The potential for local and systemic side-effects, including allergic contact dermatitis, can occasionally occur when these materials are improperly used. Allergic reactions associated with resin-based materials affect not only the patients but especially the dental personnel. In addition, wear and degradation of composite resins release components, which may cause local and systemic reactions (Yap, 2000; Munksgaard, 1990). There is data showing that resin materials, including monomers, initiators and co-initiators, are cytotoxic in cell culture systems. Whole composite resins, when placed into culture medium with cells, caused moderate to severe cytotoxic effects (Hanks, 1981; Hume, 1996; Geurtsen, 1998). Studies to evaluate cytotoxicity of dental resin materials by exposing cells to the various monomers or additives found that Bis-GMA and UDMA exhibited higher cytotoxic effects compared to hydroxylethylmethacrylate (HEMA), CQ and methyl methacrylate (MMA) (Yoshii, 1997; Geurtsen, 1998). It has been hypothesized that hydrophilic resin substances, such as TEGDMA, which may pass through cell membranes rapidly and easily, cause intracellular damage. The hydrophobic monomers, such as Bis-GMA, leach from resin materials in small
quantities and mainly accumulate in the hydrophobic membrane lipid fractions of cells (Geurtsen, 2001).

Minute amounts of formaldehyde may form as a degradation product of unreacted monomers in dentures made from resin based composite materials and their release decreases with time. The release of formaldehyde has been discussed as a possible cause of allergic reactions, since it is known to be an allergen (Oysaed, 1988).

The effect of 11 components of resin composites, including TEGDMA, Bis-GMA, BPA and UDMA, on cultured fibroblasts was determined in regard to their ability to inhibit DNA and protein synthesis. A reduction of DNA and Protein synthesis was observed, which can lead to cell death when concentrations are in the 10-100μmol/L range. Tissue culture and animal screening tests, however, do not necessarily correlate with in vivo conditions (Hanks, 1991). It has been established that TEGDMA can cause gene mutations in vitro. The molecular mechanisms leading to mutations, induced by resin monomers, are unclear at present. It has been estimated that sufficient amounts of the monomers HEMA and TEGDMA are probably eluted from clinically used bonding agents, causing cellular toxicity (Schweikl, 2006).

2.2 Glass Ionomer Cement (GIC)

Glass ionomer cements (GIC) are a group of materials developed in the late 1960's - early 1970's for use in dentistry, as a natural extension to the zinc polycarboxylate cements. Early formulations of GIC had clinical drawbacks. The initial and long-term settings were very slow and hydrolytically unstable. The newer materials that were developed, since then, are easier to manipulate clinically (Kovarik, 2005).

2.2.1 Composition of GIC

The setting reaction of GIC, an acid-base setting reaction, is typical of all cement formulations. The main components of a GIC are:
The glass is an ion leachable glass powder that is made up of three main components: Silica (SiO₂) the primary component of the glass, alumina (Al₂O₃) and calcium fluoride (CaF₂). The mixture, which also contains sodium and aluminum fluorides and calcium or aluminum phosphates, is fused at a high temperature and ground to a fine powder. The size of glass particles varies from 10-18 µm for luting to 50-80 µm for restorations. The composition of the glass can vary widely, giving many different properties. The main requirement is that the glass must be acid soluble to release aluminum and calcium ions when mixed with the acid in an aqueous environment. The glass also plays a major part in the esthetics of the restoration, depending on the presence of pigments within it and its refractive index (Van Noort, 2002; Kovarik, 2005).

The polyacid is a liquid, usually polyacrylic acid or polymaleic acid, mixed with tartaric acid (which is an important hardener and controls the pH during the setting process). These are long polymeric carbon chains with carboxylic acid side groups attached. These acids are water soluble and are supplied in aqueous solutions of varying strengths. Recently there is a relatively new GIC based on a copolymer of vinyl phosphonic acid. It is a much stronger acid and is believed to give higher long-term strength and enhanced moisture resistance (Kovarik, 2005; Van Noort, 2002).

The setting reaction is via an acid-base reaction:

Fig. 2.4: Setting reaction of conventional GI (adapted from 3M Vitremer Technical Product Profile).
When powder and liquid are mixed, the acid reacts with the outer layer of the glass, releasing ions (Ca\(^+\), Al\(^+\), Na\(^+\) and F\(^-\)) from the glass into the aqueous reaction medium. The cations serve to ionically crosslink the long polyanion chains, forming polyacrylate salts. The sodium and fluoride ions do not take part in the setting process, but are released from the matrix. In the early setting reaction (during the first 15 minutes) there is a formation of the Ca\(^+\) polyacrylate salts, whereas the Al\(^+\) ions form polyacrylate salts more slowly with the setting reaction taking up to 2 weeks or longer (Kovarik, 2005). Eventually the calcium ions and the aluminum ions will form the salt matrix. The outer layer of glass becomes depleted of Ca\(^+\), Al\(^+\), Na\(^+\) and F\(^-\) ions, leaving an inner solid glass core surrounded by a silica hydrogel. This layer has relatively low strength and probably contributes to the inherent weakness of glass ionomers. Although the material appears hard after 3-6 minutes, it still has not reached its final physical and mechanical properties and will continue to set for up to one month (Van Noort, 2002).

### 2.2.2 Properties of GIC

The two main features that have allowed GIC to become one of the most accepted dental materials are their ability to bond to enamel and dentine and their ability to release fluoride.

2.2.2.1 **Adhesion** - the GIC bonds directly to the dentin and enamel. An ion exchange process between the cement and the tooth structure leads to the development of an ion-enriched intermediate layer of new material at the cement-tooth interface that is firmly attached to the tooth surface. Bond failures are located in the bulk of the cement rather than at the interface. Conditioning the surface with polyacrylic acid or tannic acid can improve the bond to dentine (Van Noort, 2002; Eliades, 2003).

2.2.2.2 **Fluoride release** - the most intriguing property of glass ionomer cements is their ability to release fluoride to the adjacent tooth structure (Mount, 1999). During the setting phase the calcium and aluminum ions are involved in the formation of the matrix of the material. The sodium and fluoride ions are released from the glass powder, do not participate in the setting reaction but remain unbound in the set cement. They are released to the adjacent environment in large quantities initially, but the amount released drops rapidly after the first 2-3 days until it reaches a low level steady state release. The rapid initial release is considered to be that of the
loosely bound fluoride in the cement matrix, while the slower rate occurs with the release of fluoride from the glass particles (Burke, 2006). The overall amount of fluoride released is proportional to the fluoride content of the glass ionomer. Recharging of GIC has been referred to as the "reservoir effect". The amount of fluoride taken up from the oral environment through fluoride dentifrices and mouth rinses can be significant, and this allows the GIC to act as "fluoride reservoirs" slowly releasing the fluoride back to the environment. GICs release fluoride from a reservoir contained primarily in the polyacrylate gel matrix. High initial fluoride release rate may be positively correlated with a high recharging ability (Guida, 2002; Wiegand, 2007; Damen, 1996). The fluoride ions released are believed to be taken up by the enamel adjacent to the restoration, increasing its caries resistance. Remineralization of carious lesions has been reported in dentin adjacent to GI restorations, whereas further demineralization has been observed where the restorations have been composite resins or dental amalgam (ten Cate, 1995). Fluoride inhibits the activity of Streptococcus mutans, which plays a role in the etiology of caries. The fluoride ions can interfere with the adhesion of bacteria and their colonization. A reduction in the acidogenicity of Streptococcus mutans has also been found when placed on glass ionomer compared to composite (Seppa, 1993).

2.2.2.3 Ease of use- by incorporation of tartaric acid and changes in glass composition, the handling characteristics improved over the years. The rate of the setting reaction is controlled by the glass composition, particles size, powder/liquid ratios and temperature, so that the clinician can speed/slow the setting. Maintenance of correct mixing time and working time is relevant for the success of the restoration. Many formulations of GIC are available in a capsulated form, thus eliminating one of the variables that could lead to poor results (Mount, 1999; Kovarik, 2005; Van Noort, 2002).

The adhesive quality of GIC dictates minimal tooth substance removal when preparing the cavity and the use of dentine conditioners is dependent on the type of lesion (Van Noort, 2002).

2.2.2.4 Aesthetics- in GIC the color is produced by the glass. This can be controlled by the addition of color pigments such as ferric oxide or carbon black. The translucency of the GIC was closer to that of dentine in the earlier days than to enamel, making their aesthetic appearance inferior to that of composites. The cements appeared dull and lifeless and this limited their
application. Recent changes in the formulation have resulted in a marked improvement. Another aspect is the observation that there is a color change during the setting process. Generally, the shade is darker after the material is fully set than at the time of placement (Van Noort, 2002).

2.2.2.5 Shrinkage on setting- is considerably less for GIC than that for composite resins. GIC sets by an acid-base mediated cross-linking reaction, which inherently produces less shrinkage than polymerization. Therefore, the local interfacial stresses generated will be less and the bond stands a better chance of survival (Van Noort, 2002).

2.2.2.6 Thermal expansion- GIC have a coefficient of thermal expansion similar to that of tooth structure, which is one of their main advantages (Mount, 1999).

2.2.2.7 Tensile strength and wear- compared to composites the GIC have low tensile strength and wear resistance. A remarkable increase in strength is apparent as the cement undergoes setting. However, the final strength of the set GIC remains low compared with composites and amalgam. Because of the low tensile strength and low impact and fracture resistance (brittleness) GIC are not recommended for use in permanent posterior restorations. In contrast, the luting GIC are stronger than the zinc phosphate and zinc polycarboxylate cements. The compressive and tensile strengths are related to the powder content of the mixed cement. As any mixture that needs to be mixed prior to placement, it will inevitably contain a degree of porosity, and cracks tend to follow through these defects (Mount, 1999; Eliades, 2003; Bowen, 1992).

2.2.3 Clinical Applications of GIC

In the past, GIC were used mainly for restoration of abrasion/erosion lesions and as a luting agent for crown and bridge reconstruction. Today, their clinical application has extended to include the restoration of proximal lesions, occlusal restorations in the primary dentition, cavity bases and liners and core materials by the introduction of a wide variety of new formulations. Its ability to adhere to a wide variety of structures and materials found in the oral cavity, including enamel, dentin, porcelain, gold and cobalt chrome alloys makes it a valuable material for use in the mouth. The poor esthetic appearance eliminates their use as restorative materials in esthetic
anterior restorations and they are still not as widely used as filling materials as are composites. Their adhesive properties support their use for the restoration of cervical caries and for abrasion and erosion lesions, while their low strength and wear resistance prevent their use as restorative materials in occlusal and stress-bearing areas of the permanent teeth. Another recommendation for their use is as pit and fissure sealants because of their self adhesive potential with enamel. In orthodontics they can be applied for band cementation and bracket bonding (Bowen, 1992; Eliades, 2003; Kovarik, 2005; Van Noort, 2002).

2.2.4 Biocompatibility of GIC

Traditional GIC are the most biocompatible form of the glass ionomer cements. Attempts at strengthening the material by adding metal or resin reinforcement have resulted in higher toxicity.

2.2.4.1 Pulpal irritation- when GIC were first introduced, with just one acid (polyacrylic), pulpal response was classified as bland and less irritating than the response to zinc phosphate cement and composite resins. Residual polyacrylic acid in the set cement may have an adverse effect on the pulp integrity. However, Polyacrylic acid is much weaker than phosphoric acid and possesses a higher molecular weight than the resin monomers, which supposedly limits its diffusion to the pulp. The higher molecular mass together with the dentin buffering capacity seem to cause only a mild and short-term inflammation in the absence of bacteria. With the addition of many more acids to enhance certain characteristics and reduce setting time, GIC became more irritating, especially when used as luting agents in areas with thin layers of dentin. Direct contact with pulp tissue can cause pulp tissue necrosis and should be avoided. If the cavity is very deep it is sometimes recommended that a calcium hydroxide lining is placed prior to insertion of the GIC (Stanley, 1992; Eliades 2003; Van Noort, 2002; Bayne, 1992; Kovarik, 2005).

2.2.4.2 Toxicity- most of the clinical information about GIC toxicology has been developed using liners bases and cements, because those were the first widespread applications for this class of materials. Glass ionomer reports on post-operative sensitivity are most probably related to manipulation problems. There are no reports of long-term tooth damage from this sensitivity.
despite release of ions and polyacids from the GIC. The GIC have been widely used for a long period of time and so far there are no reports associating them with the onset of systemic diseases (Bayne, 1992; Kovarik, 2005).

The cytotoxicity of GIC, when placed in direct contact with human cells such as osteoblasts, gingival fibroblasts, mucosal fibroblasts and odontoblasts, is much more pronounced when the GIC is newly mixed and placed on the tissue. The toxicity is a result of the release of fluoride ions, aluminum ions and polyacids. Its ability to release fluoride, which makes GIC unique, also contributes to its cytotoxic effect on cells. Fluoride ions inhibit cell growth, proliferation and metabolism in the pulp tissue. It is also toxic to gingival and mucosal fibroblasts. Since all these effects of fluoride are dose dependent, they may have a clinical effect only during the first 1-3 days after initial setting reaction (Kovarik, 2005).

2.3 Polyacid Modified Composite Resins - PMCR

Polyacid-modified composite resins (PMCR), known as compomers, were introduced in the early 1990's and were presented as a new class of dental materials designed to combine the esthetics of traditional composite resins with the fluoride release and adhesion of glass-ionomer cements. The name compomer was devised from the composite "comp" combined with the ionomer "omer" (Nicholson, 2007; Van Noort, 2002).

The goal in developing compomers was to produce a material that had the strength, esthetics and workability of composite and the fluoride release of GIC.

Compomers are indicated for areas that are low stress, near the gingival-tooth junction or in primary teeth and have become popular as a restorative therapy for children. A comparison between compomers (Compoglass and Dyract) and composite resins (Prisma TPH and TPH Spectrum) revealed differences only for the criteria of marginal discoloration and wear, with compomer being inferior to the hybrid composite resin (Attin, 2001; Hse, 1997).
2.3.1 Composition of PMCR

These products are composite materials, as they are composed of an ion-leachable glass embedded in a polymeric matrix. It appears that compomers behave more like composite resins than glass-ionomers. Compomers use a dentin bonding system similar to that of composites and the procedural steps are similar and their setting reaction is an addition polymerization. They differ from the GIC in that the glass particles are partially silanized to provide direct bond with the resin matrix (Meyer, 1998; Nicholson, 2007).

The majority of components are the same as for composite resins. Typically, these are oligomers such as Bis-GMA and/or UDMA blended with diluents such as TEGDMA. The fillers are non-reactive inorganic powders, such as quartz or silicate glass particles and the filler particles are coated with silanes to promote bonding with the matrix, same as in the composite resin system. They also contain some reactive glass powder of the type used in GIC. This fluoro-alumino-silicate glass is thus susceptible to acid attack and provides the source of fluoride ions. In addition, and differently than conventional composites, they contain monomers which have two carboxylic groups and two double bond functions, so they are able to react simultaneously with the dimethacrylate monomers, by radical polymerization and with the cations, liberated from the glass particles, by an acid-base reaction. The matrix of the first commercially available compomer Dyract [DENTSPLY] has been modified with the addition of hydrophilic monomers such as TCB resin (di-ester of 2-hydroxyethyl di-methacrylate with butane tetracarboxylic acid).

![Figure 2.5: Structure of TCB resin.](image)

TCB resin consists of a new monomer of dual functionality, made up of a butane tetracarboxylic acid backbone with a polymerizable HEMA side chain. The resultant new monomer contains two
methacrylate groups, which can cross link with other methacrylate terminated resins, as well as two carboxyl groups, which can undergo an acid-base reaction to form a salt with metals and water (DyraC Xtra, DENTSPLY technical product profile).

In F2000 [3M] the resin matrix consist of the CDMA oligomer, (dimethacrylate functional oligomer derived from citric acid) which has a high ratio of methacrylic groups to carboxyl groups, allowing greater cross linking of the resin matrix. It also contains glycercyl dimethacrylate (GDMA) which is chemically and functionally similar to HEMA. Like HEMA, it contains a hydrophilic hydroxyl group and acts as a diluent for the CDMA and copolymerizes with the oligomer. In addition, according to the manufacturer, the F2000 compomer contains an essential and unique ingredient, a high molecular weight, hydrophilic polymer which rapidly takes up a controlled amount of water from the oral cavity, facilitating the transport of fluoride. Since it is a large and flexible polymer, it contributes to the handling characteristics of F2000, according to the manufacturer (HSE, 1999; 3M F2000 technical product profile).

![Fig. 2.6: Structure of GDMA.](image)

The final ingredient required to provide the fluoride release characteristic of compomers is water. Compomers do not contain water, but it is absorbed from the oral environment. The water sorption allows an acid-base reaction and provides the mechanism for a slow, continuous release of fluoride (Nicholson, 2007; Van Noort, 2002).

### 2.3.2 Properties of PMCR

2.3.2.1 Setting- compomers lack the ability to set in the absence of light. They set by a polymerization reaction and only once set do the minority hydrophilic components draw in a limited amount of water to promote a secondary reaction. The post-polymerization component of the setting reaction is acid-base in nature with some features in common with the GIC chemistry, most notably the release of fluoride. Polymerization in compomers is associated with
a contraction stress, as it is in conventional composites (Nicholson, 2007; Meyer, 1998; Burke, 2006; Chen, 2003).

2.3.2.2 Bonding to tooth structure- compomers are similar to composite resins in that they are fundamentally hydrophobic, though less than composites. Compomers do not have the ability to bond to dentin or enamel, so bonding agents are required, as in conventional composite resins. Some are used with single-bottle bonding agents that contain acidic primers. However, most manufacturers recommend phosphoric acid etching before priming to improve bond strength (Ruse, 1999).

2.3.2.3 Fluoride release- compomers are designed to release fluoride in clinically beneficial amounts. Fluoride is present in the reactive glass filler and becomes available following reaction of this glass with the acid functional groups, triggered by moisture uptake. Their fluoride release levels are significantly lower than those of GIC and of resin modified GIC and replenishment with fluoride is not possible, irrespective of the pH of the environment. As in other fluoride releasing materials, it has not been proven clinically yet whether the incidence of secondary caries is significantly reduced (Attin, 1999; Craig, 2002; Wiegand, 2007).

2.3.2.4 Water sorption- Following polymerization, the compomers take up small amounts of moisture and this triggers an acid-base reaction between the reactive glass filler and the acid groups of the functional monomer. This process causes fluoride to be released from the glass filler to the matrix, from where it can readily be released into the mouth and act as an anticariogenic agent. This water sorption may play some part in reducing the contraction stresses in vivo (Ruse, 1999; Nicholson, 2007).

Although compomers are designed to take up water, it has been shown to have an adverse effect on many of their mechanical properties. The flexural strength of Dyract AP [DENTSPLY] and Compoglass [Vivadent] declined on storage in water. This behavior differs from that of composite resins, which have been found to take up modest amounts of water, but to show no significant changes in mechanical properties (Van Noort, 2002).
2.3.2.5 Polymerization shrinkage- is similar to that of composite resins and water sorption is also quite the same. The difference is that the rate of water intake in compomers is higher than in composite resins. In the compomer the hydrophilic resin matrix provides a more rapid pathway for the absorption of water, with equilibrium water uptake being reached in a matter of days rather than months or even years in composite resins (Van Noort, 2002).

2.3.2.6 Ease of use- compomers' popularity is due, in part, to their ease of placement. No mixing is necessary and they have excellent handling characteristics. They are easy to polish and they give relatively good esthetic results (Van Noort, 2002). Their easy manipulation is especially beneficial in treating children because restorations can be completed faster and within the tolerance of a child patient (Hse, 1999).

2.3.2.7 Mechanical properties- in general, the mechanical properties of compomers do not differ much, if at all, from those of conventional composite resins, except for one property that differs significantly – fracture toughness. In a study comparing compomers with conventional composite resins the fracture toughness for composites fell in the range of 1.75-1.92 MPa m$^{1/2}$, whereas for compomers it was 0.97-1.23 MPa m$^{1/2}$. It was suggested that compomers should not be used in stress-bearing areas (Yap, 2004).

2.3.2.8 Buffering- compomers have been found to change the pH of lactic acid storage solutions in the direction of neutral. This buffering behavior has been also observed for GIC but was not found for conventional composites. Buffering has been suggested as being desirable under clinical conditions, since the ability to reduce the acidity of caries-producing acids (mainly lactic) would be expected to reduce the development of caries in vivo (Nicholson, 2007).

2.3.3 Clinical applications of PMCR

Compomers are designed for the same sort of clinical application as conventional composites. However, since their mechanical properties and wear resistance tend to be inferior to those of composites, but superior to those of GIC and resin modified GIC, their use should be limited to low bearing situations. These include class V and abrasion restorations, fissure sealants and
bonding of orthodontic bands, together with permanent restorations in the primary dentition and long-term temporaries in the permanent dentition (Van Noort, 2002).

2.3.4 Biocompatibility of PMCR

Several studies on the biocompatibility of compomers have shown that residual monomers and additives are leaching from them even after adequate polymerization (Michelsen, 2003; Michelsen, 2007; Geurtsen, 1998; Lygre, 1999). The elution from various materials, when placed in either ethanol or Ringer’s solution, differs significantly not only on the types of elutes but also on the amounts, therefore, the materials have different potential for causing adverse effects. In a study comparing a compomer (Dyract AP) to a resin modified GIC and two composites, HEMA elution, as well as 2-hydroxy-4-methoxybenzophenone (HMBP), was the highest for the compomer. HEMA causes cell apoptosis and HMBP, which is a photostabilizer, was recently found to have estrogenic activity in vitro (Becher, 2006; Michelsen, 2007). Concentration related apoptosis and necrosis were found in macrophages after exposure to two compomer extracts (F2000 and Freedom [SDI]) and three of their constituents (GDMA, TEGDMA and HEMA). GDMA appeared to be the most cytotoxic material of the tested constituents (Becher, 2006). In another study it was observed that a compomer (Dyract Cem) leached high amounts of TEGDMA into an aqueous medium, which may contribute to pulpal irritation and promote secondary caries (Geurtsen, 1998).

2.4 Resin Modified Glass Ionomer Cements (RMGIC)

In the mid 1980's an attempt was made to strengthen GIC by adding a polymerizable, hydrophilic resin to the polyacrylic acid. Depending upon what percentage of the carboxylate groups are substituted by methacrylate groups, the material is more or less like a GIC or a composite resin. RMGIC or hybrid ionomers, is a two component system that cures by means of an acid-base reaction, supplemented by light-activated polymerization.
RMGIC have become popular in dentistry as an aid in preventing recurrent caries beneath cemented restorations such as crowns, bridges or veneers. They are recommended for patients with high caries risk (Kovarik, 2005; Smith, 1998).

2.4.1 Composition of RMGIC

The powder of RMGIC is similar to that of conventional GIC. The liquid contains hydrophilic monomers, such as HEMA, polyacids, tartaric acid and a photo-initiator in an aqueous solution.

The RMGIC set by a combined acid-base ionomer reaction and light cured resin polymerization. When the material is mixed, the glass ionomer setting reaction begins, involving the ionic interaction between positively charged ions and the carboxylate groups. These materials will set by this process alone. However, the curing light can be applied at any time, causing free radicals formation and covalent cross-linking of the methacrylate groups. The RMGIC behave quite differently from one another, depending on the amount and type of the resin element incorporated (Kovarik, 2005).

2.4.2 Properties of RMGIC

2.4.2.1 Adhesion- RMGIC can bond to tooth structure without the use of a dentin bonding agent. Typically, the tooth is conditioned with polyacrylic acid or a primer before inserting the restoration. The bond to enamel and dentine is as good, if not superior, to that of the traditional GIC (Craig, 2002).

2.4.2.2 Strength and wear- RMGIC are significantly stronger than traditional GIC. The tensile strength is about double of a standard GIC (Kovarik, 2005).

2.4.2.3 Fluoride release- RMGIC release more fluoride than compomers and composites, but almost the same as conventional GIC. There is an early period of high release, which tapers after
about 10 days. RMGIC have the same ability to recharge with fluoride when exposed to fluoride dentifrices, as GIC (Craig, 2002).

2.4.2.4 *Ease of use*- their manipulation is like that of standard GIC. Unlike conventional GIC, RMGIC set immediately when light cured (4 minutes for GIC vs. 20 seconds for RMGIC). The advantage to this is the shorter waiting time for setting to occur and that the restoration can be polished soon after. The RMGIC are also less sensitive to moisture, therefore making their handling more convenient (Craig, 2002; Kovarik, 2005; Van Noort, 2002).

2.4.2.5 *Prolonged working time*- the acid-base reaction is the same as for the GIC with the only difference of it being slower, thus allowing a longer working time (2 minutes for GIC vs. 3.45 minutes for RMGIC). The rapid set is provided by the light activation mechanism (Van Noort, 2002).

2.4.2.6 *Aesthetics*- these restorations are more aesthetic than glass ionomers because of their resin content.

2.4.3 *Clinical applications of RMGIC*

RMGIC have been designed specifically as direct restorative materials or as bases and liners for use under composites, amalgams and ceramic restorations. When used in conjunction with composites, a strong bond is obtained between the liner and the composite and there is no need to etch the surface of the liner. They are very popular and perform better than traditional GIC as posterior restorative material in the primary dentition. RMGIC are an increasingly important part of operative dentistry for both the aging population with high incidence of root caries and children who have minimal dental care but high caries risk factors (Craig, 2002; Van Noort, 2002).
2.4.4 Biocompatibility of RMGIC

RMGIC are far less biocompatible than traditional GIC. Depending upon the amount of resin in the formulation, biocompatibility studies report a range of low toxicity to high toxicity. As a restorative material it is tolerated well. However, more pulpal inflammation has been noted than with the traditional GIC. The biocompatibility improves as the cement reaches full maturation. In addition to the same chemicals that are leached from the traditional GIC, other extracted components were Bis-GMA, Bis-EMA, UDMA, TEGDMA, HEMA and some initiators and other additives, when incubated in water, ethanol, acetic acid or artificial saliva. One of these photo-initiators, diphenylliodoniumchlorite (DPICI), has been mentioned as a toxic chemical released from one RMGIC (de Souza Costa, 2003; Schedle, 1998; Geurtsen 2000; Rogalewicz, 2006).

2.5 Human saliva

Saliva is a clear glandular secretion that constantly bathes the teeth and the oral mucosa and is critical to preservation and maintenance of healthy oral tissues. It is a mix of secretions of the three paired major salivary glands, the parotid, submandibular and sublingual, the minor salivary glands and the gingival fluid (Humphrey, 2001; Edgar, 2004).

2.5.1 Composition of saliva

Saliva is a very dilute, hypotonic fluid, composed of more than 99% water. Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, urea and ammonia. These components generally occur in small amounts, varying with changes in flow, yet provide important functions. The composition of saliva is affected by many factors, such as the type of salivary gland producing the saliva, flow rate and circadian (daily) and circannual (yearly) rhythms.
The normal pH of saliva is 6 to 7, making it slightly acidic. The pH can range from 5.3 (low flow) to 7.8 (peak flow). Major salivary glands contribute most of the secretion volume and electrolyte content, whereas minor glands contribute the blood group substances (Mandel, 1989).

2.5.2 Salivary flow

There is a great variability in individual salivary flow rates. The secretion of saliva is controlled by a salivary center in the medulla, but there are specific triggers for this secretion. There are mechanical (chewing), gustatory and olfactory triggers, as well as psychic factors (such as pain), medications or various systemic diseases affecting salivary flow.

*Unstimulated whole saliva* is the mixture of secretions that enter the mouth in the absence of exogenous stimuli. The average value for whole saliva in healthy individuals is about 0.3-0.4 ml/min, but the normal range is very wide and only unstimulated flow rate below 0.1 ml/min is considered hypofunction. Whether the flow rate is high or low is less important than whether it has changed adversely in a particular individual. Many factors affect the unstimulated flow rate, such as degree of hydration, body posture (lying/standing), smoking, circadian rhythms and drugs.

*Stimulated saliva* is secreted in response to masticatory or gustatory stimulation, or to other exogenic stimuli. Stimulated flow rate has an average maximum value of 7ml/min. It is reported to contribute as much as 80-90% of the daily salivary production.

*Whole saliva flow volume* is the mix of stimulated and unstimulated saliva, gingival crevicular fluid, oral bacteria, food debris and traces of drugs/chemicals. The average daily flow of whole saliva is 500-1500 ml (Humphrey, 2001; Edgar, 2004).

2.5.3 Salivary functions

The functions of saliva fall into the following five major categories:
2.5.3.1 *Lubrication and protection*- saliva coats mucosa and helps protect against mechanical, thermal and chemical irritation. The irritants include proteolytic and hydrolytic enzymes produced in plaque and potential carcinogens from smoking and chemicals. The best lubricating components of saliva are mucins that are excreted from the minor salivary glands.

2.5.3.2 *Buffering and cleansing*- saliva helps neutralize plaque pH after eating, clears foods and aids in swallowing. The components involved in the buffering action are bicarbonate, phosphate, urea, proteins and enzymes. The buffering action of saliva works more efficiently during stimulated high flow rates and is almost ineffective during periods of low flow with unstimulated saliva.

2.5.3.3 *Maintenance of tooth integrity*- saliva modifies the pH of plaque surrounding the enamel, reducing demineralization, thereby inhibiting caries progression. Plaque thickness and the number of bacteria present determine the effectiveness of salivary buffers. Saliva also promotes the remineralization process. Supersaturation of minerals (calcium and phosphate) in saliva is critical to this process. The presence of fluoride in saliva speeds up crystal precipitation, forming fluoroapatite, which is more resistant to caries than the original tooth structure (hydroxyapatite).

2.5.3.4 *Antibacterial activity*- specific and non-specific antimicrobial mechanisms help control oral microflora. Salivary glands secrete fluid containing immunologic and non-immunologic agents for protection of oral tissues. Immunological agents include secretory IgA, IgG and IgM. Non-immunological agents are selected proteins, mucins, peptides and enzymes.

2.5.3.5 *Taste and digestion*- saliva acts as a solvent, thus allowing interaction of foodstuff with taste buds to facilitate taste. The hypo-tonicity of saliva enhances the tasting capacity. Salivary enzymes originate from minor and major salivary glands, oral tissues, oral microorganisms, inflammatory responses and ingested substances. The levels and types of enzymes can vary between individuals and are classified into five major groups: Carbohydrases; Esterases; Transferring enzymes; Proteolytic enzymes; other enzymes (Chauncy, 1961). Cholinesterases (ChE) are a group of esterases found in whole saliva and its activity is to hydrolyze choline esters. Salivary ChE activities seem to be very low compared to those of
serum. There are two main types in humans: acetylcholinesterase (ACE), which is highly specific to acetylcholine and pseudocholinesterase (PCE). PCE has been shown to have a strong degradative activity toward the TEGDMA monomer and to reduce the surface micro-hardness of commercial dental restorative composites, compared to a control (Jaffer, 2002). In a salivary PCE study, its activity in male subjects was found to be twice as much as that of females. It also showed diurnal variation, being about three times greater at 4 a.m. than at 4 p.m. No correlation was found between the salivary flow rate and PCE activity. Enzyme PCE activity in gingival crevicular fluid was much higher than that of whole saliva, and was not found in sonicated samples of plaque, indicating that crevicular fluid is the source of enzyme activity in saliva (Ryhanen, 1983). A relationship between salivary enzymatic activity and periodontal disease has been shown. High levels of salivary PCE were found in patients with periodontitis and they were decreased with periodontal therapy (Yamalik, 1991). Cholesterol esterase (CE) is one of the enzymes produced by mature macrophages during acute inflammation and it may be used as a model enzyme for the biodegradation of dental composites with some relevance to in vivo conditions (Santerre, 2001). The process of resin degradation was shown to exhibits greater sensitivity to CE than to PCE and may be related to their different reactivity to natural and synthetic substrates (Finer, 2004).

2.5.4 Saliva as a diagnostic fluid

Saliva has become useful as a noninvasive systemic sampling measure of medical diagnosis and research. It can be used to monitor the presence and levels of hormones, antibodies, microorganisms and ions. It is used for caries risk assessment, identification markers for periodontal disease, diagnosis of viral diseases, cancers, ulcers and Sjogren's syndrome (Malamud, 1992).

2.6 Biodegradation of resin based restorative materials

Biodegradation, in the mouth, is a complex process, since it involves dissolution and disintegration in saliva, wear and erosion by food and other chemical substances, chewing forces
and bacterial activity. The stress bearing restoration has to survive the rigors of a wet environment, rapid temperature changes, as well as regurgitated stomach fluids that are highly acidic. Most of the studies of by-products elution from the biodegradation process of resin composites were conducted in vitro. No in vitro test is capable of reproducing a similar complex system and the correlation between in vitro data and what really happens in vivo is unknown, in many cases (Yap, 2000; Oilo, 1992).

After the different degradation products have left the surface of the resin composite restoration, they may continue degrading as they are transported through the body and may participate in various biologic reactions. These reactions can cause health problems and have an impact on the biocompatibility of composite restorations. Components may be eluted into salivary fluids and also may be extracted into dentin where they may diffuse toward the pulp (Ferracane, 1994; Eliades, 2003).

The nature of the degradation process of composites in the mouth is either mechanical or chemical or a combination of both. The mechanical wear is influenced by many factors including chewing load, occlusal contact area, type of food intake, efficiency of curing and polishing procedures. A factor contributing to the degradation of microfill composites is the fact that the pre-polymerized resin fillers are not bonded well to the polymer matrix. The resin fillers are heat-cured and do not form covalent chemical bonds with the polymerizing matrix, due to the lack of available methacrylate groups on their surfaces. Therefore they become dislodged under high stresses (Ferracane, 1995). In addition to the mechanical abrasive wear, chemical processes are also involved in the degradation of resin composites.

Composites can undergo leaching to release compounds and also are prone to enzymatic hydrolysis. Several studies determined that in all of the polymerized composite resins specimen that were investigated for leachable components, monomers and various additives, as well as contaminants from manufacturing processes, were identified (Spahl, 1998; Ortengren, 2001; Soderholm, 1984). It has been shown that residual monomers and other components are released from polymerized composites depending on the monomer-polymer conversion rate (Hansel,
Leaching of components from resin composites may occur at two points in time: during the setting period of the resin, and later when the resin is degraded. Leaching during the first process is related to the degree of conversion or the chaining of the oligomer into a polymer. The elution of unreacted components is a diffusion rate-dependent process (Hanks, 1991). Many studies evaluated the amount and toxicity of components released from dental composites into various extraction media, such as water, ethanol, methanol, artificial saliva and culture medium (Ortengren, 2001; Tanaka, 1991; Spahl, 1998; Moharamzadeh, 2007; Al-Hiyasat, 2005). It has been shown that more than 30 different components, with residual monomers as the main components, were released from cured dental composites. The elution of monomers appears to be mainly within the first days after placement of the restoration (Oysaed, 1988; Mazzaoui, 2002). TEGDMA has been found to be the major monomer eluted from composite resins, but also identified were HEMA, UDMA and Bis-GMA (Spahl, 1998). TEGDMA is a low molecular weight monomer, hence has higher mobility which allows it to be eluted faster than larger molecules, such as Bis-GMA and UDMA (Tanaka, 1991; Moharamzadeh, 2007). The bioavailability of TEGDMA in the saliva and pulp has been reported minutes and days after placement of a restorative resin material (Yourtee, 2001). The various additives can be extracted easily by methanol, due to their low molecular weight and them not being part of the polymerized monomer network. These substances are only present in the composite resins in very small quantities (Spahl, 1998).

The polymerization of composite resins is never complete. It has been verified that 25-55% of the methacrylate groups remain unreacted after polymerization. Light curing has increased the possibilities for incomplete conversion during clinical work, since it added another component that is dependent on clinical manipulation. Most of the unreacted carbon-carbon double bonds are on molecules that have reacted at one end and are thus bound to the polymer chain and are not free to elute. They are referred to as pendant methacrylate groups. Nearly 90% of the unreacted methacrylate groups are present on pendant molecules. The polymer matrix also contains a small portion of residual monomer. The incomplete polymerization leads to material degradation and release of components of the resin-based materials that may cause reactions both locally and systemically (Oilo, 1992; Ferracane, 1994).
Restorations of incompletely polymerized resin composites are more likely to undergo degradation than more fully polymerized restorations. Also, the outer surface of a composite restoration and the surfaces of entrapped air bubbles inside the filling may be poorly polymerized because of the inhibiting effect of oxygen on polymerization (Bowen, 1992; Ferracane, 1994). Inhibition of polymerization in surface layers exposed to oxygen presents a problem with the use of dental composites. If not removed, such layers will release an amount of monomers or degradation products from the composite corresponding to the thickness of the un polymerized layer (Oysead, 1988).

Composite resins also include flowable resins, which have lower filler volumes than the conventional composites. They are made flowable by the addition of resin diluents and may therefore exhibit increased leaching. Core materials are composites with higher filler content and may have metals added to them to enhance their strength. When assessed in vitro and compared to conventional composites for cytotoxicity, flowable and core composites were highly cytotoxic upon direct contact with fibroblasts. Based on high performance liquid chromatography (HPLC) results it could be presumed that the cytotoxicity of the materials could be related to the amount of TEGDMA that was leached into an aqueous solution (Al-Hiyasat, 2005; Wataha, 2003). Release of monomers has also been shown to occur from resin-modified GIC and compomers (Hamid, 1998; Mazzaoui, 2001).

After polymerization, it can be assumed that saliva, food components and beverages may affect dental composites. Ester bonds are susceptible to hydrolytic degradation. When the monomer molecules leach into the water, the water molecules diffuse into the resin. During this process, the matrix will swell and there will be an increase of the distance between the polymer chains. The increased distance results in weaker polar interactions between the separated chains, which in turn results in a softer and more wear-susceptible material. The chemical degradation of the polymer is triggered by the water, leading to the creation of oligomers and finally monomers that are released through pores in the polymer bulk (Eliades, 2003; Ortengren, 2001; Gopferich, 1996). It has been shown that not only ethanol, but also two organic acids of plaque (acetic and propionic acids) induced a leaching and/or mechanically weathering of Bis-GMA and UDMA based polymer. The oral cavity probably presents an environment somewhere between the more
aggressive solvents used in this study, and water, which is less aggressive (Lee, 1998). Degradation of composites can also occur as a result of overheating, as when the restoration is polished. If the frictional heat exceeds 200ºC, methyl methacrylate-based resins start depolymerizing and form monomer segments. These segments can leach out, leaving the surface porous, which in turn facilitates degradation caused by other compounds (Eliades, 2003).

In addition, it has been shown that enzymes, such as esterase, present in the oral cavity, may hydrolyze and break ester links found in composite resins. Changes in composition and chemistry of the monomers and fillers can have an effect on the degradation of the composites. Esterase enzymes can hydrolyze Bis-GMA and TEGDMA based composite resins to give Methylacrylic acid (MA), bishydroxypropoxyphenylpropane (Bis-HPPP), triethylene glycol methacrylate (TEGMA) and ethoxylated bisphenol A (E-BPA) (Lin, 2005). Results seem to indicate that the dimethacrylates, such as TEGDMA and UDMA, exhibit greater tendencies to hydrolyze than the long chain methacrylates, such as Bis-GMA (Munksgaard, 1990). Softening of the Bis-GMA/TEGDMA copolymer also caused a significant loss in the wear resistance of the resin composites (Freund, 1990). Once a layer is softened by action of hydrolysis, it is easier for mechanical forces to remove the softened layer and expose a new surface for enzymatic attack (Wu, 1982).

The surface hardness of the Bis-GMA/TEGDMA polymer after hydrolysis treatment with porcine liver esterase (in a concentration equivalent to the mean hydrolase activity found in saliva) may be reduced by about 15% in five days as a result of hydrolytic degradation (Larsan, 1992). While HEMA was shown to be resistant to hydrolysis by ACE and CE, TEGDMA was susceptible to both enzymes. A possible use for these findings is to predict resistance of polymers to surface hydrolytic degradation (Yourtee, 2001). Urethane-modified Bis-GMA/TEGDMA (ubis) based monomer system showed an increase in its chemical stability over that of the traditional Bis-GMA/TEGDMA (bis) system, when exposed to CE (Finer, 2004).

An interesting finding was the observation of a significant decrease in the amount of TEGDMA leached into serum containing and serum-free culture mediums. This finding demonstrated the
importance of the extraction medium used in toxicity tests. TEGDMA elution into culture media was limited and proteins present in the serum, such as albumin, bind to TEGDMA, reducing its toxic effect by lowering the concentration of free TEGDMA (Moharamzadeh, 2007). When hydrolyzed at the ester bond, MA is a common end-product of all methacrylate-based monomers such as Bis-GMA/TEGDMA resins (Freund, 1990).

Not all esterases have the same specificity for the different resin monomer components. PCE preferentially hydrolyzes TEGDMA over Bis-GMA, while CE's activity with respect to Bis-GMA is about 13 times greater than that of PCE (Finer, 2003). There is a synergistic effect between CE and PCE for the degradation of resin composites. Since each enzyme has been shown to have a distinct specificity for defined composite resin components, there might be a cooperative effect with respect to the cleavage sites (Finer, 2004). It has been suggested that, since two enzyme activities were isolated and characterized to have distinct specificity with respect to the monomer components of dental composites, evaluation of enzyme(s) content in an individual's saliva may pre-determine their susceptibility to having synthetic materials undergo biodegradation (Lin, 2005).

The amount of filler particles could also influence the degradation process and its end-products. When two model composite systems were incubated with CE, the release of biodegradation products (Bis-HPPP and TEGMA) was significantly higher in the first 8 days for the lower filler model material than the higher filled composite. The opposite effect was observed between the 8 and 16 days, indicating that the higher filled composite underwent a significant surface disintegration following chemical degradation of the resin phase. In the oral cavity, mastication forces could enhance this effect (Shajii, 1999). A later study found that the higher filler content system did show an increase in its stability with time over the lower filler content group, when incubated with CE. The higher filler content composite leached less unreacted monomer (TEGDMA) as well as generated less biodegradation products (MA and Bis-HPPP) (Finer, 2006).
2.6.1 Biodegradation of PMCR

In most previous studies, compomers were studied *in vitro* by elution analysis of leached monomer components and not for biodegradation products released as a result of exposure to cells and biological fluids (Michelsen, 2003; Michelsen, 2007; Guertsen, 1998; Becher, 2006; Lygre, 1999). HPLC analysis of two compomers' degradation products, following incubation with CE, reported qualitative and quantitative differences between the two materials (Revuelta, 2006). The findings of that study suggested that Dyract AP is degraded more extensively than Z250 and F2000. Dyract AP samples released significantly higher amounts of Bis-HPPP, TEGDMA and MA than the F2000 and Z250 samples. Dyract AP also released more fluoride for each incubation period in the presence of CE as compared with F2000. However, the compomers' full interaction with human saliva is yet unknown. Their capacity to take up water may increase hydrolysis rate, enhance the penetration of salivary esterases and resin matrix degradation product transport.

2.6.2 Clinical relevance

Degradation of compomers and composite resins will lead to reduced mechanical properties, discoloration of the restorations and accelerated wear. Furthermore, the incomplete conversion of monomers and the biodegradation processes release numerous substances that have been shown to have an effect on the surrounding tissues and maybe influence distant tissues as well. As pointed out above, biodegradation compounds of composite resins and compomers and leachable substances have been associated with adverse reactions such as post-operative sensitivity (Stanely, 1992), hypersensitivity/allergy (Yap, 2000; Munksgaard, 1990; Oysaed, 1988), cytotoxicity (Hanks, 1981; Hanks, 1991; Hume, 1996; Becher, 2006), stimulation of cariogenic bacteria (Hansel, 1998; Khalichi, 2004) and mutagenic effects (Schweikl, 2006). The risk of having estrogenic or mutagenic responses to these monomers might be considered insignificant in the short term, but due to the growing tendency to have resin based restorations in the anterior, as well as the posterior teeth one cannot overlook the effects of long term exposure. With the total amount of resin-based restorations on the rise, they may be considered as contributing to the
exposure to organic substances responsible for allergic and toxic effects. Safety and durability tests and studies of new resin-based restorative materials should include enzyme resistance tests.
CHAPTER 3 – MATERIALS AND METHODS

3.1 Sample preparation:

Materials used in this experiment were Dyract eXtra (DENTSPLY), F2000 and Z250 (3M). Dyract AP (DENTSPLY) was used only for the fluoride analysis. Standardized samples were prepared using Mylar® strips and a Teflon® mould of 4mm diameter by 4mm depth (Finer, 2004). Samples were photo-cured using a light-curing lamp (THE MAX, DENTSPLY) for 20 seconds on each side for a total of 40 seconds, as per the manufacturer's instructions. Cured samples were post-cured for 48 hours at 60°C in a vacuum oven and pre-incubated in PBS solution, pH 7.0 at 37°C for 48 hours to reduce the fraction of unreacted leachable monomers and increase the degree of monomer conversion (Ferracane, 1992; Bagis, 1997; Finer, 2006).

3.2 Human saliva samples:

Unstimulated whole human saliva was collected (University of Toronto Human Ethics Protocol # 15548) and processed as described previously (Lin, 2005). The saliva samples were collected and processed by Dr. Babak Shokati. Each whole saliva sample was first homogenized for 15 seconds and bulk debris was separated from the whole saliva by centrifugation, using a clinical model centrifuge at 2400 rpm for 20 minutes at 4°C. The supernatant was collected and then filtered using 0.8/0.2 μm syringe filters (Supor® Acrodisc®, PALL Gelman Sciences, Ann Arbor, MI). The filtrates from patients were pooled and freeze-dried at -78°C until required and later reconstituted with PBS to desired HSDE activity level.

3.3 CE-like activity assay:

The CE-like activity of the thawed filtered saliva samples was measured by using a spectrophotometer. The enzyme activity was determined by using para nitrophenol butyrate (p-NBP) as the enzyme substrate.
4 mM P-NBP substrate solution was prepared in advance by adding 17.7µl of p-NBP to 5.5 acetonitrile. The solution was vortexed and diluted by 19.5 ml of PBS, divided into aliquots and stored at -80°C in 5ml amber glass vials until needed. An Ultrospec II spectrophotometer unit (LKB Biochrom, Cambridge England) was used at wavelength of 410 nm and temperature set to 37°C.

One unit of CE is defined as the amount of CE capable of generating 1 nmole /min of para nitrophenol.

CE catalysis

\[
\text{p-NBP} \quad \rightarrow \quad \text{p-nitrophenol (yellow)} + \text{butyrate}
\]

CE activity was measured at a wavelength of 401nm, by adding the following reagents to a 1.5 ml optical plastic cuvette:

950 µl of 50 mM sodium phosphate buffer

500 µl of 0.4 mM p-NBP

50µl of a saliva sample

Spectrophotometric measurements were obtained every 30 seconds for 300 seconds, using a cuvette with 50 µl PBS instead of saliva, as the reference. CE activity was calculated using the following equation:
CE activity (units/ml) = \( \frac{O.D \times T.V}{E.C \times L.P \times S.V} \)

Where:

- **O.D** = change in absorbance per minute at 401 nm
- **T.V** = total volume (1.5ml)
- **E.C** = molar absorptivity of pNBP at 401 nm (16000 M\(^{-1}\) cm\(^{-1}\))
- **L.P** = length path (1cm)
- **S.V** = sample volume (0.5ml)

Each saliva sample was measured in triplicates.

### 3.4 Enzyme Stability Assays:

Enzyme activity levels were measured at time intervals of 0, 1, 2, 4, 6, 10, 24 and 48 hours in the presence and absence of Z250, F2000, Dyract AP and Dyract eXtra samples when incubated in PCE/CE media with a measured activity level of ~11 units at starting point. A CE-activity protocol was used with para-nitrophenol butyrate (p-NBP) as the substrate. An Ultrospec II spectrophotometer unit (LKB Biochrom, Cambridge England) was used at wavelength of 410 nm and temperature set to 37\(^\circ\)C. 50\(\mu\)l of PCE/CE incubation media, 950 \(\mu\)l PBS and 500 \(\mu\)l of P-NPB (brought to 37\(^\circ\)C in a water bath) were mixed in a 1.5 ml optical plastic cuvette. The optical density (OD) was recorded every 30 seconds for 300 seconds, with a blank cuvette containing 1000 \(\mu\)l of PBS and 500 \(\mu\)l of p-NPB used as reference. Enzyme activity levels are reported as a percentage of the initial measured activity.

The same method was used to measure enzyme activity levels in the presence and absence of Z250, F2000, Dyract AP and Dyract eXtra samples incubated in HSDE media with a measured activity level of ~11 units at starting point at the same time intervals. Enzyme activity levels are reported as a percentage of the initial measured activity.
3.4.1 Preparation of PCE/CE media with a measured activity level of ~11 units:

PCE-like activity of saliva sample and of PCE/CE solution was determined by using butyrylthiocholine (BTC) and 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) as the enzyme substrate (Sigma diagnostics, catalog No. 421-10, St. Louis MO).

BTC/DTNB substrate solution was prepared in advance by dissolving 39.6 mg BTC and 2.48 mg of DTNB in 25 ml of PBS. The solution was vortexed, divided into aliquots and stored at -80°C in 5ml amber glass vials until needed.

One unit of PCE is defined as the amount of PCE capable of generating 1 mmol/min of butyrate.

PCE catalysis

BTC+DTNB+H₂O → butyrate+thiocholine+thio-2-nitrobenzoic acid (yellow)

PCE activity was measured at a wavelength of 405nm, by adding the following reagents to a 1.5 ml optical plastic cuvette:

1000 µl of BTC/DTNB solution

500 µl of saliva sample
Spectrophotometric measurements (Ultropec II spectrophotometer unit (LKB Biochrom, Cambridge England) were obtained every 30 seconds for 600 seconds, using a cuvette with 500 µl PBS instead of saliva, as the reference. PCE activity was calculated using the following equation:

\[ \text{PCE activity (units/ml)} = \frac{\text{O.D} \times \text{T.V} \times 1000}{\text{E.C} \times \text{L.P} \times \text{S.V}} \]

Where:

O.D = change in absorbance per minute at 405 nm
T.V = total volume (1.5ml)
E.C = molar absorptivity of TC at 405 nm (136000 M⁻¹ cm⁻¹)
L.P = length path (1cm)
S.V = sample volume (0.5ml)

Each saliva sample was measured in triplicates.

PCE-like and CE-like activities were determined for the saliva sample (1.76 units/ml and 11.45 units/ml, respectively). PCE solution was prepared in a concentration tested to have the same PCE-like activity level as recorded for the saliva (1.81 units/ml). CE solution was prepared and added to the PCE solution to match the level of CE-like activity that was recorded for the saliva sample (11.44 units/ml).

### 3.5 Biodegradation experiment:

PMCR and resin samples (N=3/material) were incubated in 1ml of HSDE in levels found in human saliva (~11 units of activity) (Jaffer, 2002) or PBS (control) for 14 days at 37°C, pH=7.0. Incubation media was collected and replaced at 2, 4, 7 and 14 days. Incubation media samples
were filtered (Millipore centrifuge filter device (Ultrafree®-CL, UFC4LCCOO 5000 NMWL, Millipore, Bedford, MA) at 2400 rpm for 30 minutes at 4°C, to remove high molecular weight proteins and refrigerated at 4°C until required for HPLC analysis. Non-incubated and incubated PMCR and composite samples were kept for Scanning Electron Microscopy (SEM) analysis.

3.6 Analysis of biodegradation products by HPLC:

In this study, biodegradation products of the collected incubation solutions were isolated and identified using Waters™ HPLC system (Waters™, Mississauga, Ontario).

![High-Performance Liquid Chromatography (HPLC) System](image)

**Fig 3.4: High-Performance Liquid Chromatography [HPLC] System.**

The separation process is based on injecting a sample of the solution into a column of partitioning material and eluting the solution by pumping a solvent system through the column. The different compounds migrate at different rates forming discrete bands. A reservoir holds the solvent, called the mobile phase. A high-pressure pump is used to generate and meter a specified flow rate of mobile phase. An injector introduces the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column. The column contains the
chromatographic packing material needed to effect the separation (the stationary phase). A detector is needed to see the separated compound bands as they elute from the HPLC column.

In this study, product isolation was carried out by using reverse phase HPLC which is comprised of a non-polar stationary phase and a polar eluting system. There will be a strong attraction between the polar solvent and polar molecules in the mixture being passed through the column. Polar molecules in the mixture will therefore spend most of their time moving with the solvent. Non-polar compounds in the mixture will spend less time in solution in the solvent and this will slow them down on their way through the column. In this case the polar molecules will travel through the column faster than the non-polar molecules.

The mobile phase used was HPLC grade methanol and 2mM ammonium acetate (pH 3.0) buffered solution (filtered with 0.22µm membrane filter).

The mobile phase solutions were degassed for 20 minutes prior to use for the HPLC system. Flushing of system between sample injections is carried out with purified water. 50µl of the prepared sample solution was drawn into a syringe and injected into the HPLC via the injector valve.

The HPLC system in this study utilizes a detector for scanning the UV spectra (215nm) every second during the analysis. A chromatogram is a representation of the separation that has chemically occurred in the HPLC system. A series of peaks rising from a baseline is drawn on a time axis.

The time taken for a particular compound to travel through the column to the detector is known as its retention time. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound. Different compounds have different retention times. Each peak represents the detector response for a different compound. The chromatogram is plotted by the computer data station.

For this study, separation of the composite resins biodegradation products was achieved using a gradient method developed by Finer and Santerre, 2004.

Data was recorded into the computer hard-drive using a specific software (Millenium chromatography manager software Version 2.15, Waters™, Mississauga, Ontario) as a
chromatogram and was represented in terms of an absorbance signal (volts/sec) given as a function of retention time.

Once the resin composite and compomers biodegradation products from the samples were isolated using the HPLC system, major peaks were selected from the chromatograms and samples were sent for mass spectrometry analysis to verify their identity.

3.7 Mass spectrometry (MS) analysis:

Mass spectrometry is an analytical technique that identifies the chemical composition of a sample based on the mass-to-charge ratio of charged particles. HPLC fractions of interest were collected and sent for analysis MS using a Hybrid quatrupole-Time of Flight mass spectrometer, model QStar Elite (Applied Biosystems/MDS Sciex, Foster city, CA) located at the Toronto Integrated Proteomics (TIP) Laboratory, MaRS Centre.

3.8 Fluoride analysis:

Incubation solutions, collected after each incubation period (2, 4, 7 and 14 days), were analyzed for fluoride ion concentration after filtering and analysis by HPLC. The solutions were diluted with 4ml distilled water to reach a volume of ~5 ml. Then, stabilized with 5 ml Total Ionic Strength Adjustment Buffer (TISAB II), they were analyzed for fluoride content by the Ion Analyzer Detector (Orion 930 Ionaalyzer System).

Fig 3.5: The Orion 930 Ionaalyzer System (www.thermo.com)

Fluoride concentration is expressed as parts per million (ppm).
Since the results were not as anticipated, a parallel study to investigate the effect of filtering the incubated solutions on fluoride ion concentration was conducted. Known concentrations of fluoride (1, 2.5 and 5 ppm) were prepared and added to 1 ml HSDE or PBS and incubated at 37ºC for 3 days. The incubated solutions were analyzed for fluoride ion concentration with or without filtering by the Millipore centrifuge filter device. Each fluoride concentration was run in triplicate. Fluoride concentration is expressed as parts per million (ppm).

Data from the parallel study demonstrated that fluoride release levels in the presence of human salivary esterases should be measured without filtering. Therefore, samples of Z250, F2000, Dyract eXtra and Dyract AP were incubated in 1 ml of either HSDE (~11 units of activity) or PBS over 14 days at 37ºC. Incubation solutions were collected and replaced at 2, 4, 7 and 14 days as in the original biodegradation study described above. Collected solutions were not filtered this time and fluoride ion concentration analysis was carried out as described above. Fluoride concentration is expressed as parts per million (ppm).

3.9 Surface morphology analysis:

Samples were analyzed by scanning electron microscopy prior to and following incubation for 14 days with either HSDE or PBS. Specimens from each experimental group were mounted on a holder using a double-sided adhesive carbon tape. The samples were coated with 6nm of platinum (SC515 SEM coating system, Polaron equipment LTD). Samples were analyzed at a magnification ratio of x2500, x5000 and x10000 using a Hitachi S 2500 scanning electron microscope with 10 KVolt operating voltage.

3.10 Statistical analysis:

For each experimental group, one-way ANOVA and Scheffe multiple comparison analyses were used with a significance level of p<0.05. The dependent variable was the amount of biodegradation product or fluoride released by each material, respectively for the biodegradation
and fluoride release studies. The independent variables were the material type and/or incubation time and/or incubation media.
CHAPTER 4: RESULTS

4.1 Enzyme stability assays:
It was observed that PCE/CE underwent a more rapid decrease in activity in the presence of the compomer and composite samples than without any specimen. A relative activity of 50% was reached at 10, 5, 3, 5 and 6 hours for the no specimen, Z250, Dyract AP, Dyract eXtra and F2000 incubation media, respectively (fig 4.1). When incubated in HSDE, Dyract AP reached a relative activity of 50% before the other materials, 35 hours as compared with more than 48 hours for the other materials (fig 4.2).

The activity of PCE/CE and HSDE, with and without the presence of composite or compomer samples was measured at different time points up to 48 hrs. Enzyme activity levels are reported as a percentage of the initial measured activity.

Fig. 4.1: Relative activity of PCE/CE when incubated in the presence and absence of Dyract AP, Dyract eXtra, F2000 and Z250.
4.2 Isolation and identification of degradation products:

Higher levels (p<0.05) of MA, Bis-HPPP and TEGDMA release were observed for Z250 samples as compared with PMCR samples for both HSDE and PBS conditions (Figures 4.3A, B and C, respectively).

For all materials, MA release was significantly higher in HSDE incubation solution than in PBS (p<0.05) and it decreased with time for the HSDE incubated samples (Figure 4.3A). For all time points significantly higher amounts (p<0.05) of MA were detected for HSDE incubated Z250 samples, compared with Dyract eXtra and F2000 samples. HSDE incubated F2000 samples released the lowest amounts of MA (p<0.05) for each time point (Figure 4.3A).

Bis-HPPP release for Z250 samples was significantly higher (p<0.05) than for Dyract eXtra samples, when incubated with HSDE, for all incubation periods (Figure 2B). Bis-HPPP release levels for Z250 were significantly higher for HSDE than for PBS and there was an overall increase with time for the HSDE incubated samples although statistically not significant. Bis-HPPP release was not detected in F2000 samples, in both incubation media, as previously reported (Revuelta, 2006) (Figure 4.3B).
Fig. 4.3: Incremental release of MA (A), Bis-HPPP (B) and TEGDMA (C) for Z250, Dyract eXtra and F2000 at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS.
For all materials, incubation solution (PBS or HSDE) showed no significant effect on TEGDMA release, while incubation period had a significant effect for HSDE incubated Z250 samples and PBS incubated Z250 samples (from day 4) (p<0.05) (Figure 4.3C).

Both Dyract eXtra and F2000 samples demonstrated unique degradation products as compared with the resin material. For Dyract eXtra (Figure 4.4A), incubated in either PBS or HSDE, mass spectrum analysis confirmed the identity of the product isolated at 15.8 and 16.4 minutes as di-ester of 2-hydroxyethyl di-methacrylate with butane tetracarboxylic acid (TCB).
Fig. 4.4: Incremental release of the two TCB related products (15.8 minutes and 16.4 minutes retention time) (A) and total incremental TCB release for Dyract eXtra (B) at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS.
Significantly higher amounts of TCB were detected for Dyract eXtra samples incubated with HSDE as compared with samples incubated with PBS (p<0.05) at the 15.8 minutes retention time (Figure 4.4A). Levels of TCB decreased after 14 days in HSDE and PBS as compared with earlier time points (p<0.05), except for day 7 HSDE and PBS incubated samples. At the 16.4 minutes retention time, an overall trend for higher amounts of TCB was observed for Dyract eXtra samples incubated with PBS as compared with HSDE, which was statistically significant for the second day only (p<0.05). Overall levels of TCB were the highest after 2 days in HSDE and PBS as compared with the following time points (statistically significant except for PBS incubated samples on day 7) and at each time point HSDE incubated samples released higher amounts of TCB than the PBS incubated samples (statistically significant on days 2, 4 and 14) (Figure 4.4B).

For F2000, the product released at 14 and 16 minutes was identified as glyceryl dimethacrylate (GDMA).
Fig. 4.5: Incremental GDMA release at 14 and 16 minutes for F2000 (A) and total incremental GDMA release (B) at each time point (2, 4, 7 and 14 days) following incubation in PBS and HSDE.

GDMA was identified for both HSDE and PBS incubation conditions, at each time point (Figure 4.5A). GDMA levels were significantly higher for HSDE incubated F2000 samples (p<0.05) at each time point (Figures 4.5A and 4.5B). Levels of GDMA decreased over time and were the
lowest after 14 days in HSDE and PBS as compared with earlier time points (statistically significant for HSDE incubated samples).

4.3 Fluoride release analysis:

Fluoride ion concentration analysis was carried out after the incubation solutions were filtered and analyzed by HPLC, as described in the materials and methods section.

Figure 4.6 demonstrates the fluoride release from each material following incubation in PBS or HSDE at each time point. Fluoride concentration is expressed as parts per million (ppm).

Contrary to what was expected, F2000 and Dyract eXtra samples incubated in PBS released more fluoride than the HSDE incubated samples for each time point. Since the results were not as anticipated, a parallel study to investigate the effect of filtering the incubated solutions on fluoride ion concentration was conducted.

Figure 4.7 shows the fluoride concentration measured following incubation of known fluoride concentrations with PBS or HSDE for three days, with or without filtering the incubation solutions prior to measuring.
Fig 4.7: Fluoride measured following incubation of 1, 2.5 and 5 ppm fluoride in HSDE and PBS for 3 days.

Non-filtered PBS and HSDE incubation solutions showed higher fluoride concentrations than the filtered solutions. The filtered HSDE incubation solutions showed a 30-60% decrease in fluoride concentration compared to the non-filtered HSDE incubation solutions. The higher the initial fluoride concentration, the higher relative decrease in fluoride after filtering was evident. The filtered PBS incubation solutions also showed a decrease in fluoride concentrations measured compared with the non-filtered PBS incubation solutions, but it was only a 20-25% decrease. After understanding the effect filtering the solutions has on the final fluoride concentrations measured, the incubation and measurements of fluoride concentrations were repeated.

Figure 4.8 demonstrates the fluoride release into the incubation solutions, measured for the first (1-7 days) and second (8-14 days) incubation periods.
Fig. 4.8: Fluoride release from Dyract eXtra, Dyract AP, F2000 and Z250 samples following incubation in HSDE and PBS for 1st and 2nd weeks.

Dyract AP samples released more fluoride than Dyract eXtra in the first 7 days and more than Dyract eXtra and F2000 in the second time frame (p<0.05). F2000 samples released significantly more fluoride than Dyract eXtra for both incubation periods. In the second period (days 8-14), all HSDE incubated PMCR show a trend of increased fluoride release when compared with the PBS incubated samples, (statistically significant for Dyract AP (p<0.05) only). For all PMCR, fluoride levels decreased with time (statistically significant except for HSDE incubated Dyract AP samples). Z250 had no significant fluoride release in all incubation solutions.
4.4 Scanning Electron Microscopy (SEM) analysis:

Fig. 4.9: SEM images for Dyract eXtra, F2000 and Z250 samples. Non-incubated (A); Following 14 days incubation with PBS (B); following 14 days incubation with HSDE (C).

The images of the PMCR incubated for 14 days with HSDE show an extensive change in their surface morphology when compared to the non-incubated and PBS incubated samples (compare Figure 4.9C to 4.9A and B). The samples appear uneven with denuded particles and without the
smooth layer of matrix that is evident in the non-incubated samples and the PBS incubated samples. The extent of changes in the Dyract eXtra and F2000 samples after incubation with PBS or HSDE for 14 days, as compared with the non-incubated samples, appears to be similar for the two materials. The composite sample (Z250), following incubation with HSDE for 14 days (Figure 4.9C), appears also grainier and with voids, compared with the non-incubated and PBS incubated samples (Figure. 4.9 A and B), but the change is less extensive than it is for the PMCR samples.
CHAPTER 5: DISCUSSION

Overall, HSDE incubated samples generated significantly higher amounts of degradation products as compared with the PBS incubated groups; this is demonstrated by the higher amounts of MA for all materials, and the higher amounts of Bis-HPPP, TCB and GDMA for respectively Z250, Dyract eXtra and F2000. These data demonstrate that salivary induced biodegradation is a process that has the potential to compromise the clinical value of composite resins and PMCR restorative materials.

Biodegradation:

The findings for MA release resemble a previous study (Revuelta, 2006) in that F2000 exhibited less MA release when compared with Dyract (Dyract AP in that study, Dyract eXtra in the present study). In the earlier study significantly higher amounts of MA were detected for Dyract AP as compared with Z250, while in the current study the amount of MA release for Dyract eXtra was lower than for Z250 (p<0.05).

MA can be a result of hydrolysis of unreacted or partially reacted pendant monomers such as TEGDMA, Bis-GMA, TCB resin, and GDMA. For all materials, MA release was significantly higher in HSDE incubation solutions than in PBS, and was reduced over time for all materials in both incubation solutions with the exception of PBS incubated Z250 samples. This can be related to the enzymatic hydrolysis of leached unreacted monomers and the availability of partially reacted monomers within the external layer of the resinous matrix, which decrease after the initial days of the incubation. TEGDMA release in Z250 incubated samples, in both solutions, was significantly higher than for Dyract eXtra and F2000 samples. The greater amount of TEGDMA in Z250 can be related to the higher MA release, as reported in previous studies (Finer, 2006).

Biodegradation of Dyract eXtra

In Dyract eXtra despite the relatively low release of TEGDMA there was relatively high release of MA. One possible source for MA in this material could be the TCB resin, leached into the aqueous solution, as demonstrated by the reduction of TCB.
Another possible source could be attributed to the degradation of partially reacted monomers within the resinous matrix (Finer, 2004). These monomers can provide the source for MA, while their backbone is still attached into the resinous matrix. The fact that Dyract eXtra contains a multi-functional monomer, trimethylpropane trimethacrylate (TMPTMA), supports that view. While cleavage of some of the ester bonds between a partially reacted TMPTMA monomer and the matrix can result in leaching of significant amount of MA, the remaining reacted methacrylate groups can prevent the diffusion of the monomer into the incubation solution. As well, the presence of multifunctional monomers, in the resin matrix can enhance its cross-linking and rigidity, ultimately reducing the rate of diffusion of TEGDMA from the matrix into the incubation solution.

It should be noted that the exact composition and amounts of monomers in the PMCR are not completely known, a fact that results in challenges to determine the sources for the MA production.

As in the previous study (Revuelta, 2006), Bis-HPPP was not detected for F2000. The previous study reported higher amounts of Bis-HPPP for Dyract AP as compared with Z250. In contrast, in the present study Dyract eXtra released negligible amounts of Bis-HPPP as compared with Z250. The changes in the biodegradation release pattern of Dyract eXtra as compared with Dyract AP indicate that these materials are significantly different in their chemical composition and/or filler properties.

TCB release in Dyract eXtra samples was higher in HSDE than in PBS, for the 15.8 minutes peak (p<0.05) and the opposite for the 16.4 minutes peak (statistically significant for day 2 only, (Figure 4.4A). The two peaks, which are less than one minute apart, represent two TCB resin isomers, which interact with the mobile phase and HPLC column differently, hence their slightly different retention times. The differences in their release into the solutions could be explained by their different incorporation rates into the polymerised matrix and by their potentially different susceptibility to salivary esterase (Ferracane, 1994). Since TCB degradation products were not detected in the current and previous study (Revuelta, 2006), it is possible that unreacted TCB is released into the incubation media as a result of matrix degradation, but is not susceptible to the esterases found in human saliva as are other components of the matrix monomers. This is further
supported by the finding that Dyract eXtra samples exhibited less MA release than Z250 samples and by the fact that high amounts of TCB were detected in the pre-incubation solution (appendix 2). The Higher release of TCB isomers in HSDE incubated samples as compared with PBS incubated samples can be explained by the fact that the resin matrix is degraded more extensively when placed in HSDE (as evident from the SEM images, Figure 4.9), producing unreacted (with its di-methacrylate functional groups) TCB readily available to leach into the media. TCB, with its dual functionality quality, is polymerized via free radical and acid-base reactions. The additional acid-base reaction results in further cross-linkage of the matrix, which can provide its relative stability to the salivary esterases. The fact that TCB degradation products were not identified in this study, as well as in the previous degradation study (Revuelta, 2006), can be attributed also to the limitations of the HPLC analysis utilised in both studies.

Comparing Dyract eXtra and Dyract AP for their relative degradation patterns and Fluoride release reveals significant differences. The relatively lower amounts of TEGDMA, MA and Bis-HPPP released for Dyract eXtra, (also compared with Z250 samples), together with the lower fluoride release levels of Dyract eXtra when compared with F2000 and Dyract AP, as well as the enzyme stability assays indicate that there are significant changes in the formulations of Dyract eXtra as compared with Dyract AP. Apparently, Dyract eXtra contains less Bis-GMA and TEGDMA and it is either more resistant to degradation and/or contains lower levels of fluoride than Dyract AP. The changes may not be limited only to the chemistry and composition of the matrix but may involve filler type, size and distribution. In the newer versions of the PMCR, the reduction in size of the filler particles, as well as changes to their composition, increase cross linking, improve the materials’ resistance to mechanical and chemical degradation and affect their fluoride release properties. SEM analysis in the present study reveals that filler particles for both PMCR samples are significantly larger and less packed than those of Z250 samples, therefore possibly increasing the surface area available for degradation (Finer, 2006). The size, shape and distribution of the filler particles in the Dyract eXtra samples resembled those that were presented for Dyract AP in the previous study (Revuelta, 2006) indicating that the observed differences between the two versions of Dyract were likely due to changes to the matrix composition and not to the filler. SEM images of Dyract eXtra samples following incubation
with HSDE for 14 days showed an extensive change in their surface morphology when compared to the samples before incubation and after 14 days of PBS incubation. As well, HPLC analysis of Dyract eXtra revealed overall more degradation products than Z250, therefore both SEM and HPLC analyses may indicate that, in spite of the change in formulation, Dyract eXtra still has a higher degradation rate than Z250.

**Biodegradation of F2000**

Similarly to Dyract eXtra, there were two HPLC peaks, (14 and 16 min retention time), associated with GDMA release for F2000. The two peaks represent two GDMA isomers, that are hypothesized to interact with the mobile phase and HPLC column differently, hence their slightly different retention times. As in TCB resin release, the differences in their release into the solutions could be explained by their different incorporation rates into the polymerised matrix and by their potentially different susceptibility to salivary esterase (Ferracane, 1994). GDMA release in the F2000 samples was higher in HSDE than in PBS, for both peaks (14 and 16 min., Figure 4.5) (p<0.05) and can be explained by the fact that the resin matrix is degraded more extensively when placed in HSDE (as evident from the SEM images, Figure 4.9), producing unreacted GDMA readily available to leach into the media. No degradation products were detected for GDMA. This can be due to its resistance to esterases, similarly to HEMA and possibly to TCB (Ferracane, 1994) or to the limitation of the current HPLC system. In both PBS and HSDE incubated samples, the amount of GDMA decreases over time. GDMA is a small monomer which acts as a diluent in F2000 and can reach the surface and leach out fairly quickly when it is in the form of an unreacted monomer. When incubated in HSDE, the matrix is degraded more extensively, as evident from SEM images, allowing the GDMA monomer to leach out faster than in the PBS incubated samples. It is also possible that the relative hydrophilicity of the dual functionality monomers in F2000, by facilitating the penetration of the salivary enzymes closer to cleavage sites, allows for the unreacted monomers to be leached faster into the incubation solution. The fact that GDMA levels are decreasing with time indicates that there is less available unreacted GDMA monomer available for diffusion into the incubation solutions.
In both present and previous studies (Revuelta, 2006), not all components of the PMCR’s matrices or their degradation by-products were detected. These include urethane dimethacrylate (UDMA) for Dyract eXtra and citric acid di-methacrylate oligomer (CDMA) for F2000. CDMA contains multifunctional methacrylate groups as well as carboxyl groups, allowing greater cross-linking and enhancing its resistance to enzymatic degradation and transport out of the matrix. It is also possible that some components are in low amounts, are resistant to esterases and/or in levels below the detection threshold of the HPLC system used in the study.

**Fluoride release:**

For Dyract eXtra and F2000, there was no significant difference between the fluoride release for HSDE and PBS conditions following incubation for 7 days and 14 days. For both materials, fluoride release in the second week was lower than for the first week of incubation in both incubation conditions (p<0.05) (Figure 4.8). This might indicate that in a period of 14 days most of the measured fluoride is the result of leaching and solubility more than a result of material degradation.

Similarly to F2000 in the first week of incubation, Dyract AP released similar amounts of fluoride for both incubation conditions. However in the second week of incubation, HSDE incubated Dyract AP samples released more fluoride than the PBS incubated samples (p<0.05). In addition, both incubation conditions resulted in higher amount of fluoride release from Dyract AP, as compared with all other materials. This may indicate that after the release of freely available fluoride ion in the first incubation period, the HSDE incubated samples are degrading, resulting in increased fluoride levels. It can be expected therefore that the difference in fluoride release levels between HSDE and PBS incubated samples would increase with time since enzymatic degradation is a temporal process.

Overall (14 days incubation) Dyract AP samples released more fluoride than Dyract eXtra samples (p<0.05) and F2000 (statistically not significant). This finding is consistent with the previous study (Revuelta, 2006) in which Dyract AP samples released significantly higher levels of fluoride than F2000 samples in the presence of PBS or esterase (CE) and is another indication for the change in the latest version of Dyract (Dyract eXtra) as compared with the previous one
(Dyract AP). The lower fluoride release from Dyract eXtra, as compared with Dyract AP, may be related to the material’s reduced fluoride contents, its lower solubility in aqueous media and its enhanced biostability as compared with its predecessor (Dyract AP).
CHAPTER 6 – CONCLUSIONS AND RECOMMENDATIONS

- Both previous (Revuelta, 2006) and current study show that the latest formulation of Dyract (Dyract eXtra) released less degradation products and may have been more resistant to salivary enzymes than the previous formulation (Dyract AP).
- While the latest formulation of Dyract (Dyract eXtra) is more biochemically stable than its previous version (Dyract AP) in the presence of human salivary enzymes, SEM images and the overall greater number of degradation products for the compomers, as compared with Z250, suggest that Dyract eXtra and F2000 samples are degraded more extensively than Z250 samples. TCB resin for Dyract eXtra and GDMA for F2000 were detected at higher concentrations when incubated with HSDE. This corroborates the previous study (Revuelta, 2006) findings and could be attributed to the composition of the PMCR which contain hydrophilic monomers in addition to the monomers used in composites. By being designed for aqueous solutions, the hydrophilicity of the PMCR allows salivary enzymes and water easier access to cleavage sites, thus increasing the degradation rate of the resin matrix. The faster and more extensive degradation of Dyract eXtra and F2000 samples compared with Z250 samples may have considerable clinical relevance on the longevity of restorations in vivo.
- The fact that no degradation products for both TCB and GDMA were detected in this study, as well as in the previous CE degradation study (Revuelta, 2006), can be attributed to the limitations of the analysis that could not identify the degradation products, if there were any.
- Previous studies (Finer and Santerre, 2004) show that the presence of high levels of degradation products reduces enzymes stability, resulting in shorter half-lives. Therefore, the longer half-life of HSDE when incubated with Dyract eXtra, compared with Dyract AP is another indication for an improvement in the matrix biochemical stability in the latest version of Dyract.
- Fluoride analysis results suggest that the latest formulation of Dyract (Dyract eXtra) is different than the previous version of the material (Dyract AP).
- For Dyract eXtra and F2000 the incubation medium had no effect on the amounts of fluoride release. The lower fluoride release from Dyract eXtra, in relation to Dyract AP and F2000 samples may be related to the material’s reduced fluoride content, its lower
solubility in aqueous media or its enhanced biostability as compared with its predecessor (Dyract AP).

- Based on the findings of this study, fluoride release levels, in the presence of human salivary esterases, should be analyzed without filtering the solutions prior to the analysis.

Conclusion to hypothesis #1:
As presented by the overall greater amounts of isolated degradation products and SEM images PMCR are degraded more extensively in the presence of human salivary esterases than in buffer solution. Therefore, the hypothesis that PMCR (Dyract eXtra and F2000) are more susceptible to hydrolytic degradation by human saliva derived esterases as compared with buffer was found to be true.

Conclusion to hypothesis #2:
When comparing the amounts of degradation products with the amounts of fluoride release in the two versions of Dyract (Dyract AP and Dyract eXtra) it appears that the lower levels of fluoride release from the latest version (Dyract eXtra) were associated with its increased biochemical stability, as demonstrated by its lower overall release of biodegradation products. Therefore for Dyract materials, the hypothesis can be accepted.

SEM images of F2000 and Dyract eXtra samples show similar degradative effects of HSDE and PBS on them. However, since the degradation products of F2000 and Dyract eXtra are different their amounts could not be directly compared. Yet the fluoride release amounts were significantly higher for the F2000 samples than for the Dyract eXtra samples. Therefore it is impossible to conclude at this point that there is a correlation or proportional relationships between the level of released fluoride and the amount of degradation products from F2000. The second hypothesis that the levels of fluoride release from the PMCR are proportional with the amount of the degradation products for F2000 has to be rejected.

Longer incubation periods might give a better picture of the compomers’ degradation process as more degradation products might be released and identified. When indicators of compomer matrix degradation are identified (as Bis-HPPP serves as a degradation indicator for traditional composite resins), in vivo studies of the compomers’ degradation in the oral environment are the next step.
PMCR gained popularity, especially due to their fluoride releasing property and easy manipulation, but their relative susceptibility to degradation compared with composite in the presence of human salivary enzymes, combined with a decreased fluoride release, should be considered among all other factors when choosing an aesthetic restoration material. The greater degradation rate of PMCR may not be a critical factor when choosing a restoration material for deciduous teeth, since the life expectancy of the restoration and the teeth is much shorter than the longevity of the restoration.
CHAPTER 7 - REFERENCES


Stanley HR. Local and Systemic Responses to Dental Composites and Glass Ionomers. 1992; Adv Dent Res 6:55-64.


APPENDIX 1: REPRESENTATIVE CHROMATOGRAMS AND MASS SPECTRA

Fig 8.1.1: Representative HPLC chromatogram of MA standard (Fluka CAS 79-4-41).

Fig 8.1.2: Representative HPLC chromatogram of Bis-HPPP standard (Fluka CAS 5581-32-8).

Fig 8.1.3: Representative HPLC chromatogram of TEGDMA standard (Aldrich CAS 109-16-0).
Fig 8.1.4: Representative HPLC chromatogram of Dyract eXtra following incubation in PBS.

Fig 8.1.5: Representative HPLC chromatogram of Dyract eXtra following incubation in HSDE.

Fig 8.1.6: Representative HPLC chromatogram of Dyract eXtra following 48 hours of pre-incubation in PBS.
Fig 8.1.7: Representative HPLC chromatogram of F2000 following incubation in PBS.

Fig 8.1.8: Representative HPLC chromatogram of F2000 following incubation in HSDE.

Fig 8.1.9: Representative HPLC chromatogram of F2000 following 48 hours of pre-incubation in PBS.
Fig 8.1.10 Mass spectrum of GDMA monomer isolated at retention times of 14 and 16 minutes in F2000 samples incubated in PBS and HSDE.

Fig 8.1.11 Mass spectrum of TCB resin isolated at retention times of 15.8 and 16.4 minutes in Dyract eXtra samples incubated in PBS and HSDE.
**APPENDIX 2: PREINCUBATION TCB AND GDMA RELEASE**

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**Fig 8.2.1:** Total incremental TCB release for Dyract eXtra at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS and following 48 hr. preincubation in PBS at 37°C.

**Fig 8.2.2:** Total incremental GDMA release for F2000 at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS and following 48 hr. preincubation in PBS at 37°C.
APPENDIX 3: CALIBRATION CURVES

Fig. 8.3.1: Calibration curve for MA

\[ y = 5 \times 10^{-6}x - 0.0333 \]
\[ R^2 = 0.9998 \]

Fig. 8.3.2: Calibration curve for TEGDMA

\[ y = 2 \times 10^{-6}x + 0.0009 \]
\[ R^2 = 0.9992 \]
Fig 8.3.3: Calibration curve for Bis-HPPP

\[ y = 5 \times 10^{-7} x + 0.0082 \]
\[ R^2 = 0.9924 \]
APPENDIX 4: CALCULATION OF MA, BIS-HPPP AND TEGDMA RELEASE (µg) FROM Z250, F2000 AND DYRACT EXTRA USING CALIBRATION CURVES

Fig 8.4.1: Incremental release (µg) of MA for Z250, Dyract eXtra and F2000 at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS.

Fig 8.4.2: Incremental release (µg) of Bis-HPPP for Z250, Dyract eXtra and F2000 at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS.
Fig 8.4.3: Incremental release (μg) of TEGDMA for Z250, Dyract eXtra and F2000 at each time point (2, 4, 7 and 14 days) following incubation in HSDE and PBS.