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RIGID ROD POLYMER FILLERS IN
ACRYLIC DENTURE AND DENTAL
ADHESIVE RESIN SYSTEMS

by

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“More than anything in life
we need a person,
who encourages us to do,
what we can do.”

Ralph Waldo Emerson

ABSTRACT

Anne-Maria Vuorinen. Rigid rod polymer fillers in acrylic denture and dental adhesive resin systems. Department of Biomaterials Science, Institute of Dentistry, University of Turku. *Annales Universitatis Turkuensis*, Turku, Finland 2010.

Polymeric materials have been used in dental applications for decades. Adhesion of polymeric materials to each other and to the tooth substrate is essential to their successful use. The aim of this series of studies was two-folded. First, to improve adhesion of poly(paraphenylene) based rigid rod polymer (RRP) to other dental polymers, and secondly, to evaluate the usability of a new dentin primer system based on RRP fillers.

Poly(paraphenylene) based RRP would be a tempting material for dental applications because of its good mechanical properties. To be used in dental applications, reliable adhesion between RRP and other dental polymers is required. In this series of studies, the adhesion of RRP to denture base polymer and the mechanical properties of RRP-denture base polymer-material combination were evaluated. Also adhesion of BisGMA-TEGDMA-resin to RRP was determined. Different surface treatments were tested to improve the adhesion of BisGMA-TEGDMA-resin to RRP. Results were based on three-point bending testing, Vickers surface hardness test and scanning electron microscope analysis (SEM), which showed that no reliable adhesion between RRP and denture base polymer was formed. Addition of RRP filler to denture base polymer increased surface hardness and flexural modulus but flexural strength decreased. Results from the shear bond strength test and SEM revealed that adhesion between resin and RRP was possible to improve by surface treatment with dichloromethane (DCM) based primer and a new kind of adhesive surface can be designed.

The current dentin bonding agents have good immediate bond strength, but in long term the bond strength may decrease due to the detrimental effect of water and perhaps by matrix metalloproteinases. This leads to problems in longevity of restorations. Current bonding agents use organic monomers. In this series of studies, RRP filled dentin primer was tested in order to decrease the water sorption of the monomer system of the primers. The properties of new dentin primer system were evaluated *in vitro* by comparing it to commercial etch and rinse adhesive system. The results from the contact angle measurements and SEM showed that experimental primer with RRP reinforcement provided similar resin infiltration to dentin collagen and formed the resin-dentin interface as the control primer. Microtensile bond strength test and SEM revealed that in short term water storing, RRP increased bond strength and primer with BMEP-monomer (bis[2-(methacryloyloxy)-ethyl]phosphate) and high solvent concentration provided comparable bonding properties to the commercial control primers. In long term water storing, the high solvent-monomer concentration of the experimental primers decreased bond strength. However, in low solvent-monomer concentration groups, the long-term water storing did not decrease the bond strength despite the existence of hydrophilic monomers which were used in the system.

These studies demonstrated that new dentin primer system reached the mechanical properties of current traditional etch and rinse adhesive system in short time water storing. Improved properties can be achieved by further modifications of the monomer system. Studies of the adhesion of RRP to other polymers suggest that adhesion between RRP and other dental polymers is possible to obtain by certain surface treatments.

Keywords: Adhesion, polymers, dentin bonding, rigid rod polymer

TIIVISTELMÄ

Anne-Maria Vuorinen. Rigid rod polymeeri fillerit akryylisissä pohjalevypolymeereissä ja hammaslääketieteen adhesiiviresiini systeemeissä. Biomateriaalitiede, Hammaslääketieteen laitos, Turun yliopisto. Annales Universitatis Turkuensis, Turku, Suomi 2010.

Polymeerejä on käytetty hammaslääketieteessä vuosikymmeniä. Polymeerien kiinnittyminen toisiin polymeereihin ja hampaaseen on välttämätöntä niiden kliiniselle käytölle. Tällä väitöskirjatyöllä oli kaksi tavoitetta. Ensinnä kehittää menetelmiä poly(paraphenyleeni)-pohjaisen rigid rod -polymeerin (RRP) kiinnittämiseksi muihin hammaslääketieteessä käytettyihin polymeereihin ja toiseksi arvioida uudentyyppisen RRP-vahvistetun dentiiniprimerin ominaisuuksia hammaspaikka-aineen kiinnittämiseksi hampaaseen.

Poly(paraphenyleeni)-pohjainen RRP on houkutteleva materiaali hammaslääketieteelliseen käyttöön hyvien mekaanisten ominaisuuksien vuoksi. Jotta RRP:ä voitaisiin hyödyntää kliinisessä käytössä, olisi löydettävä keino kiinnittää RRP muihin hammaslääketieteen polymeereihin. Väitöskirjatyön tutkimuksissa arvioitiin RRP:n kiinnittymistä proteesin pohjalevypolymeeriin ja samalla tutkittiin RRP-pohjalevypolymeeri-materiaaliyhdistelmän mekaanisia ominaisuuksia. Niin ikään tutkittiin BisGMA-TEGDMA-resiinin kiinnittymistä RRP:in. Tutkimuksissa arvioitiin myös erilaisten pintakäsittelymenetelmien vaikutusta resiinin ja RRP:n väliseen sidokseen. Kolmipistetaivutuskokeista, pintakovuustesteistä ja pyyhkäisyelektronimikroskooppianalyyseista saadut tulokset osoittivat, että RRP ei kiinnity luotettavasti proteesin pohjalevypolymeeriin. RRP-täyteaineen lisääminen kasvatti taivutusmoduulia ja pintakovuutta, mutta laski taivutuslujuutta. Tulokset sidoslajuustesteistä ja elektronimikroskooppianalyyseistä osoittivat, että sidos resiinin ja RRP:n välille on mahdollista saavuttaa. Dikloorimetaani-pohjainen primeri paransi sidoslajuutta ja muodosti uudenlaisen sidospinnan RRP:n pinnalle.

Nykyisillä dentiinisidosaineilla on saatavissa hyvä välitön sidoslajuus hampaan ja paikka-aineen välille, mutta pitempiaikainen sidoslajuus heikkenee veden imeytymisen ja mahdollisesti matriksimetalloproteiinaasien toiminnan myötä. Tämä aiheuttaa ongelmia kliinisessä työssä. Nykyiset dentiinisidosaineet sisältävät orgaanisia monomeereja. Tämän väitöskirjatyön tavoitteena oli yrittää kehittää RRP-vahvistettu dentiiniprimeri, joka vähentäisi vedenimeytymisen haitallisia vaikutuksia. Kokeellisen primerin ominaisuuksia verrattiin kaupalliseen "etch-and-rinse" sidosaineeseen. Tulokset kontaktikulmamittauksista ja elektronimikroskooppianalyyseistä osoittivat, että kokeellinen primeri edesauttaa adhesiivin tunkeutumista dentiinin kollageeniverkoston kaupallisen primerin tavoin. Mikrovetolujuustesti ja elektronimikroskooppianalyysi paljastivat, että lyhyessä vesisäilytyksessä RRP nosti sidoslajuutta ja hydrofiilinen korkean liuotinpitoisuuden omaava primeri tarjosi samanlaiset mekaaniset ominaisuudet kuin kaupallinen kontrolli materiaali. Pitkässä vesisäilytyksessä korkea liuotinpitoisuus laski kuitenkin sidoslajuutta, kun taas pienen liuotinpitoisuuden omaava primeri säilytti mekaaniset ominaisuutensa käytetyistä hydrofiilisistä monomeereistä huolimatta.

Väitöskirjatyön tutkimukset osoittivat, että kokeellisella primerilla saavutettiin lyhyessä vesisäilytyksessä samat ominaisuudet kuin kaupallisella kontrolliprimerilla. Kokeellisen primerin ominaisuuksia voitaneen edelleen parantaa modifioimalla monomeerikoostumusta. Lisäksi tutkimukset osoittivat, että RRP voidaan kiinnittää muihin hammaslääketieteen polymeereihin pintakäsittelymenetelmien avulla.

Avainsanat: Kiinnittyminen, polymeeri, dentiinisidostus, rigid rod polymeeri

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ABBREVIATIONS

| | |
|--------------------------------|--|
| 4-AETA | 4-acryloyloxyethyl trimellitate anhydride |
| Al ₂ O ₃ | aluminium trioxide |
| ANOVA | analysis of variance |
| BisGMA | bisphenol-A-glycidyl methacrylate |
| BMEP | bis[2-(methacryloyloxy)-ethyl]phosphate |
| DCM | dichloromethane |
| DMAEMA | dimethyl amino ethyl methacrylate |
| EGDMA | ethylene glycol dimethacrylate |
| EGMP | ethylene glycol methacrylate phosphate |
| FEPA | Federation of European Producers of Abrasives |
| FRC | fiber reinforced composite |
| GF | glass fiber |
| GPa | gigapascal |
| HEMA | hydroxyethyl methacrylate |
| IPF | interfacial porosity formation |
| IPN | interpenetrating polymer network |
| ISO | International Standardization Organization |
| 10-MAC | 11-methacryloyloxy-1,1-undecanedicarboxylic acid |
| 10-MDP | 10-methacryloyloxydecyl dihydrogenphosphate |
| 4-META | 4-methacryloyloxyethyl trimellitate anhydride |
| MMA | methylmethacrylate |
| MMP | matrix metalloproteinase |
| MPa | megapascal |
| MPS | methacryloxypropyltrimethoxysilane |
| M _w | weight average molecular weight |
| n | number of specimen |
| NaOCl | sodiumhypochlorite |
| NPG-GMA | N-phenylglycine glycidyl methacrylate |
| PMMA | poly(methylmethacrylate) |
| RRP | rigid rod polymer |
| SD | standard deviation |
| SEM | scanning electron microscope |
| Semi-IPN | semi-interpenetrating polymer network |
| SiC | silicone carbide |
| SiO ₂ | silicon dioxide |
| SPSS | Statistical Package for Social Science |
| TEGDMA | triethyleneglycol-dimethacrylate |
| THF | tetrahydrofuran |
| UDMA | urethane dimethacrylate |
| VHN | Vickers hardness number |
| Vol% | volume percentage |
| Wt% | weight percentage |
| ZrO ₂ | zirconium dioxide |

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-IV. The original publications are reproduced with the permission of the copyright holders.

- I Vuorinen A-M, Dyer SR, Lassila LVJ, Vallittu PK. Effect of rigid rod polymer filler on mechanical properties of poly-methyl methacrylate denture base material. *Dent Mater* 2008; 24: 708-713.
- II Vuorinen A-M, Dyer SR, Lassila LVJ, Vallittu PK. Adhesion of BisGMA-TEGDMA resin to poly(paraphenylene) based rigid rod polymer. *Dent Mater*. Submitted.
- III Vuorinen A-M, Dyer SR, Vallittu PK, Lassila LVJ. Bonding of composite resin to dentin using rigid rod polymer modified primers. *J Adhes Dent* 2010; 12: 175-182.
- IV Vuorinen A-M, Dyer SR, Vallittu PK, Lassila LVJ. Effects of water storage on the microtensile bond strenght of composite resin to dentin using experimental rigid rod polymer modified primers. *J Adhes Dent* 2010. In press.

1. INTRODUCTION

Synthetic polymers have been used in dentistry since the introduction of poly(methylmethacrylate) (PMMA) in the 1930's. PMMA was first utilized as denture base material (Cook et al. 1985). Development of modern dental polymers started in the 1960's when Bowen invented substituted dimethacrylates – particularly substituted bisphenol-A-glycidyl methacrylate (BisGMA) (Bowen 1965a, Bowen 1965b). Present day polymeric materials are widely used in dentistry as dentures, composite filling materials, adhesive materials, cements, sealants, et cetera.

All of the polymeric materials in dentistry are not ideal for their clinical use. For example, a common clinical problem is fractures of prosthesis or fractures of composite restorations (Collins et al. 1998, Danbar et al. 1994, Deligeorgi et al. 2000, Palotie and Vehkalahti 2002, Vallittu et al. 1993). Therefore, it is essential to find new materials and develop techniques in dentistry. Rigid rod polymers (RRP) could be used as new materials in dentistry in many applications, because of their good mechanical properties (Chae and Kumar 2006, Hu et al. 2003). Some problems with the traditional RRP relate to their crystallinity and difficulty to dissolve them into solvents. These features have limited usage of RRP eg. as fillers in composite resins. Poly(paraphenylene) based backbone with benzoyl sidegroups is a new kind of RRP. Its rigid backbone with high proportion of paraphenylene linkages restricts the rotational movement and ensures the chain stiffness and good mechanical properties. The benzoyl side groups make the polymer dissolvable and amorphous (Connolly and Karasz 1995, Ha et al. 2001, Marocco et al. 1993, Morgan et al. 2006, Wangt and Quirk 1995). If this kind of RRP would be used in dentistry, it would be essential to find a way to attach this polymer to other polymers and to the tooth substrate.

Reliable adhesion between tooth substrate and polymeric materials is essential for the durability of a composite restoration. Enamel bonding via acid etching is widely accepted and has been used successfully in clinical work for decades. Dentin bonding, however, is more challenging. In the last decades, a substantial number of dentin bonding studies have been published and various improvements for dentin bonding agents have been suggested (Peumans et al. 2005, Van Meerbeek et al. 1998). Contemporary dentin adhesives have good immediate bond strength but the long-term bond strength may be insufficient (De Munck et al. 2005, Peumans et al. 2005, Van Meerbeek et al. 1998). It has been suggested that the degrading effects of water sorption on hybrid layer polymers and the action of matrix metalloproteinases (MMPs) are the matters that decrease the long-term bond strength of dentin adhesives (Carrilho et al. 2005, Carrilho et al. 2007a, Carrilho et al. 2007b, Carrilho et al. 2009, Tay and Pashley 2003, Yiu et al. 2004). Possibilities to enhance the long-term bond strength have been presented. One suggestion

has been to change the hydrophilic monomers into hydrophobic monomers and use ethanol-wet bonding instead of water-wet bonding (Cadenaro et al. 2009a, Carrilho et al. 2008, Sadek et al. 2010). Other means is to improve more stable monomers for wet-bonding [acrylic phosphonic acids and bis(acrylamides)] (Moszner et al. 1999, Moszner et al. 2001, Moszner 2004, Moszner et al. 2006). Carrilho et al. have proposed the use of chlorhexidin on dentin at bonding stage to prevent the action of MMPs (Carrilho et al. 2007a, Carrilho et al. 2007b, Carrilho et al. 2009). Current dentin bonding agents use organic monomers. No previous studies about the use of RRP fillers in dentin bonding have been published.

In this series of studies, the aim was to evaluate the usability of a new kind of RRP filled dentin primer system. Another aim was to examine the attachment of poly(paraphenylene) based RRP to some other dental polymers and to evaluate the reinforcing capability of RRP while being used as fillers in denture base polymer.

2. REVIEW OF LITERATURE

2.1 Polymers in dentistry

Polymeric materials in dentistry can be divided into linear polymers (eg. PMMA) and cross-linked polymers (eg. BisGMA). Linear polymers are composed of long carbon chains which are linked together by weak chemical bonds (Figure 1). Linear polymers can be heated and reformed and they are also dissolvable to certain solvents. Cross-linked polymers are composed of carbon chains which are linked together by strong chemical covalent bonds (Figure 1). Cross-linked polymers can not be reformed by heat and it is also more difficult to dissolve them into solvents (O'Brien WJ 2008).

Most polymeric materials used in dentistry are based on chemistry of methacrylates. Methacrylates are esters of methacrylic acid and they have carbon-carbon double bond which can react in free radical polymerisation. A simple methacrylate monomer is methylmethacrylate (MMA) (Figure 2). MMA is used in its polymerised form, poly(methylmethacrylate) (PMMA) (Figure 2), eg. as a denture base material. Restorative filling materials and adhesive materials are composed of cross-linking methacrylates such as bisphenol-A-glycidyl dimethacrylate (BisGMA), triethyleneglycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) (Figure 2). Hydroxyethyl methacrylate (HEMA) is hydrophilic monofunctional monomer (Figure 2) (Garcia et al. 2006, O'Brien WJ 2008).

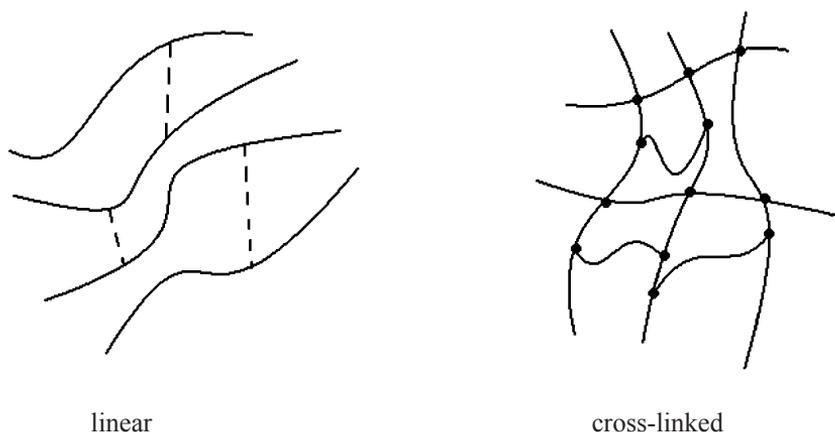


Figure 1. Two-dimensional schematic representations of linear and cross-linked polymers.

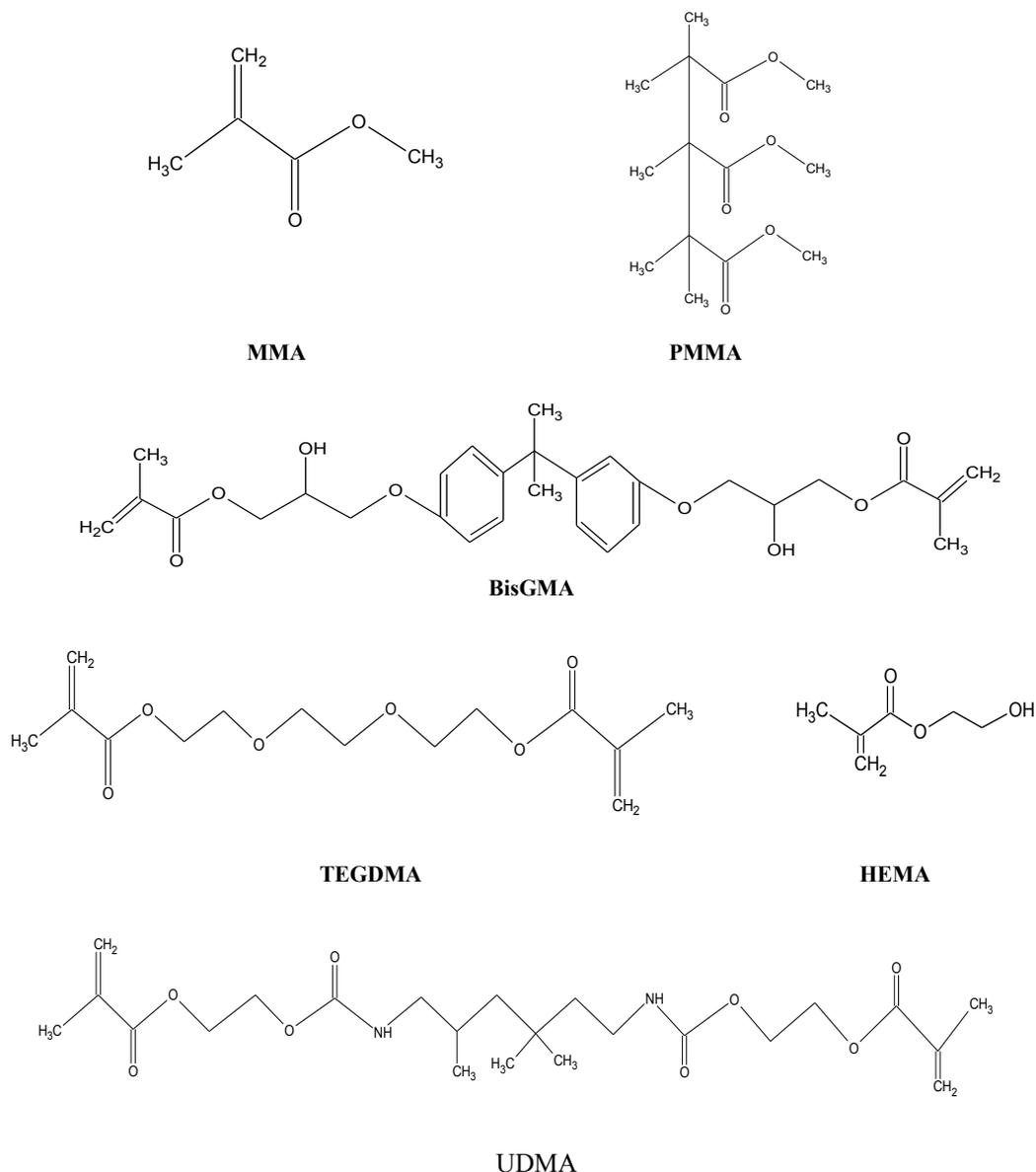


Figure 2. The structural formulas of common methacrylates in dentistry.

2.2 Rigid rod polymers

Rigid rod polymers (RRP) are macromolecules known for their good mechanical properties and usability in high temperatures. Efforts to synthesize these high temperature resistant polymers started in the 1960s in the United States and Russia. Based on many decades' research work, many kinds of RRP have been introduced and their properties have been studied. One well-known RRP is Kevlar® fiber (Figure 3a). RRP are aromatic carbocyclic or heterocyclic structures. The problems with the traditional rigid rod polymer

fibers have been, despite good mechanical properties, their crystallinity and difficulty to dissolve these polymers into solvents (Chae and Kumar 2006, Hu et al. 2003). These features have limited their use as fillers of composites. An example of limitation of RRP Kevlar fiber in PMMA matrix was shown by delaminations of RRP fiber from the matrix polymer (Vallittu and Narva 1997). A number of recent methods have been developed to synthesize substituted RRP to improve their solubility. Poly(paraphenylene) backbone with benzoyl side-chains has been introduced. RRP with benzoyl side-chains has rigid backbone with high proportion of paraphenylene linkages which restricts the rotational movement and produces chain stiffness. At the same time, the benzoyl side groups make the polymer dissolvable and amorphous (Marocco et al. 1993). Commercial polymers Parmax 1000® and Parmax 1240® have been developed (Figures 3b, 3c) (Ha et al. 2001, Morgan et al. 2006, Wangt and Quirk 1995). Parmax 1000® is a linear polymer of benzoyl-1,4-phenylene. Parmax 1240® is a copolymer of 1,3-phenylene and benzoyl-1,4-phenylene. Incorporation of 1,3-phenylene results in improved melt processability, but somewhat reduced modulus (Marocco et al. 1993). Parmax 1240® has proved to be two to three times as hard as polycarbonate (Morgan et al. 2006).

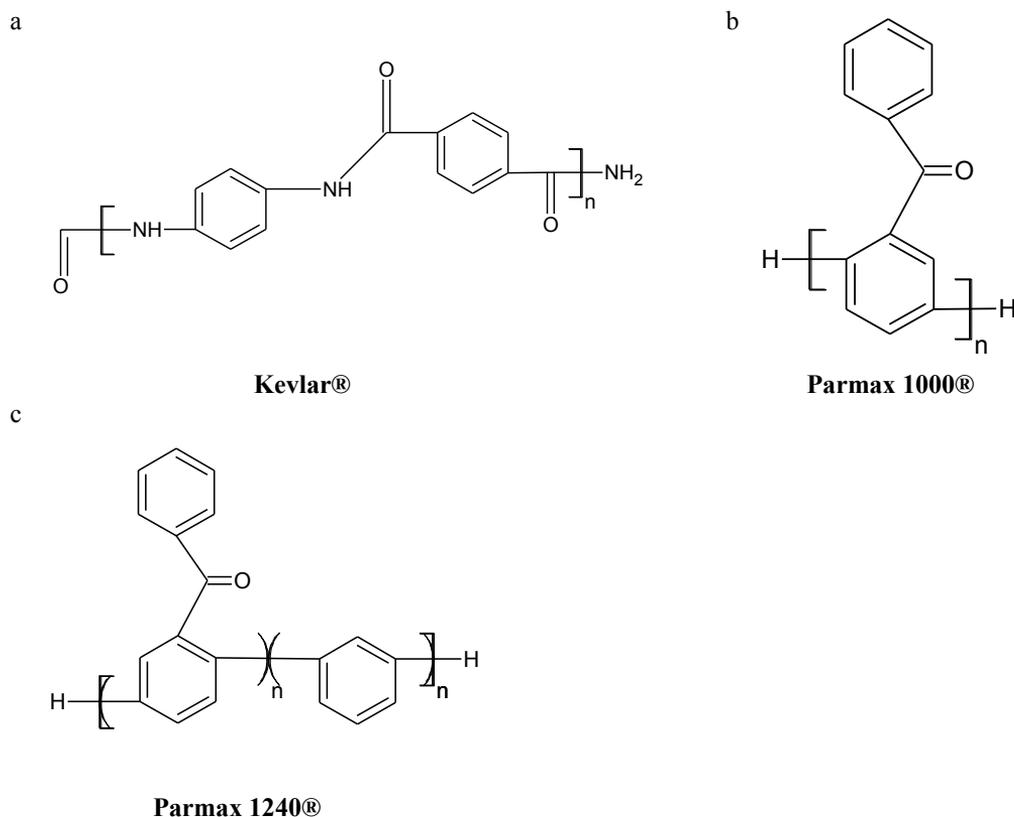


Figure 3 a-c. Structural formulas of rigid rod polymers a) Kevlar® b) Parmax 1000® [poly(benzoyl-1,4-phenylene)] and c) Parmax 1240® (copolymer which contains approximately 15% meta-linkages which do not contain any side-chains).

2.3 Reinforcements in polymers

The mechanical properties of currently used dental polymers are necessarily fulfilling the demands by the loading conditions by the masticatory system. The regular problems with patients, however, are fractures of prosthesis or cracks of composite restorations (Collins et al. 1998, Danbar et al. 1994, Deligeorgi et al. 2000, Palotie and Vehkalahti 2002, Vallittu et al. 1993). Also wear of composite fillings, marginal leakage and marginal discolorations are matters in clinical work. The most common reasons for replacement of composite restorations are reported to be secondary caries and bulk fractures (Collins et al. 1998). Denture base studies have shown detached teeth and midline fractures, more commonly seen in upper complete dentures, to be the most common reasons for the repair of dentures (Danbar et al. 1994, Vallittu et al. 1993).

To improve the mechanical properties of polymers different reinforcements for polymers have been introduced. The most successful reinforcements are based on various kinds of fibers (eg. glass fibers (GF) and carbon graphite fibers) and inorganic filler reinforcements (Garcia et al. 2006, Vallittu 1996). In dentistry, the fiber reinforcements have been typically used in the field of prosthetic dentistry. The review of fiber reinforcements in denture base resin concluded that the highest transverse strength at the time of the publication of article with PMMA based fiber composite was 265 MPa and was obtained by incorporating 58 wt% GF into denture base resin (Vallittu 1996). In 10 years water storing, the reduction in flexural strength and modulus of E-glass fiber reinforced composite (FRC) was 24% and 21%, respectively; for silica FRC, reduction was 47% and 46%, respectively; and for controls, reduction was 24% and 11%, respectively (Vallittu 2007). In recent years, also fiber reinforced filling composite resins have been developed (Garoushi et al. 2006, Garoushi et al. 2007, Garoushi et al. 2008).

Inorganic fillers are typically used in filling composites and sometimes also in adhesive resins. Most commonly used fillers are silicon dioxide (SiO_2), zirconium dioxide (ZrO_2) and different silicates (Garcia et al. 2006). Filler particles increase some mechanical properties, decrease the polymerisation shrinkage and support the good handling (Garcia et al. 2006, Labella et al. 1999).

2.4 Resin adhesion to other polymers and reinforcements

In the clinical use, the adhesion of polymeric materials to each other and to the reinforcements is essential. The adhesion is important to transfer load from the polymer matrix to the reinforcing fibers, fillers, or to the stronger component of multiphasic structures. General principles of adhesion are mechanical interlocking, chemical bonding through ionic- or covalent bonds, interaction of Van der Waals or similar dispersion forces or formation of interpenetrating polymer network (IPN) at the interface of bonded polymers.

2.4.1 Mechanical interlocking

Mechanical interlocking is used when chemical or other adhesion mechanics are not possible to obtain. Traditionally mechanical interlocking has been used eg. in amalgam restorations. Amalgam can not attach properly to tooth substrate and the way to attach amalgam to tooth is to create cavity with mechanical retention (Marshall et al. 2010, Vaught 2007). The term micromechanical retention is used when large rough surface is created. Example of this is the acid etching of enamel. Attachment of composite restorations to enamel is therefore based on micromechanical retention (Buonocore 1955).

2.4.2 Chemical adhesion

Chemical adhesion means either formation of covalent bonds or ionic bonds between atoms or formation of weak dispersion forces (eg. Van der Waals forces) or dipole-dipole bonds between molecules of substrate and adhesives. Covalent bond builds up between non-metal atoms. Consequently bonds between carbon atoms are of covalent nature. Covalent bond can be polar or non-polar depending on the electronegativity of atoms. Covalent bond between two similar atoms is non-polar and as the difference in electronegativity between atoms increases the bond becomes more polar. If the electronegativity difference between atoms is over 1.7 the bond is of ionic nature. Covalent and ionic bonds are classified into strong chemical bonds (Darvell 2002, Marshall et al. 2010).

Dispersion forces hold together non-polar molecules. Dispersion forces are weak bonds which are quite easy to break. Dipole-dipole bonds hold polar molecules together and they are stronger than dispersion forces. A special case of dipole-dipole bond is a hydrogen bridge. A hydrogen bridge forms between a hydrogen atom and a highly electronegative non-metal atom. Hydrogen bridge is the most durable bond between molecules, but it is still weaker than covalent bonds between atoms (Darwell 2002, Marshall et al. 2010).

2.4.3 Interpenetrating polymer networks

Interpenetrating polymer networks, IPNs, are combinations of two or more polymers in network form, with at least one such polymer polymerized and/or cross-linked in the immediate presence of the others. Polymers are at least partially interlaced at molecular level but are not covalently bonded to each other. IPNs differ from polymer blends which are less homogenous structures. They differ from copolymers which contain chemical bonds between polymers. IPN can be formed between cross-linked polymers (IPN) or between linear and cross-linked polymer (semi-IPN) (Figure 4) (Klempner et al. 1994, Sperling and Mishra 1995, Vallittu 2009). Sequential IPN is formed when first polymer network I is polymerised and after this monomer II is swollen into this network and

polymerised. Simultaneous IPN forms when two monomers are mixed and polymerised at the same time (Sperling and Mishra 1995).

IPNs are utilized in dentistry in denture base polymers, denture teeth, fiber-reinforced composites (FRCs) and also in restorative composite resins (Garoushi et al. 2008, Jagger and Huggett 1990, Manocci et al. 2008, Ruyter and Sjövik 1981, Vallittu 1995, Vallittu et al. 2001, Vallittu 2009). The dental IPN structures are semi-IPNs. The cross-linked part of dental IPNs is formed by dimethacrylate monomers or multifunctional monomers and dendrimers and monofunctional MMA forms the non-cross-linked part of the system (Vallittu 2009). The best and oldest example of dental IPN formation is the formation of semi-IPN structure between PMMA beads and MMA-EGDMA-monomer matrix in denture base polymers. Before polymerization, the MMA-EGDMA dissolves the PMMA bead from the surface or entire beads (Vallittu 2009). When polymeric dental devices made of IPN polymers are bonded to the resin systems, the bonding is also based on the formation of IPNs. To make a difference between the IPNs of the substrate and that of bonding interface, the latter has been defined as “secondary IPN” (Vallittu 2009).

The capability of monomers to dissolve polymers in order to bond to each other through IPN bonding system can be evaluated, for instance, by using solubility parameter values. The better the match (i.e. the smaller the difference) of the solubility parameters of polymer and monomer, the better the formation of IPN bond (Barton 1991, Klempner et al. 1994). Solubility parameters have been developed to provide a simple method of correlating and predicting the cohesive and adhesive properties of materials. Solubility parameters are of greater value when considered along with other chemical and physical data (Barton 1991). The numerical value of solubility parameter illustrates the amount of energy required to separate molecules. The basis of the solubility parameter approach to interactions may be stated as follows: A material with high solubility parameter value requires more energy for dispersal than is gained by mixing it with a material of low solubility parameter, so immiscibility results. On the other hand, two materials with similar solubility parameter values gain sufficient energy on mutual dispersion to permit mixing (Barton 1991). The term solubility parameter, which has been used widely, has been claimed to be too restrictive for a quantity that may be used to correlate such a wide range of physical and chemical properties. This is why a term cohesion parameter has also been used (Barton 1991).

Several groups have published information about cohesion parameters and because of this several different cohesion parameters are in use. Cohesion parameters can be divided into one-component and several-component parameters. Hildebrand cohesion parameter is perhaps the best known parameter. Hildebrand parameter is a one-component parameter which was originally intended for non-polar non-associating systems, but later the concept has been extended to all types of systems. Hildebrand

parameter is sometimes also called the total cohesion parameter. Hansen proposed a practical extension of the Hildebrand parameter to polar and hydrogen-bonding systems. Hansen's cohesion parameter is a several-component parameter. Hansen assumed that dispersion, polar and hydrogen-bonding parameters are valid simultaneously. Hansen's total cohesion parameter equals to Hildebrand parameter (Barton 1991).

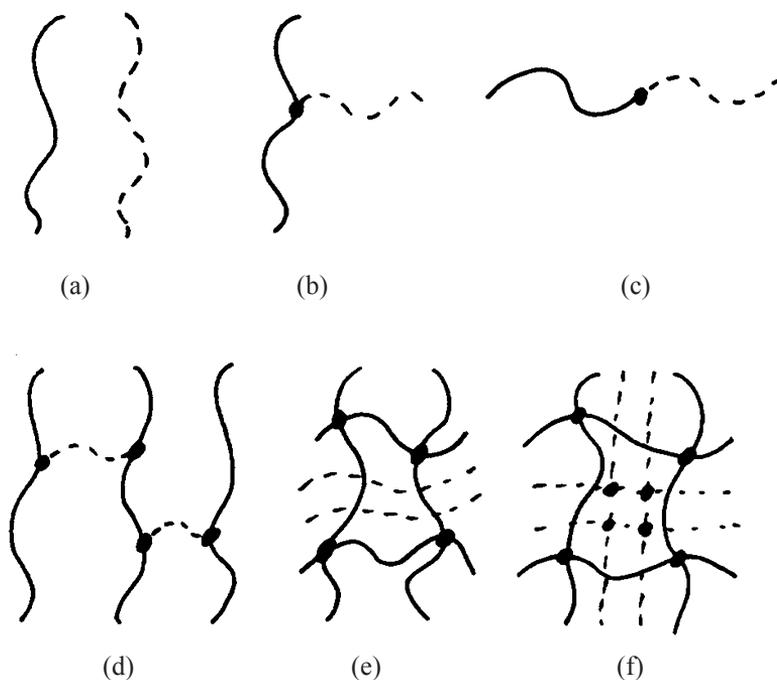


Figure 4. 4a shows a basic polymer blend. 4b illustrates a graft copolymer, wherein the end of a side chain is grafted to part of a backbone chain. 4c illustrates a block copolymer, differing from a graft copolymer in that the junction is between the ends of the two participating polymer chains. 4d represents single network composed of two kinds of chains, known as an AB cross-linked copolymer. Figure 4e shows a semi-IPN composed of two polymers, one linear and one cross-linked. 4f illustrates an IPN composed of two cross-linked polymers (Figure from Sperling and Mishra 1995).

2.4.4 Surface treatments used in dentistry to improve attachment

By certain surface treatments the adhesion between two materials can be improved. The improvements are based on the ability of surface treatments to increase the surface area, to lower contact angle, thus improve wetting between two materials and to provide opportunity for chemical bond formation.

The best known surface treatment in dentistry is perhaps the phosphoric acid etching of enamel. Phosphoric acid etching was invented by Buonocore in 1955. By etching the enamel, the surface area of enamel increases and etching creates surface for

micromechanical retention (Buonocore 1955). Etching is also in clinical use while bonding oxide-ceramics to adhesive resins. Oxide-ceramics are etched with hydrofluoric acid and this also creates large microretentive surface. Non-oxide ceramics (ZrO_2 , Al_2O_3) are often sandblasted to increase the surface area (Blatz et al. 2003, Özcan et al. 1998). Matinlinna et al. and Aboushelib et al. have studied the possibility to use etching also in zirconia based ceramics (Aboushelib et al. 2008, Matinlinna et al. 2007).

Silanes are used in dentistry in different applications to lower the contact angle and to provide the opportunity for chemical bonding. Silanes form a large group of compounds. They typically contain two functional parts. One end contains an organic group which can polymerize with the adhered resin system. The other end contains groups which can react with inorganic groups of substrate. Silanes can function as mediators between dissimilar, inorganic and organic, matrices through dual activity. The silane most commonly applied in dental laboratories and chairside is a monofunctional methacryloxypropyltrimethoxysilane (or 3-trimethoxysilylpropylmethacrylate [MPS]) (Figure 5). MPS is used to optimize and promote the adhesion, through chemical and physical coupling, between metal-composite, ceramic-composite, and composite-composite. Any resin system that contains methacrylate groups in the molecules can be used, since the methacrylate end of the monomers copolymerizes to the methacrylate groups of the siloxane (Matinlinna et al. 2004).

For an effective bond of resin to porcelain and metal, the use of silane in combination with surface conditioning has been used. The Rocatec air particle abrasion system (3M/ESPE), based on Al_2O_3 abrasive particles with coating of SiO_2 (eg, with 50- μ m diameter) has been used to roughen the substrate surface. This increases the bond strength because of the increase in silica content on the substrate surface that provides a basis for silanes to enhance resin bonding. At the same time also the surface area increases. This is called the tribochemical bonding method. For better clinical success, the use of silane coupling agents is crucial in creating long-lasting bonds of polymers and composites to ceramic or to metal (Matinlinna and Vallittu 2007).

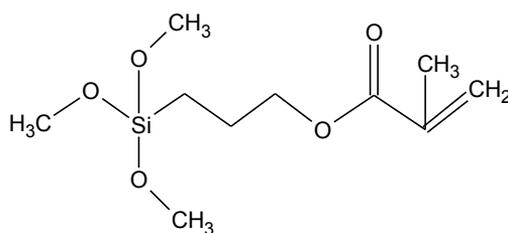


Figure 5. The structural formula of MPS.

2.5 Attachment of resins to tooth substrate

Buonocore has been proposed to be the father of adhesive dentistry. In 1955, Buonocore published a paper in which he focused on enamel etching with phosphoric acid (Buonocore 1955). Buonocore found that enamel etching led to microscopic porosities which enhanced the retention of dental materials.

After Buonocore had presented the idea of enamel etching also dentin bonding agents were started to develop. Dentin bonding agents are traditionally divided into generations. Until today, seven generations of dentin bonding agents have been developed (Kugel and Ferrari 2000, Söderholm 2007). The first generation of dentin bonding agents was launched in the 1950s and 1960s. Buonocore with his colleagues claimed glycerophosphoric acid dimethacrylate to enhance dentin bond strength (Buonocore et al. 1956). Bowen et al. tried to use N-phenylglycine and glycidyl methacrylate (NPG-GMA) as bifunctional molecule in dentin bonding. These first generation dentin bonding agents had only bond strengths of 1 to 3 megapascals (Bowen 1965c, Bowen 1965d, Bowen 1965e, Bowen 1965f).

In the 1970s, second generation dentin bonding agents were introduced. BisGMA and HEMA were launched in dentin bonding agents. However, dentin was not acid etched and since this much of the bond strength was due to bonding of smear layer (Kugel and Ferrari 2000, Söderholm 2007). Major breakthrough in dentin bonding happened in the late 1970s and early 1980s when third generation dentin bonding agents were presented. In these agents, acid etching of dentin was used. However, the used etching pattern was mild. Smear layer was treated with a conditioner that was rinsed off before the hydrophilic primer and unfilled resin were applied (Fusayama et al. 1979, Kugel and Ferrari 2000, Söderholm 2007).

In the 1980s and 1990s, fourth generation dentin bonding agents were introduced. These fourth generation materials are still considered as the golden standard in dentin bonding. In the fourth generation materials aggressive dentin acid etching is used. In fourth generation materials dentin is etched with phosphoric acid and after this rinsed and left moist (wet-bonding), and after this hydrophilic primer is added and hybrid layer is formed (Kanca J 1991, Kanca J 1992, Kugel and Ferrari 2000, Söderholm 2007). Nakabayashi et al. reported the formation of a hybrid layer already in 1982 (Nakabayashi et al. 1982).

In the 1990s and in the ongoing decade, generations five to seven of dentin bonding agents have been introduced. These generations have focused on simplifying the dentin bonding procedure. In fifth generation agents, the hydrophilic primer and hydrophobic resin were combined and used after acid etching of dentin (two-step etch-and-rinse system). In the sixth generation agents, self-etching hydrophilic primer is used together with

hydrophobic resin in separate steps (two step self-etch system). In these sixth generation agents, the technique sensitive etching step is avoided. The seventh generation bonding agents have combined all the work steps in one bottle and use only one hydrophilic self-etching solution (one step self-etch system) (Kugel and Ferrari 2000, Söderholm 2007).

2.5.1 Enamel bonding

Enamel is composed of inorganic minerals mainly of hydroxyapatite crystals (96%). Enamel contains hardly any water (4%) and this is why enamel bonding is easier to perform than dentin bonding (O'Brien 2008). Enamel bonding is based on acid etching which produces large microretentive surface with high surface energy (Buonocore 1955). High energy permits good wetting by adhesive resin and penetration to microretentive surface. Since enamel lacks water, hydrophobic resin monomers can be used and this is why enamel bonding agents are not prone to water sorption (O'Brien 2008). Also the lack of water enables polymerization of adhesive resin to well polymerized layer of adhesive without the detrimental effect of water or residual solvents in the resin layer.

2.5.2 Dentin bonding

Dentin is composed of organic and inorganic materials. The inorganic part consists of hydroxyapatite (45%) and the organic part consists of water (25%) and organic matrix which is mainly collagen (30%). Dentin bonding is based on acid etching of dentin surface which partially decalcifies dentin and opens dentinal tubules. After this, hydrophilic primer is used and the hydrophilic primer enables penetration of adhesive resin to decalcified dentin and forms a so called hybrid layer together with the organic dentin matrix. This hybrid layer is the main supporting microstructure behind dentin bonding. On the surface of polymerized adhesive resin, there is a non-polymerized, so called oxygen inhibition layer, which provides a free radical polymerization site for overlaying filling composite (O'Brien 2008).

2.5.2.1 Monomers used in dentin bonding

Two kinds of monomers are used in dentin primers: cross-linkers and functional monomers. Functional monomers typically have only one polymerizable group whereas cross-linkers have two polymerizable groups. Functional monomers contain a functional group which will impart monomer-specific functions. This group may enhance wetting or demineralising of dentin. It can also release fluoride or influence the antibacterial properties of monomers. Functional groups typically have hydrophilic nature. The most common functional groups are phosphate, carboxyl acid and alcohol groups. Functional monomers will form linear polymers and cross-linkers will form cross-linked polymers. Cross-linked polymers have better mechanical properties compared to linear polymers.

Both functional monomers and cross-linkers contain spacer component between polymerizable groups and functional groups. The spacer part influences in many properties of monomers. Spacers have an effect on eg. solubility, viscosity, hydrophilicity, mechanical properties and wettability. The spacer is usually an alkyl chain but it can also contain several other groups (Van Landuyt et al. 2007).

Countless number of monomers has been used in dentin adhesives. Traditionally, three-step etch-and-rinse adhesives and two-step self-etch adhesives have contained hydrophilic monomers in primers and more hydrophobic cross-linked monomers in adhesive resin. Simplified two-step etch-and-rinse adhesives and one-step self-etch adhesives have combined hydrophilic and hydrophobic monomers in the same blend (Van Landuyt et al. 2007). Latest research results have shown that hydrophilic monomers create membrane prone to water degradation, hence the use of hydrophobic monomers has been suggested (Cadenaro et al. 2009a, Carrilho et al. 2005, Malacarne et al. 2006, Nishitani et al. 2006, Reis et al. 2004, Van Landuyt et al. 2008a, Yiu et al. 2006).

To mention some commonly used monomers hydroxyethylmethacrylate (HEMA), 4-META (4-methacryloyloxyethyl trimellitate anhydride), 4-AETA (4-acryloyloxyethyl trimellitate anhydride), 10-MDP (10-methacryloyloxydecyl dihydrogenphosphate), MAC-10 (11-methacryloyloxy-1,1-undecanedicarboxylic acid), BisGMA, UDMA and TEGDMA are good examples. HEMA is a small hydrophilic monomer, which also dissolves other monomers and lowers the viscosity of primer system. HEMA enhances wetting of dentin and this way significantly improves the bond strength. The drawback of HEMA is the high affinity of water and tendency to water degradation. High amounts of HEMA in dentin primers can lead to reduced co-polymerization due to both water-attraction and oxygen inhibition and to formation of droplets inside hybrid layer due to osmosis reaction. In addition, as a linear polymer, HEMA has low mechanical properties (Carvalho et al. 2003, Ikeda et al. 2008, Van Landuyt et al. 2008a). 4-META and 4-AETA are functional monomers capable of acid etching due to acidic group. However, the aromatic group of these monomers is hydrophobic and will moderate the acidity of the carboxyl groups. 10-MDP is also an acidic monomer with dihydrogenphosphate group. The long carbonyl chain makes this monomer at the same time quite hydrophobic. 10-MDP has shown to chemically bond to tooth substrate. MAC-10 is also an acidic monomer and differs from MDP-10 only in a functional group (Van Landuyt et al. 2007). BisGMA, UDMA and TEGDMA are most frequently used as cross-linkers in adhesive systems. Cross-linked monomers offer mechanical strength and better resistance to water degradation compared to hydrophilic monomers. BisGMA is a highly viscous and rigid monomer. Other monomers, such as TEGDMA and UDMA, are needed as diluents for BisGMA (Cook et al. 1985, Van Landuyt et al. 2007).

2.5.2.2 Solvents used in dentin bonding

Solvents are used in dentin bonding to replace water in dentin collagen network and to enable adhesive monomers to penetrate into this network. Solvents should evaporate and monomers should replace them before polymerisation happens in the hybrid layer (O'Brien 2008, Van Landuyt et al. 2007). Another function of solvents is to maintain dentin collagen matrix, which has been formed in acid etching (Agee et al. 2006, Pashley et al. 2001). The effect of different solvents on dry and wet dentin surface has been studied (Agee et al. 2006, Carvalho et al. 2003, Garcia et al. 2005, Pashley et al. 2001, Perdigao et al. 1999). It has been concluded that the ability of adhesive systems solvent to maintain the demineralised dentin collagen matrix is essential for the bonding procedure and the resulting bond strength (Carvalho et al. 2003). A solvent needs to be capable of creating hydrogen-bonding with a higher affinity for the dentin collagen peptide hydrogen-bonds than do adjacent peptides in order to maintain the matrix (Agee et al. 2006, Pashley et al. 2001). Hydrogen bonding capacity of solvents can be ranked by using the solubility parameters for hydrogen-bonding, as calculated by the methods of Hansen (Agee et al. 2006, Barton et al. 1991, Pashley et al. 2001). Water has been proposed to be the best solvent to maintain the dentin matrix. The hydrogen bonding cohesion parameter of water is (40.4 MPa^{1/2}) (Barton et al. 1991). In self-etch adhesives solvent is also needed to ensure ionization of the acidic monomers. The solvent used for this purpose is water (Van Landuyt et al. 2007).

The most used solvents in dentin primers are ethanol, acetone and water. All these three solvents have been used in etch and rinse adhesives. In self-etch adhesives, water is the typical solvent. Ethanol has been proposed to have the best features as a solvent. Ethanol, with the hydrogen cohesion parameter of 20.0 MPa^{1/2}, maintains the dentin collagen matrix almost as efficiently as water (Agee et al. 2006, Barton et al. 1991, Pashley et al. 2001). Usually ethanol is used in conjunction with water as a co-solvent. Ethanol is able to replace water and it can be used with hydrophobic resin monomers, which are not so prone to water sorption over time. A comparison of contemporary adhesives revealed that the ethanol-water based etch-and-rinse adhesives are the “golden standard” in terms of adhesion durability (De Munck et al. 2005).

The problem with acetone as a solvent is the high technique-sensitivity. Since acetone, with the hydrogen cohesion parameter of 11.0 MPa^{1/2}, is not capable of maintaining the dentin collagen network efficiently, a “wet bonding” technique is needed (Agee et al. 2006, Barton et al. 1991, Pashley et al. 2001). Wet bonding technique is challenging, especially in clinical work, since it is hard to know when dentin is too wet or too dry. Acetone's vapour pressure, however, is about four times as high as that of ethanol and that is the main advantage of acetone as a solvent (Van Landuyt et al. 2007). Together with water, acetone forms an “azeotropic” mixture. This means that hydrogen bonds are formed between water and acetone molecules and result in better evaporation of water

compared to pure water. Wet bonding etch-and-rinse systems usually contain acetone to facilitate water removal (Van Landuyt et al. 2007).

Water is used as solvent especially in simplified two-step etch-and-rinse adhesives and in self-etch adhesives. Even if water has optimal hydrogen cohesion parameter to maintain collagen matrix as solvent, water also has many problems (Van Landuyt et al. 2007). Together with water, hydrophilic monomers must be used, and after polymerisation semi-permeable membrane is formed as hybrid layer. This semi-permeable membrane attracts water faster than hydrophobic adhesives (Nishitani et al. 2006, Yiu et al. 2006). The vapour pressure of water is low and it is difficult to obtain adhesive resin layer free from residual solvents (Yiu et al. 2005). Residual solvent makes the hybrid layer porous and prone to water sorption. During the bonding stage, the monomer/water ratio changes and this can lead in phase separations and blistering. Because of these drawbacks, water is typically used with ethanol or acetone as a co-solvent (Van Landuyt et al. 2007). Also, it has been assumed that residuals of solvents hinder polymerization of monomers in primers and adhesives, and make the hybrid layer prone for weakening over time (Vallittu, personal communication).

2.5.2.3 Fillers used in dentin bonding

Composite resins contain always fillers, but this is not the case concerning adhesive resins (Van Landuyt et al. 2007). The reasons to add fillers into adhesive resins are several and partly the same as for composite resins. Some authors have suggested fillers to increase the mechanical properties of adhesive layer, to prevent overly thinning of the adhesive layer and to provide good relief of contraction stresses produced by the restorative resin (Frankenberger et al. 2002, Kim et al. 2005, Miyazaki et al. 1995, Van Landuyt et al. 2007, Van Meerbeek et al. 1993a, Van Meerbeek et al. 1993b). Some fillers also provide fluoride release and radio-opacity (Van Landuyt et al. 2007). The amount of fillers in adhesive resins is lower than in composite resins, in order to maintain the wetting properties of adhesive resins. Also, the size of fillers is smaller than in composite resins, in order to let the fillers to penetrate into dentin tubules and possible also demineralised collagen network. After etching, the interfibrillar spaces of the demineralised collagen network have been shown to be in the range of 20 nm. However, debate exists whether fillers can actually infiltrate demineralized collagen network (Van Landuyt et al. 2007).

Most filled adhesive resins for bonding composites contain pure silicon dioxide. Also silicate glasses containing heavy metal atoms such as barium and strontium tailored to provide radio-opacity have been used. Fluorine-containing reactive silicate glasses are sometimes added with the intention to release fluoride. However, beneficial effects of fluorine release from adhesive resins still need to be established (Van Landuyt et al. 2007). In general, fillers in adhesive resins are silanized to improve adhesion of fillers to resin (Van Landuyt et al. 2007, Yoshida et al. 2002).

2.5.2.4 Challenges and recent developments in dentin bonding

Current dentin bonding agents still have many known and perhaps also unknown problems. The biggest problem at the moment is that long term bond strength by dentin bonding agents decreases over time. Many reasons for this have been presented. It has been suggested that water absorbs over time into hybrid layer and plastizizes it. It has been claimed that the more hydrophilic monomers are used in dentin bonding the more water is absorbed into hybrid layer (Carrilho et al. 2008, Malacarne et al. 2006, Tay and Pashley 2003, Van Landuyt et al. 2008a, Yiu et al. 2004, Yiu et al. 2006). Also, it has been argued that the retained solvent in hybrid layer increases water sorption and similarly the plastification process (Ikeda et al. 2008, Yiu et al. 2005). Water has been proposed to be the most problematic solvent concerning this because of low vapour pressure. It has also been suggested that the action of matrix metalloproteinases (MMP) decreases the long term bond strength. MMPs are a group of 23 mammalian enzymes capable of degrading all extracellular matrix components. Human dentin contains at least collagenase (MMP-8), gelatinases MMP-2 and MMP-9 and enamelysin MMP-20. It has been claimed that MMPs degrade the collagen network revealed in acid etching and this leads to degrading of hybrid layer. Incomplete hybridization of collagen network by adhesive monomers accelerates the action of MMPs (Carrilho et al. 2007a, Carrilho et al. 2007b, Carrilho et al. 2009, Martin-De Las Heras et al. 2000, Mazzoni et al. 2006, Sulkala et al. 2002, Sulkala et al. 2007). Presently, some correlation between activity of MMPs and weakening of dentin bond has been shown and the caused relationship requires future investigation.

Possibilities to enhance the long-term bond strength have been presented. One suggestion has been to change the hydrophilic monomers prone to water degradation into hydrophobic monomers and use ethanol-wet bonding instead of water-wet bonding (Cadenaro et al. 2009a, Carrilho et al. 2008, Sadek et al. 2010). Another means is to improve more stable monomers for wet-bonding [acrylic phosphonic acids and bis(acrylamides)] (Moszner et al. 1999, Moszner et al. 2001, Moszner et al. 2004, Moszner et al. 2006, Van Landuyt et al. 2008c). Several authors have also published articles about minimizing the amount of HEMA and water in adhesives (Furukawa et al. 2008, Kanehira et al. 2009, Mine et al. 2008, Van Landuyt et al. 2008b).

Carrilho et al. have proposed the use of chlorhexidin on dentin at bonding stage to prevent the action of MMPs (Carrilho et al. 2007a, Carrilho et al. 2007b, Carrilho et al. 2009). The use of chlorhexidin has offered promising results (Breschi et al. 2009, Carrilho et al. 2007a, Carrilho et al. 2007b, Carrilho et al. 2009, Erhardt et al. 2008, Gendron et al. 1999, Zhou et al. 2009). However, De Munck et al. have concluded that MMP inhibitors appeared effective in reducing bond degradation only for the etch and rinse adhesives and not for self-etch adhesives. They concluded also that water sorption remains as the principal mechanism of bond degradation, while endogenous enzymes

appear to contribute to bond degradation of only etch-and-rinse adhesives (De Munck et al. 2009).

Also the use of chemical cross-linkers to cross-link dentin collagen network before bonding has been proposed as a new reinforcement method. The use of glutaraldehyde, grape seed extract and tannic-acid has been introduced (Bedran-Russo et al. 2009, Cilli et al. 2009, Macedo et al. 2009). The use of term cross-linking in this context can be questioned due to lack of evidence of strong chemical bonds.

In recent years, also articles about the formation of acid-base resistant zone under hybrid layer have been published. It has been concluded that by using self-etching bonding agents, dentin adjacent to the adhesive-dentin interface is different from normal dentin, which has a potential to resist an acid attack from the microorganisms. It has been suggested that the formation of the reinforced dentin may become a key strategy in preventive dentistry in the future (Waidyasekera et al. 2009).

3 AIMS OF THE PRESENT STUDY

The purpose of this series of studies was to evaluate the suitability of poly(paraphenylene) based rigid rod polymer (RRP) as a polymeric material in dentistry. The hypothesis of this study was that RRP fillers enhance the mechanical properties of currently used denture base polymer and that the adhesion between RRP and some other dental polymers is possible to obtain. The second purpose was to evaluate the properties of RRP filled experimental dentin primer system. The specific aims and hypotheses were:

1. To investigate the flexural properties, surface hardness, water sorption, solubility and surface structure of denture base polymer with fillers of RRP. The hypothesis was that RRP increases flexural strength, flexural modulus and surface hardness. Second hypothesis was that RRP will decrease water sorption and solubility of denture base polymer. Third hypothesis was that RRP will create an IPN bond with denture base polymer. (I)
2. To evaluate the effect of different surface treatments of RRP substrate in bonding with BisGMA-TEGDMA-resin. The hypothesis was that substrate surface treatment including primers would affect the adhesion of BisGMA-TEGDMA-resin and RRP. (II)
3. To determine the microtensile bond-strength of short-term water stored RRP-modified dentin primer system compared to commercial control and to examine the structure of the interface between resin and dentin of RRP modified primer by scanning electron microscope. The null hypothesis was that monomer type, solvent:monomer ratio and RRP of the experimental primers have no effect on bond strength. (III)
4. To investigate the effect of long term, six months and twelve months, water storing on microtensile bond strength and on the interface between resin and dentin of RRP modified dentin primer. The null-hypotheses were that water storage has no effect on the bond strength of experimental primers and the long-term bond strength of experimental primers does not differ from the bond strength of the control group. (IV)

4. MATERIALS AND METHODS

4.1 Materials

Materials used in this series of studies can be seen in Table 1.

Table 1. Materials used in these studies.

| Trade name | Type of material | Manufacturer | Study |
|--|----------------------------|---|----------------|
| Palapress® powder | PMMA polymer powder | Heraus Kulzer, Wehrheim, Germany | I, II |
| Palapress® liquid | MMA monomer, cross-linker | Heraus Kulzer, Wehrheim, Germany | I, II |
| Parmax 1240®* | Rigid rod polymer | Mississippi Polymer Technologies, Inc, St. Louis, Missouri, USA | I, II, III, IV |
| Tetrahydrofuran (THF) | Solvent | Sigma-Aldrich, Laborchemicalien GmbH, Seelze, Germany | I |
| PMMA** | Polymer | Sigma Aldrich | II |
| BisGMA | Resin monomer | Degussa chemicals | II |
| TEGDMA | Resin monomer | Fluka Chemika | II |
| DMAEMA | Activator | Fluka Chemika | II |
| Camphorquinone | Initiator | Sigma Aldrich | II |
| Dichloromethane (DCM) | Solvent | Fluka Chemika | II, III, IV |
| Vocoacid | Etching gel | Voco, Cuxhaven, Germany | III, IV |
| Voco Solobond Plus Primer | Bond primer | Voco, Cuxhaven, Germany | III, IV |
| Voco Solobond Plus Adhesive | Light-curing bond adhesive | Voco, Cuxhaven, Germany | III, IV |
| Voco Grandio Caps | Composite resin | Voco, Cuxhaven, Germany | III, IV |
| Ethylene glycol methacrylate phosphate (EGMP) | Monomer (primer component) | Sigma Aldrich, Steinheim, Germany | III |
| Bis[2-(methacryloyloxy)-ethyl]phosphate (BMEP) | Monomer (primer component) | Sigma Aldrich, Steinheim, Germany | III, IV |
| 3-(trimethoxysilyl)-propylmethacrylate (MPS) | Silane (primer component) | Sigma Aldrich, Steinheim, Germany | III, IV |

* M_w of RRP has been reported to be 26.900 – 30.000 (Connolly and Karasz 1995, Ha et al. 2001)

** M_w of used PMMA was 350.000

4.1.1 Materials and specimen preparation in denture base polymer-RRP material combination study (I)

Groups of the Study I are seen in Table 2. Bar shaped test specimens ($65 \times 10 \times 3 \text{ mm}^3$) were fabricated. Group 1 specimens contained only rigid rod polymer (Parmax 1240®). Specimens were made by compression moulding system with the pressure of ca 13.8

MPa, temperature of 315°C for 30 minutes by the manufacturer of the RRP (manufacturer Mississippi Polymer Technologies, Inc.). Specimens in groups 2–5 were fabricated from autopolymerised poly(methylmethacrylate) (PMMA) denture base resin (ISO 1567). Powder-to-liquid ratio for the resin mixture in Group 2 was 10g / 7ml, in Group 3 it was 10g / 8ml, in Group 4 10g / 9ml and in Group 5 10g / 13ml. The powder was first weighed and monomer liquid was mixed with the powder. In Groups 3, 4 and 5 part of the PMMA-powder was replaced with RRP-powder (Parmax 1240®). Higher monomer liquid ratio was used with increased RRP quantity to ensure proper blending of powder mixture with liquid. Test specimens in Groups 2–5 were polymerised in a mould under a pressure of 300 kPa at a temperature of 80 °C ± 5°C for 15 minutes in a pressure-curing device (Ivomat, Typ IP 2, Ivoclar AG., Schaan, Liechtenstein).

Test specimens' surfaces were ground with 1200 and 2400 grit silicon carbide grinding paper (Struers A/S, Rodovre, Denmark) using a grinding machine (LaboPol-21, Struers A/S, Rodovre, Denmark). Specimen's dimensions (width, height and length) were measured at three points on each side in order to ensure the dimensions of the test specimens. The number of specimens in each group was twelve. The test specimens were either stored dry at room temperature for two days (n=6) or in water at 37°C for 44 days (n=6) before a three point bending test. After the three point bending test the same fractured test specimens were used in Vickers surface microhardness test, in solubility and sorption determination studies and in SEM studies.

Table 2. The groups and the amounts of the used materials in the Study I.

| Group | Amount of PMMA (g) | Amount of RRP (g) | Amount of MMA (ml) |
|--------------|--------------------|-------------------|--------------------|
| 1 (100%-RRP) | 0 g | 100% | 0 ml |
| 2 (0%-RRP) | 10.0 g | 0 g | 7.0 ml |
| 3 (10%-RRP) | 9.0 g | 1.0 g | 8.0 ml |
| 4 (20%-RRP) | 8.0 g | 2.0 g | 9.0 ml |
| 5 (30%-RRP) | 7.0 g | 3.0 g | 13 ml |

The amounts of PMMA-powder (g), RRP-powder (g) and MMA-EGDMA-monomer liquid (ml) in the groups of Study I.

4.1.2 Preparation of samples for SEM (I)

Bar shaped test specimens fabricated for three point bending test were used in SEM analysis after testing until fracture in three point bending test. One half of test specimen from each group was used. Samples were wet polished with 4000 grit silicon carbide grinding paper, followed by diamond paste polishing (Struers polishing cloths, diamond paste ≤ 1 µm, Struers A/S, Rodovre, Denmark). Specimens were cleaned in water in ultra-sound cleaning device for 10 minutes. After polishing and cleaning, specimens were immersed into solvent tetrahydrofuran (THF) for 30 seconds to separate cross-

linked and non-cross-linked polymer phases on the surface and to show possible signs of IPN structures. Specimens were sputter-coated with gold using a coater (BAL-TEC SCD 050 Sputter Coater, Balzer, Liechtenstein) (Vallittu and Ruyter 1997).

4.1.3 Materials and specimen preparation in BisGMA-TEGDMA-resin-RRP adhesion studies (II)

Seventeen groups test specimens for shear bond strength test were prepared (Table 3). Each group contained eight specimens (n=8). Schematic presentation of test specimen is seen in Figure 6.

Before the different bonding procedures, the surface of the RRP substrate was ground in all groups with silicone carbide (SiC) grinding paper (Struers A/S, Rodovre, Denmark) up to paper no. 1000 grit (FEPA) under water cooling with a grinding machine (LaboPol-21, Struers A/S, Rodovre, Denmark) to obtain a flat substrate surface.

In Groups 1–7, the ground surface of the RRP substrate was treated with BisGMA-TEGDMA resin system. The BisGMA-TEGDMA-resin system contained 60% of BisGMA, 40% of TEGDMA and 0.7% of DMAEMA (dimethyl amino ethyl methacrylate) as an activator and 0.7% of camphoroquinone as an initiator. Resin system was led to influence on the surface of RRP under a light coverage for different periods of time. The treatment times in Groups 1–7 were 10, 60, 180, 300, 600, 900 and 1800 seconds, respectively.

In Groups 8–10, the ground surface of the RRP substrate was heated before the application of resin. In the heat treatment, the substrate temperature at bonding surface was heated up to circa 140 °C. In Group 8, the heat treatment time was 10 seconds, in Group 9 it was 60 seconds and in Group 10, 180 seconds.

In Group 11, the ground surface of the RRP substrate was treated with solvent dichloromethane (DCM) for 180 seconds and followed by air-blew drying for 5 seconds.

In Groups 12–14, the ground surface of the RRP substrate was treated with a primer of PMMA/DCM. The primer contained 9.1 wt% of dissolved PMMA in the solvent DCM. In Group 12 the treatment time was 10 seconds, in Group 13, 60 seconds and in Group 14 the time was 180 seconds. After the surface treatment, the surface of RRP was air-blown for 5 seconds.

In Groups 15–17, the ground surface of the RRP substrate was treated with primer system of RRP/BisGMA-TEGDMA-resin/DCM. The primer system contained 0.96 wt% RRP and 0.96 wt% BisGMA-TEGDMA-resin dissolved in solvent DCM. The BisGMA-TEGDMA-resin contained 60% of BisGMA, 40% of TEGDMA and 0.7% of DMAEMA as an activator and 0.7% of camphoroquinone as an initiator. In Group 15, the treatment

time was 10 seconds, in Group 16 the time was 60 seconds and in Group 17 it was 180 seconds. After the treatment, the treated surface was air-blown for 5 seconds.

Immediately following the different surface treatments at the RRP substrates BisGMA-TEGDMA-resin adherent cylinders diameter of 3.6 mm and height of 4.0 mm were light polymerised for 40 seconds (Optilux 501, Sds Kerr, Danbury CT) with the help of a polyethylene mould. The BisGMA-TEGDMA-resin contained 60% of BisGMA, 40% of TEGDMA and 0.7% of DMAEMA as an activator and 0.7% of camphoroquinone as an initiator.

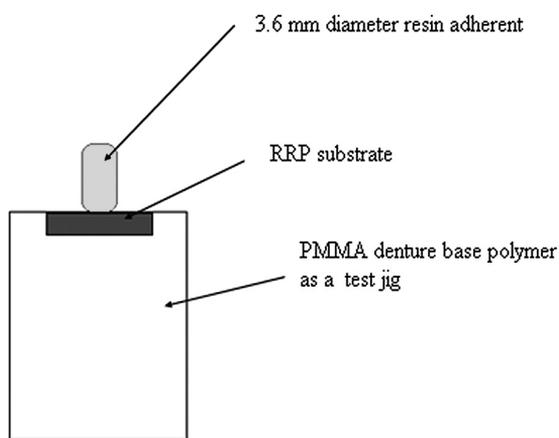


Figure 6. Schematic presentation of the test setup.

Table 3. The groups in the Study II.

| Group | Treatment of the RRP substrate | Time |
|-------|------------------------------------|-----------------|
| 1 | BisGMA-TEGDMA-resin dissolving | 10 s |
| 2 | BisGMA-TEGDMA-resin dissolving | 60 s |
| 3 | BisGMA-TEGDMA-resin dissolving | 180 s = 3 min |
| 4 | BisGMA-TEGDMA-resin dissolving | 300 s = 5 min |
| 5 | BisGMA-TEGDMA-resin dissolving | 600 s = 10 min |
| 6 | BisGMA-TEGDMA-resin dissolving | 900 s = 15 min |
| 7 | BisGMA-TEGDMA-resin dissolving | 1800 s = 30 min |
| 8 | Heat treatment | 10 s |
| 9 | Heat treatment | 60 s |
| 10 | Heat treatment | 180 s = 3 min |
| 11 | Solvent DCM dissolving | 180 s = 3 min |
| 12 | PMMA/DCM-primer | 10 s |
| 13 | PMMA/DCM-primer | 60 s |
| 14 | PMMA/DCM-primer | 180 s = 3 min |
| 15 | RRP/BisGMA-TEGDMA-resin/DCM-primer | 10 s |
| 16 | RRP/BisGMA-TEGDMA-resin/DCM-primer | 60 s |
| 17 | RRP/BisGMA-TEGDMA-resin/DCM-primer | 180 s = 3 min |

4.1.4 Preparation of samples for SEM (II)

For scanning electron microscope (SEM) evaluation representative, test specimens were fabricated to simulate the bonding surface of the substrate after the surface treatments. The SEM samples were rinsed with acetone for 30 seconds (in Groups 13 and 16) or for 60 seconds (in Groups 2, 3, 9, 10 and 11) to clean the surface of the RRP substrate after surface treatments. Additional SEM samples were prepared to simulate the interface between RRP and BisGMA-TEGDMA-resin (Group 6) to find possible signs of the IPN formation between the RRP substrate and BisGMA-TEGDMA. The interface of RRP and resin was rinsed with solvent DCM for 30 seconds to separate cross-linked and non-cross-linked polymer phases on the surface and to demonstrate possible signs of IPN structures.

For the SEM samples, the surface treatments were performed in a similar way as for shear bond strength test specimens, with the exception that the BisGMA-TEGDMA-resin used in surface treatments had the composition of 70% of BisGMA, 30% of TEGDMA and 0.7% of DMAEMA as an activator and 0.7% of camphoroquinone as an initiator. All the specimens were sputter-coated with gold (BAL-TEC SCD 050 Sputter Coater, Balzer, Liechtenstein).

4.1.5 Materials and specimen preparation in dentin bonding studies (III, IV)

The groups of these studies are presented in Table 4. In Study III, Groups 1–9 were tested. In Study IV, only Groups 1–3 and 9 were tested. In these studies, experimental primers were fabricated to be used as a component of commercial three-step etch-and-rinse adhesive system (Voco Solobond Plus). Table 4 shows the primer compositions of the experimental groups. In Group 9 (control group) Voco Solobond Plus primer was used.

Test specimen preparation for microtensile test

Human molar teeth (the typical age range of donors was 18–30 years) were used as test specimen. Teeth were used within 3 months after extraction. Before using, the teeth were stored at the temperature of -1° and -5 ° C (Pereira et al. 2006, Tezvergil et al. 2003, Uekusa et al. 2007). Prior the specimen preparation, the teeth were rinsed under tap water and excess hard and soft tissues were removed with a scalpel. The specimen teeth were then randomly divided into nine groups. The number of teeth and test specimens can be found in Table 5. Each tooth was ground occlusally with silicone carbide (SiC) grinding paper (Struers A/S, Rodovre, Denmark) up to paper no. 1000 grit under water cooling with a grinding machine (LaboPol-21, Struers A/S, Rodovre, Denmark) to obtain a flat dentin surface.

Composite resin build-up was bonded in 1 mm layers onto the dentin using Voco Solobond Plus adhesive system to a 4 mm height. Optilux 501 light curing unit (Sds Kerr, Danbury CT) was used and the curing time was 40 seconds for polymerization of each 1 mm

composite layer. In the control group, Voco Solobond Plus adhesive system was used. In the study groups the Voco Solobond Plus primer was replaced by one of the eight experimental primers. Manufacturer's directions in the case of Voco Solobond Plus were followed during bonding procedures. Figure 8 shows the experimental design for these studies. Experimental RRP modified primers were fabricated first by dissolving RRP (1.4 wt %) into dichloromethane (DCM). Then mixing it to mixture of 3-(methacryloyloxy)-propyltrimethoxysilane (MPS) and bis[2-(methacryloyloxy)-ethyl]phosphate (BMEP) or either ethylene glycol methacrylate phosphate (EGMP) (Figures 5 and 7). In all of the experimental primers, the same volumetric mixture ratio of MPS-silane and organophosphate-methacrylate was used (1:1). Two solvent-monomer ratios were used. The volume proportions of primer materials in each group are reported in Table 4.

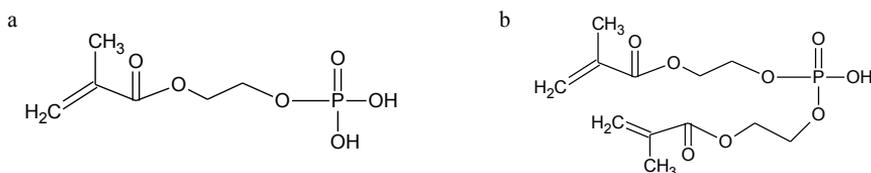


Figure 7. The chemical structure of EGMP (a) and BMEP (b) monomers.

After bonding procedures were conducted, all the teeth were stored in distilled water (grade 3) at 37°C for 48 hours, 6 months or 12 months. After 48 hours' storage, 5 to 6 teeth from each group were cut into rectangular bar shape 1.00mm x 1.00mm ± 0.14 mm thick specimens using a diamond disc (Leitz Wetzlar 1600, Ernst Leitz GMBH, Wetzlar, Germany) under water irrigation. The outermost layers from each tooth were excluded from testing to ensure that only composite bonded to dentin was tested. After 6 months' storage, 3 teeth from Groups 1–3 and 9 were cut into test beams and after 12 months' storage, another 3 teeth were cut from Groups 1–3 and 9. The specimens were kept moist during preparation. Figure 9 represents the test specimen preparation setup.

4.1.6 Preparation of samples for SEM (III, IV)

Bonding procedures for SEM-examination teeth in Studies III and IV were the same as for microtensile testing. In Study III, the teeth for SEM-studies were cut in half with diamond disk and after this they were ground with silicone carbide (SiC) grinding paper up to paper no. 4000 grit under water cooling with a grinding machine.

In Study IV, similar rectangular bars as for microtensile bond strength studies were fabricated for SEM studies. These beams were dehydrated in ascending ethanol series and after this casted in epoxy resin. Beams were further dyed with toluidin blue. After this, they were ground with silicone carbide (SiC) grinding paper up to paper no. 4000 grit under water cooling with a grinding machine.

In Study III, as also in Study IV, to reveal the interface between resin and dentin, the composite-dentin interface was etched with phosphoric acid for 120 seconds, rinsed with water, after this treated with NaOCl for 120 seconds and rinsed after this with water. All the specimens were sputter-coated with gold (BAL-TEC SCD 050 Sputter Coater, Balzer, Liechtenstein).

Table 4. The primer compositions of the groups of Studies III and IV. In Study III, all of the listed groups were tested, whereas in Study IV only Groups 1–3 and 9 were tested.

| Group | BMEP (vol%) | MPS (vol%) | DCM (vol%) | DCM/ RRP (vol%) | DCM treatment |
|-------|-------------|------------|------------|-----------------|---------------|
| 1 # | 25 | 25 | 50 | | |
| 2 # | 25 | 25 | | 50 | |
| 3 & | 8.3 | 8.3 | | 83.3 | |
| 4 & | 8.3 | 8.3 | | 83.3 | x |
| Group | EGMP (vol%) | MPS (vol%) | DCM (vol%) | DCM/ RRP (vol%) | DCM treatment |
| 5 # | 25 | 25 | | 50 | |
| 6 & | 8.3 | 8.3 | | 83.3 | |
| 7 & | 8.3 | 8.3 | 83.3 | | |
| 8 & | 8.3 | 8.3 | | 83.3 | x |
| 9 | control | control | control | control | control |

Dichloromethane (DCM), ethylene glycol methacrylate phosphate (EGMP), bis[2-(methacryloyloxy)-ethyl]phosphate (BMEP), 3(methacryloyloxy)propyltrimethoxysilane (MPS), rigid rod polymer (RRP). Mixture of RRP and DCM contained 1.4wt% RRP. # represents solvent-monomer ratio 1:1, & represents solvent-monomer ratio 5:1.

Table 5. The number of teeth and test specimens (in brackets) in each group after 48 hours', 6 months' or 12 months' water storing.

| Group | 48 hours | 6 months | 12 months |
|-------|----------|----------|-----------|
| 1 | 6 (72) | 3 (32) | 3 (40) |
| 2 | 6 (74) | 3 (43) | 3 (53) |
| 3 | 6 (100) | 3 (42) | 3 (57) |
| 4 | 6 (83) | - | - |
| 5 | 6 (59) | - | - |
| 6 | 5 (53) | - | - |
| 7 | 6 (64) | - | - |
| 8 | 6 (92) | - | - |
| 9 | 5 (66) | 3 (33) | 3 (41) |

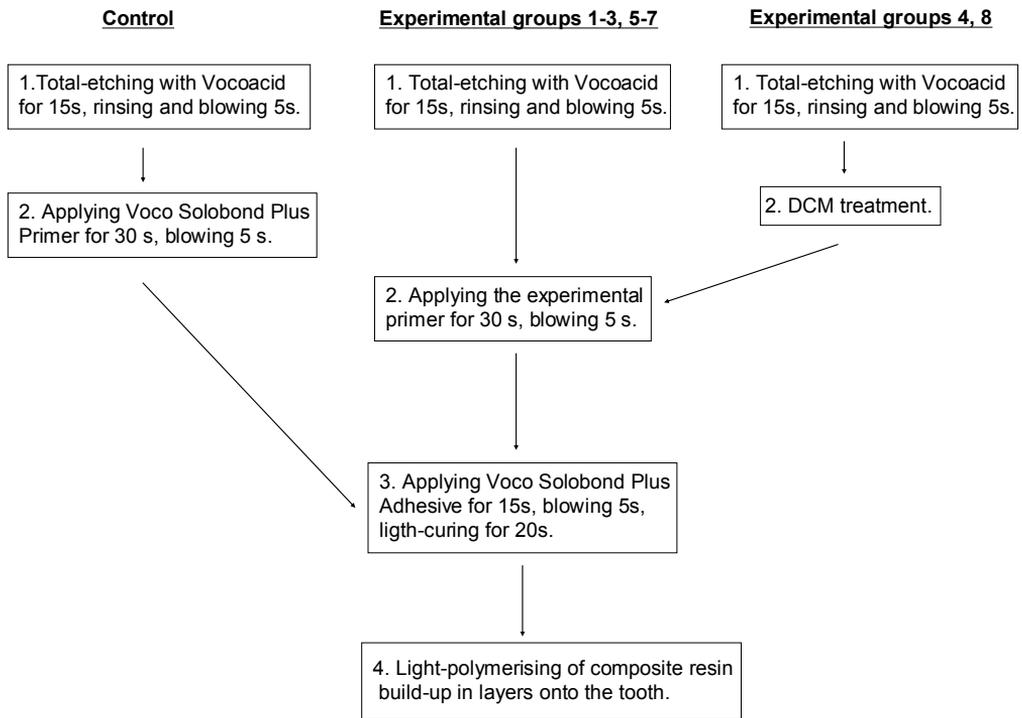


Figure 8. Study design for the experiments of Studies III and IV.

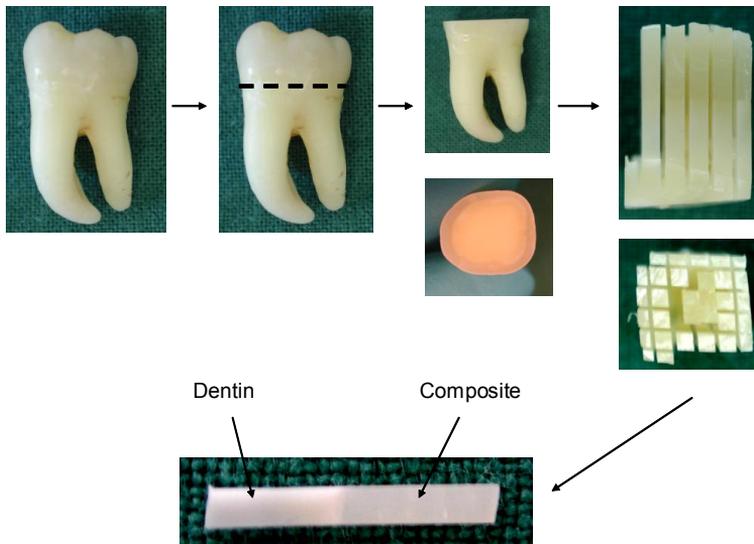


Figure 9. Test specimen preparation for microtensile strength testing of bond strength between dentin and composite resin.

4.1.7 Preparation of samples for contact angle measurements (III)

The contact angles of adhesive resin (Voco Solobond Plus) on the dentin substrates were measured after dentin had been conditioned with different primers. The measurements were

conducted for the control group (Voco Solobond Plus), for one bis[2-(methacryloyloxy)-ethyl]phosphate (BMEP) based primer (Group 3), and for one ethylene glycol methacrylate phosphate EGMP based primer (Group 6). In addition, contact angles were measured on teeth surfaces without any conditioning. For the contact angle measurements 1.0 mm high dentin discs were prepared by sectioning them with the diamond disc.

4.2 Methods

4.2.1 Flexural testing of denture base polymer-RRP material combination (I)

The flexural strength and flexural modulus of the RRP filled denture base polymer and the control polymer were measured according to ISO 1567 standards using three-point bending test with span length of 50 mm and crosshead speed of 5.0 mm/min (Figure 10). All specimens were tested until failure in air in a Lloyd material testing machine (model LRX; Lloyd Instruments, Fareham, England) and the results were recorded with PC-computer software (Nexygen, Lloyd Instruments, Fareham, England). Flexural strength (S), i.e. highest stress, and flexural modulus (E) were calculated from the formulas:

$$S = \frac{3FL}{2bd^2}$$

where S = stress (MPa), F = load or force at break or at yield (N), L = span of specimen between supports (50 mm), b = width (10 mm), d = thickness (3.3 mm)

$$E = \frac{F_1 L^3}{4bd^3 D_1}$$

E = modulus (GPa), F_1 = force at point D_1 (N), L = span of specimen between supports (50 mm), b = width (10 mm), d = thickness (3.3 mm), D_1 = deflection at linear region of load-deflection curve (mm)



Figure 10. Test setup for the three point bending test.

4.2.2 Surface hardness testing of denture base polymer-RRP material combination (I)

The surface microhardness test (Vickers test) was made after the three point bending test, followed by wet polishing the test surface with 4000 grit silicon carbide grinding paper. The microhardness measurements were performed with Duramin-1 Hardness Tester (type 565, Struers A/S, Rodovre, Denmark) at room temperature. The Vickers microhardness test uses a square based pyramidal indenter with an apex of 136°. Vickers hardness number (VHN) was calculated from the formula of:

$$\text{VHN} = 1.854 F/d^2$$

Where F is the applied load (N) and d (mm) is the mean diagonal length of the diamond-shaped indenter.

In the microhardness test, the force used was 19.6 N and loading time 5 seconds (Figure 11). In each group three halves of test specimens were used and 10 measurements for each halves were done.



Figure 11. Test setup for Vickers surface microhardness test.

4.2.3 Solubility and sorption of the denture base polymer-RRP material combination (I)

To calculate weight change in water storing of test specimens six (n=6) specimens from each group were immersed into water. Before immersing the dry-weight (m_d) of specimens was measured with a precision balance (AT261 DeltaRang® Mettler Toledo). The water-sorption of the test specimens was measured after 1, 2, 5, 7, 14, 21, 30, 37 and 44 days with the precision balance. To calculate weight change (%) plotted against storage of time in water with various quantities of RRP fillers in denture base polymer following formula was used:

$$\text{wt}\%_{so} = \frac{m_w - m_d}{m_d} \cdot 100\%$$

where

m_d is the mass of the specimen before immersing in water, in micrograms (μg).

m_w is the mass of the specimen, in micrograms (μg), after immersion in water in different time points.

Test specimens that had been stored in water were tested in three point bending test until fracture for flexural properties and surface hardness values were further analyzed. Two pieces of each specimen were dehydrated in air in an oven (Ehret) at 120°C for 43 hours to calculate sorption and solubility.

Sorption was calculated from the formula below:

$$wt\%_{so} = \frac{m_w - m_{dh}}{m_w} \bullet 100\%$$

where

m_w is the mass of the specimen, in micrograms (μg), after immersion in water.

m_{dh} is the reconditioned or dehydrated mass of the specimen, in micrograms (μg).

Solubility was calculated by comparing the weight change of untested specimens at 44 days time-point to calculated sorption values after dehydration in oven. This calculation method is compared to the use of the formula below:

$$wt\%_{sl} = \frac{m_d - m_{dh}}{m_{dh}} \bullet 100\%$$

where

m_d is the mass of the specimen before immersing in water, in micrograms (μg).

m_{dh} is the reconditioned or dehydrated mass of the specimen, in micrograms (μg).

4.2.4 Shear bond strength testing of BisGMA-TEGDMA-resin-RRP material combination (II)

The shear bond strength specimens were stored at room temperature for one day before testing. The specimens were tested in air until fracture with the universal testing machine (model LRX; Lloyd Instruments, Fareham, England) and the results were recorded with PC-computer software (Nexygen, Lloyd Instruments, Fareham, England). The specimens were secured in a mounting jig (Bencor Multi-T shear assembly, Danville Engineering Inc., San Ramon, CA) with the shearing rod against and parallel to the flat prepared bonding sites. Figure 12 represents the test setup. The crosshead speed was 1.0 mm/min. The shear bond strengths were calculated by dividing the highest fracture force (N) with the contact surface area of the adherent to the substrate (diameter 3.6mm).



Figure 12. Test setup in shear bond strength testing.

4.2.5 Microtensile testing (III, IV)

Test specimens were attached to a microtensile testing machine (Dillon Quantrol Bisco, Schaumburg, IL, USA) with cyanoacrylate adhesive glue and subjected to microtensile testing until fracture with the crosshead speed of 1.0 mm/min (Figure 13). Results were reported in MPa (Pashley et al. 1999, Phrukkanon et al. 1998, Sano et al. 1994b).



Figure 13. Test jig for microtensile measurement.

4.2.6 Contact angle measurements (III)

Ten measurements for each group, one measurement from each sample, were performed (KSV Cam100, KSV Instruments LTD, Helsinki, Finland). The measurements were performed with the time interval of 2000 milliseconds and number of frames was 10.

4.2.7 Assessment of failure mode (II-IV)

In Study II, the fracture types were analyzed visually after shear bond strength test and classified as adhesive between resin adherent and RRP substrate, cohesive in resin adherent or mixed mode. In Studies III and IV fractured specimens were observed under

a light microscope (Wild M3B, Heerburg, Switzerland) at 16X and 40X to determine the type of fracture after microtensile testing. Modes of failure were classified as cohesive in dentin, cohesive in composite, adhesive between resin and dentin, adhesive between resin and composite, or mixed mode.

4.2.8 Scanning electron microscopy (I-IV)

In Studies I–IV, samples for the SEM were examined using scanning electron microscope (SEM, model 5500, JEOL Ltd. Tokia, Japan). In Study I, one test specimen per group was examined to find signs of IPN formation between RRP fillers and denture base polymer matrix. In Study II, the bonding surfaces after the surface treatments were evaluated to find out whether surface treatments altered the bonding surface of the RRP substrate. One specimen from Groups 2, 3, 9, 10, 11, 13 and 16 was examined. Additionally, one specimen from Group 6 was examined to find signs of IPN at the interface of the RRP substrate and resin adherent. In Studies III-IV, one to three samples were examined from Groups 2, 3 and 9, both after 48 hours' and 12 months' water storing, to evaluate the structure of the interface between resin and dentin.

4.2.9 Statistical methods (I-IV)

In all studies, data were statistically analyzed with analysis in the first instance of variance (ANOVA) with statistical software SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA).

In Study I, two-way ANOVA was used, and in Study II, one-way ANOVA was used. Tukey post hoc test was used to determine the differences between the groups ($p < 0.001$ in study I, $p < 0.05$ in study II). In Study I, the factors of quantity of RRP and storage condition were used as independent variables and flexural strength, flexural modulus, surface microhardness, water sorption and solubility as dependent variables. In Study II, the factor of surface treatment was used as independent variable and shear bond strength was used as dependent variable.

In Studies III and IV, both one-way ANOVA and three-way ANOVA were used. In one-way ANOVA, the factor of primer type was used as independent variable and the factor of microtensile bond strength was used as dependent variable. Monomer type, solvent concentration and existence of RRP in primer were used as a fixed factor in three-way ANOVA to evaluate the effect on microtensile bond strength. Tukey post hoc test was used to determine the differences between the groups ($p < 0.05$).

Data from mechanical tests (flexural strength, flexural modulus, surface hardness, shear bond strength, microtensile bond strength) were analyzed with Kolmogorov-Smirnov test ($p < 0.05$) to evaluate normal distribution of data.

5. RESULTS

5.1 Mechanical properties of denture base polymer-RRP material combination (I)

The results from the three-point bending test (mean flexural strength and flexural modulus for dry and water stored specimens) are shown in Table 6. ANOVA revealed that quantity of RRP significantly affected ($p < 0.001$) the flexural strength and the flexural modulus. Kolmogorov-Smirnov test revealed that only flexural modulus data had normal distribution ($p < 0.05$).

Table 6. Mean flexural strength, flexural modulus, surface microhardness, water-sorption and solubility of dry and wet groups.

| | 100%-RRP Group 1 | 0%-RRP Group 2 | 10%-RRP Group 3 | 20%-RRP Group 4 | 30%-RRP Group 5 |
|------------------------------------|-----------------------------|---------------------------|----------------------------|----------------------------|----------------------------|
| Flexural strength (MPa) dry | 305.0 (4.4) ^a | 93.9 (3.5) ^b | 73.1 (4.4) ^c | 64.3 (9.5) ^c | 67.4 (5.6) ^c |
| Flexural strength (MPa) wet | 293.5 (9.4) ^a | 93.5 (9.5) ^b | 62.9 (9.4) ^c | 65.6 (5.6) ^c | 59.2 (6.7) ^c |
| Modulus (GPa) dry | 7.5 (0.2) ^a | 3.3 (0.1) ^b | 3.9 (0.3) ^b | 5.1 (0.7) ^c | 6.9 (0.5) ^a |
| Modulus (GPa) wet | 7.4 (0.2) ^a | 3.6 (0.4) ^b | 3.8 (0.6) ^{bc} | 4.7 (0.6) ^d | 4.6 (0.3) ^{cd} |
| Surface microhardness dry (VHN) | 56 (3.2) ^a | 22.0 (1.0) ^b | 23 (1.7) ^b | 28 (2.2) ^c | 26 (2.4) ^d |
| Surface microhardness wet (VHN) | 52 (1.0) ^a | 23.0 (1.9) ^b | 23 (1.3) ^b | 25 (2.2) ^c | 25 (1.5) ^c |
| Water sorption (wt%) | 0.94 (0.09) ^a | 2.30 (0.03) ^b | 2.22 (0.04) ^b | 2.08 (0.01) ^c | 2.00 (0.01) ^c |
| Solubility (wt%) | 0.08(0.06) ^a | 0.68(0.04) ^b | 0.54(0.14) ^b | 0.60(0.04) ^b | 0.60(0.01) ^b |

ANOVA was performed. Superscript letter indicates homogenous subsets by each row (Tukey). Values in parentheses represent SDs.

Group 1 with 100 % RRP revealed the highest flexural strength (dry: 305.0 ± 4.4 MPa water-stored: 293.5 ± 9.4 MPa) and the flexural modulus (dry: 7.5 ± 0.2 GPa (dry) water-stored: 7.4 ± 0.2 GPa). In Group 2, the control test specimens had the flexural strength of 93.9 ± 3.5 MPa and the flexural modulus of 3.3 ± 0.1 GPa. Addition of RRP-fillers to the denture base polymer decreased the flexural strength and increased the flexural modulus compared to unreinforced test specimens. The flexural strength and the flexural modulus had tendency to decrease slightly in the water stored specimens compared with those stored in dry, except in Group 4 for flexural strength and for Group 2 for flexural modulus. Flexural strength and flexural modulus are shown in Figure 14 a–b. Typical deflection curves of dry specimens can be seen in Figure 15.

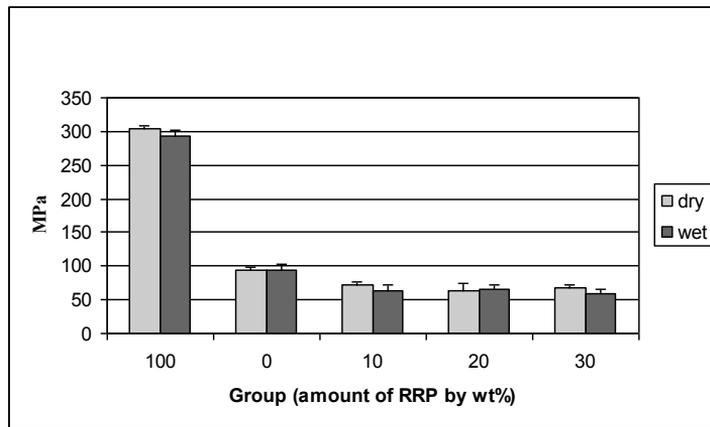


Figure 14 a. Flexural strength of test specimens in various groups. (n=6) Vertical lines represent SDs.

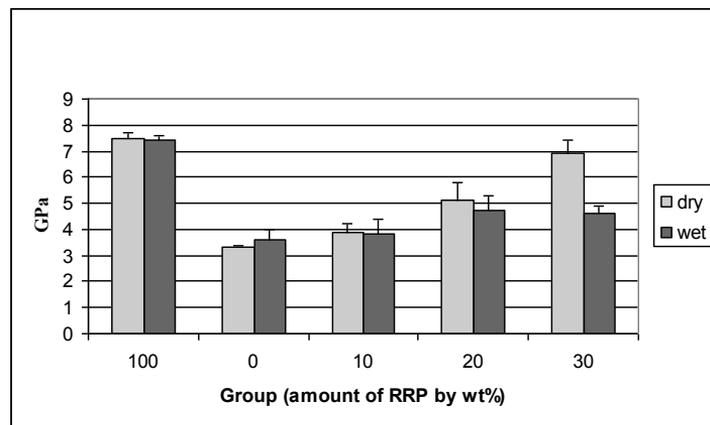


Figure 14 b. Flexural modulus of test specimens in various groups. (n=6) Vertical lines represent SDs.

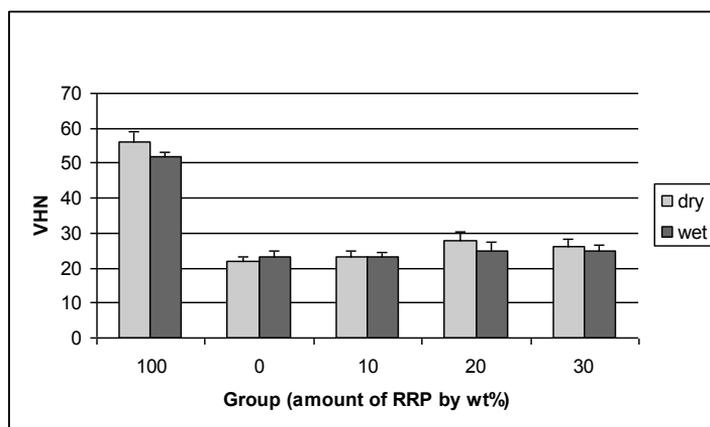


Figure 14 c. Surface microhardness (VHN) of test specimens in various groups. Vertical lines represent SDs.

Summary of microhardness values in VHN are presented in Table 6 and Figure 14 c. ANOVA revealed significant differences between the groups ($p < 0.001$). Vickers hardness values were not normally distributed according to Kolmogorov-Smirnov test ($p < 0.05$). Specimens in Group 1 were approximately two times harder than specimens made fully of denture base polymer (Group 2). Water storage had tendency to decrease the microhardness slightly except in Groups 2 and 3.

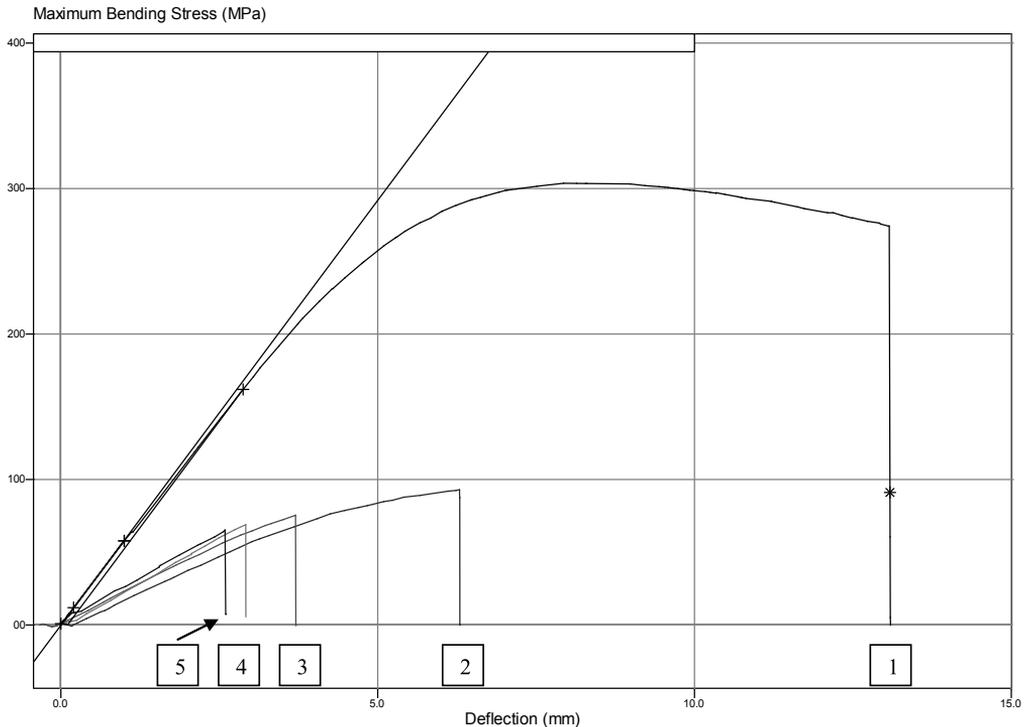


Figure 15. Typical load-deflection curves for materials tested. Numbers refer to test groups (see Table 2). + marks the part of load-deflection curve where elastic deformation takes place. * marks breaking of the specimen.

5.2 Solubility and water sorption of denture base polymer-RRP material combination (I)

RRP fillers decreased the water sorption of the denture base polymer. Water-sorption of the specimens in Group 2 was 2.30 wt% (Table 6), whereas the specimens in Group 1 presented sorption of 0.94 wt%. Significant differences were found between the groups in ANOVA ($p < 0.001$) in water-sorption. Water-immersion, which was carried until plateau (saturation), was achieved in sorption curves (1–44 days), which is shown in Figure 16. Solubility was not influenced by incorporation of RRP fillers. However, specimens made fully of RRP revealed lower solubility than other specimens of the groups. Mean solubility values are presented in Table 6.

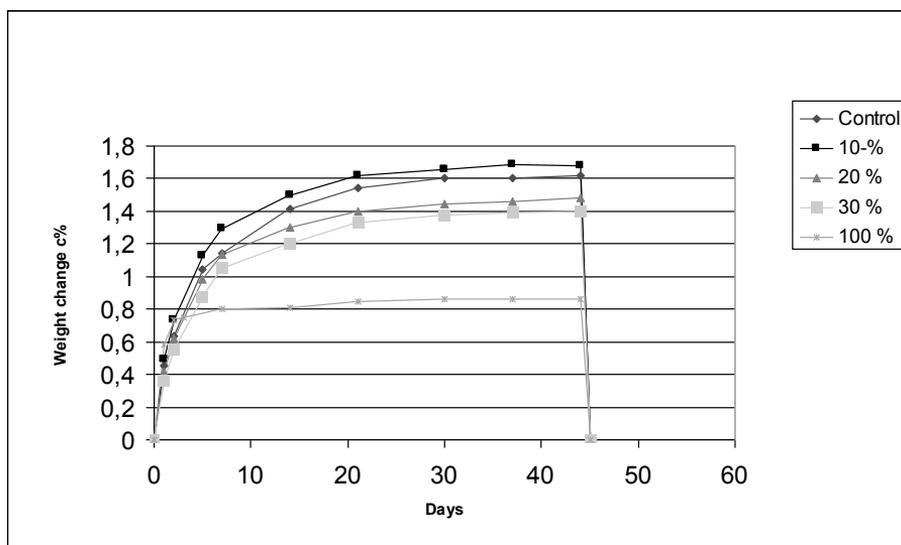


Figure 16. Weight change (%) plotted against storage of time in water with various quantities of RRP fillers in denture base polymer.

5.3 Adhesion of RRP to denture base polymer (I)

SEM-micrographs revealed signs of differently cross-linked polymer layers around PMMA beads suggesting existence of IPN-layer which was not seen between RRP fillers and denture base polymer matrix (Fig. 17).

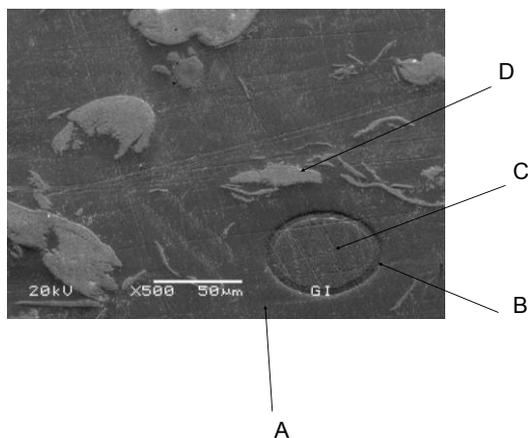


Figure 17. SEM-micrograph of surface of test material showing A: polymer matrix, B: IPN layer, C: PMMA bead and D: RRP filler. Orig. magnification x 500, bar 50 μm, tilting angulation: 45°.

5.4 Adhesion of BisGMA-TEGDMA-resin to substrate of RRP (II)

The results of the shear bond strength test are illustrated in Table 7 and in Figure 18. The highest shear bond strength values were found in Group 6. This was the group

in which the surface of the RRP substrate was treated with BisGMA-TEGDMA-resin for 15 minutes. Heat treatment of the RRP substrate and the use of RRP/BisGMA-TEGDMA-resin/DCM-primer on the RRP substrate tend to increase bond strengths. The predominant failure type in all groups was adhesive failure. Kolmogorov-Smirnov test revealed shear bond strength values not to be normally distributed ($p < 0.05$).

Table 7. Shear bond strength test results in Study II.

| Time (s) | Shear bond strength BisGMA-TEGDMA-resin dissolving (MPa) (groups 1-7) | Shear bond strength Heat treatment*** (MPa) (groups 8-10) | Shear bond strength Solvent DCM dissolving (MPa) (group 11) | Shear bond strength PMMA/DCM-primer* (MPa) (groups 12-14) | Shear bond strength RRP/BisGMA-TEGDMA-resin/DCM-primer** (MPa) (groups 15-17) |
|----------|---|---|---|---|---|
| 10 | 1 0 | 8 0 | - | 12 2.5 (0.6) ^a | 15 18.5 (3.7) ^e |
| 60 | 2 6.3 (2.1) ^{abc} | 9 11.8 (3.7) ^{cd} | - | 13 3.4 (2.3) ^a | 16 20.1 (4.7) ^e |
| 180 | 3 5.1 (1.6) ^{ab} | 10 10.6 (2.4) ^{bcd} | 11 3.4 (1.0) ^a | 14 4.7 (2.4) ^{ab} | 17 10.9 (4.0) ^{bcd} |
| 300 | 4 7.1 (1.7) ^{abc} | - | - | - | - |
| 600 | 5 6.6 (3.3) ^{abc} | - | - | - | - |
| 900 | 6 20.9 (5.5) ^e | - | - | - | - |
| 1800 | 7 15.8 (7.6) ^{de} | - | - | - | - |

DCM=dichloromethane, PMMA=poly(methylmethacrylate), BisGMA= bisphenol A glycidyl methacrylate, TEGDMA=triethyleneglycol-dimethacrylate, RRP= rigid rod polymer Parmax 1240®. Superscript letters indicate the homogenous subsets in one-way ANOVA (Tukey). Values in parentheses represent SDs.

* The amount of PMMA dissolved into DCM was 9.1 wt%.

** The amount of RRP dissolved into DCM was 0.96 wt%. The amount of BisGMA-TEGDMA-resin dissolved into DCM was 0.96 wt%.

*** Temperature around 140 °C.

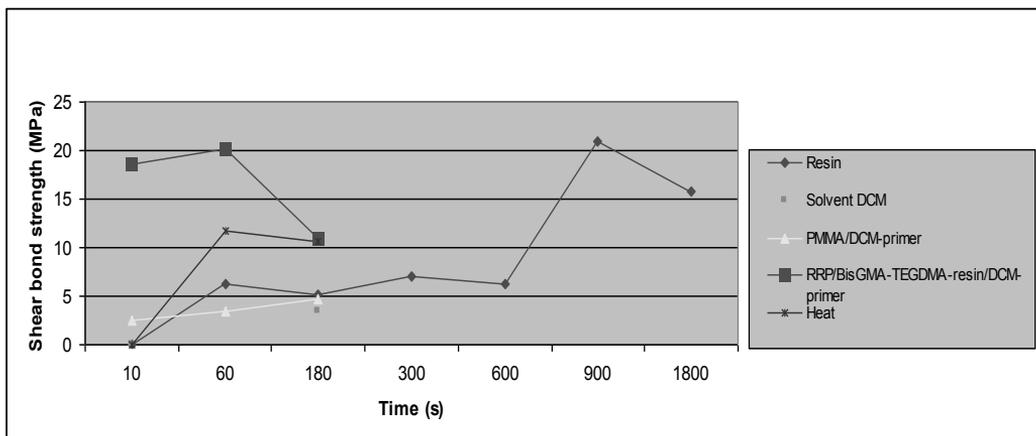
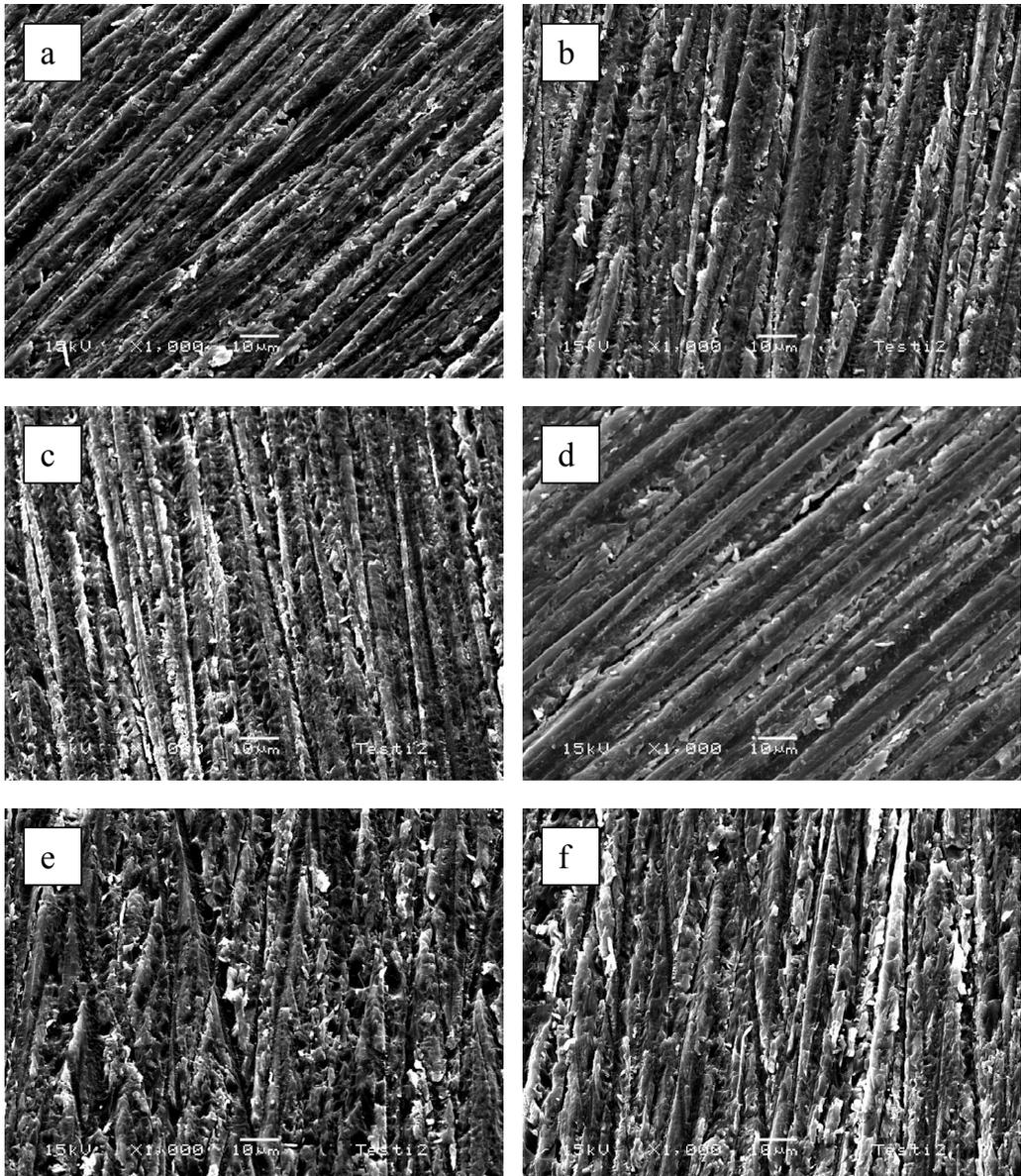
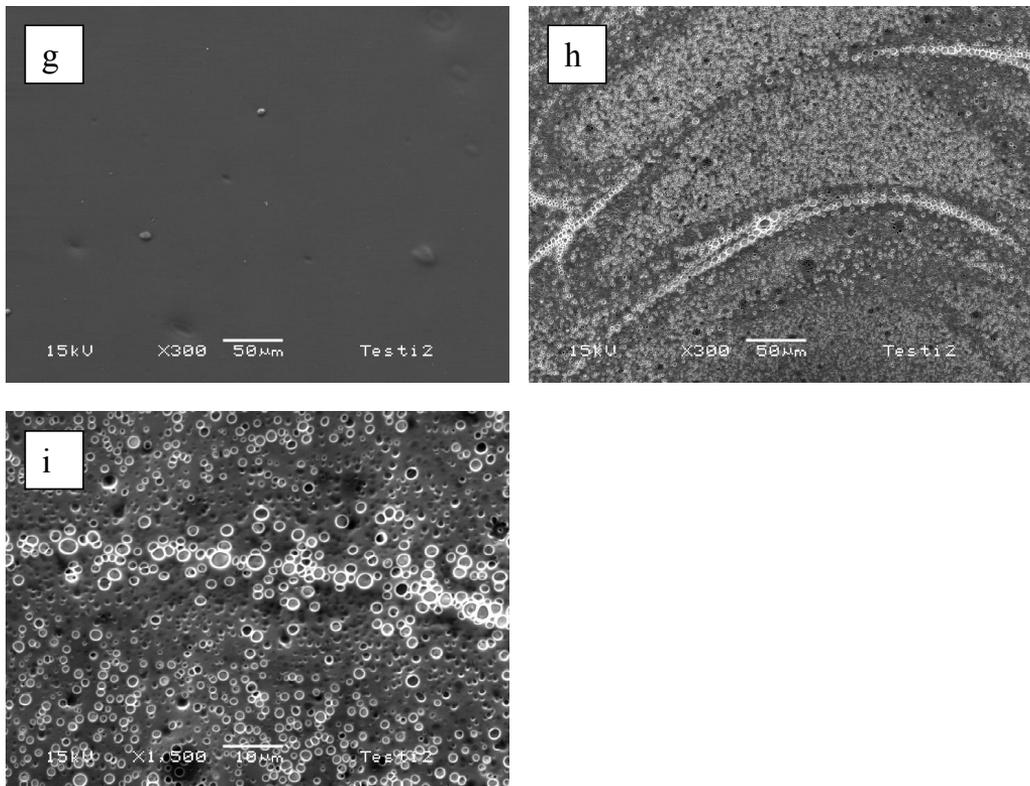


Figure 18. Shear bond strength of BisGMA-TEGDMA to RRP substrate as plotted against the background variables from Study II.

SEM micrographs of the RRP substrates after being treated with the methods described in Table 3 (Groups 2, 3, 9, 10, 11, 13 and 16) or without any treatment are illustrated in Figures 19 a–i. Scanning electron micrographs which shows the bonding surface topography after bonding procedures revealed that resin, solvent DCM or heat treatment had no effect on the topography of ground surface of the RRP substrate (Figures 19 a–f). PMMA/DCM-primer created a smooth surface with holes (Figure 19 g). RRP/BisGMA-TEGDMA-resin/DCM-primer created a surface with number of small holes (Figures 19 h–i). SEM micrographs taken from the interface of RRP and resin (Group 6) showed no signs of dissolution (Figure 20).





Figures 19 a) Ground surface of the RRP substrate, no surface treatments, **b)** Surface of the RRP substrate in Group 2 after surface treatment and acetone cleaning, **c)** Surface of the RRP substrate in Group 3 after surface treatment and acetone cleaning, **d)** Surface of the RRP substrate in Group 11 after surface treatment and acetone cleaning, **e)** Surface of the RRP substrate in Group 9 after surface treatment and acetone cleaning, **f)** Surface of the RRP substrate in Group 10 after surface treatment and acetone cleaning, **g)** Surface of the RRP substrate in Group 13 after surface treatment and acetone cleaning, **h)** and **i)** Surface of the RRP substrate in Group 16 after surface treatment and acetone cleaning.

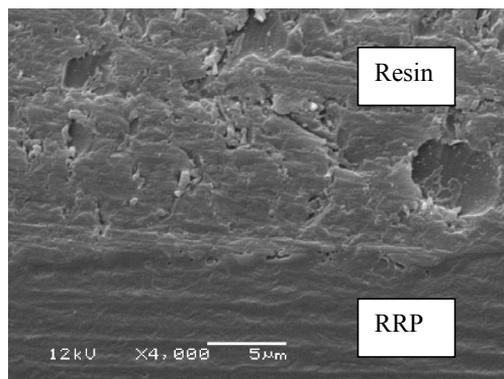
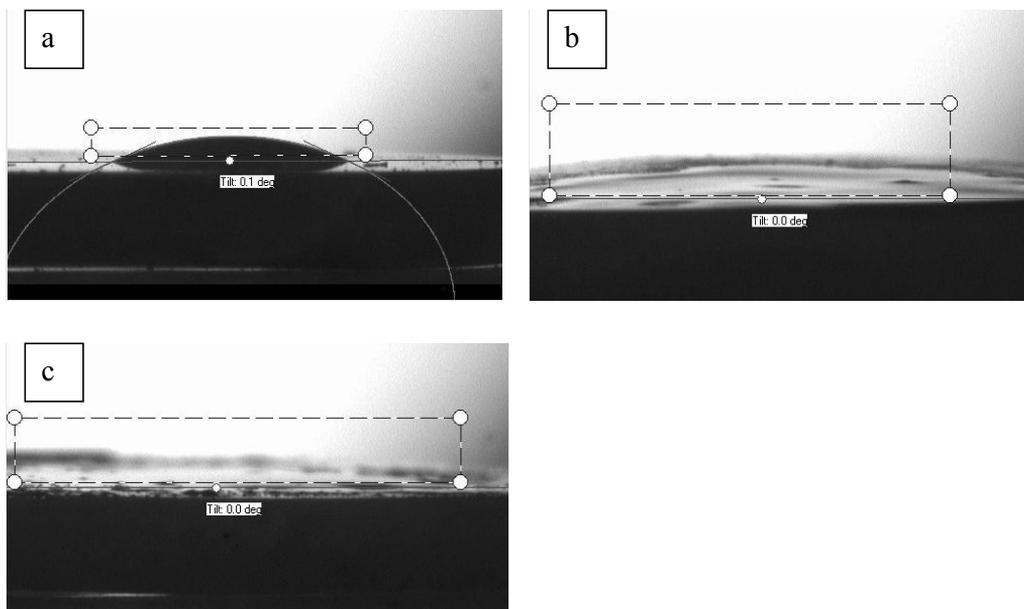


Figure 20. Cross section of the interface of the RRP substrate and resin in Group 6 after DCM treatment.

5.5 Properties of experimental dentin primer (III, IV)

The contact angle measurements showed that the contact angle of the adhesive resin applied onto the dentin without any conditioning was $18 \pm 7.9^\circ$. By conditioning the dentin surface with the control primer or BMEP based experimental primer (Group 3), better wetting of dentin was achieved (contact angle 0°). With the EGMP based experimental primer (Group 6) the average contact angle of resin was $5 \pm 11.5^\circ$. (Figures 21 a–c)

Mean microtensile bond strengths in all groups are presented in Table 8 and in Figure 22. Three-way ANOVA revealed that all factors; monomer type, solvent-monomer ratio and RRP had a significant effect ($p < 0.05$) on the bond strength in 48 hours' water storing. Primer with combination of MPS and dimethacrylate BMEP (Groups 1–4) revealed higher bond values than mixture of MPS and monomethacrylate EGMP (Groups 5–8). Increase of volumetric solvent-monomer ratio from 1:1 (Groups 1, 2, 5) to 5:1 (Groups 3, 4, 6–8) produced also higher μ TBS values. Addition of RRP into primers increased μ TBS in both MPS-BMEP and MPS-EGMP monomer combinations. Three-way ANOVA ($p < 0.05$) revealed this to be also statistically significant. In the EGMP groups the dichloromethane solvent treatment, before applying the primer, increased the bond strength (Group 8). However, in the BMEP groups the solvent treatment had no effect on bond strength (Group 4).



Figures 21 a-c. Contact angle measurement of dentin substrate. Examples of images of three groups. Group without any conditioning before applying resin (a), control group (b), BMEP group 3 (c).

Water storing of the test specimens decreased μ TBS in the high solvent-monomer ratio Group 3 (ANOVA $p < 0.05$). In the experimental Group 2 and in the control group, the water storing did not have statistically significant effect on the bond strength. In the

experimental Group 1, the 6 months' water storage increased the bond strength (ANOVA $p < 0.05$) but the result from the 12 months' water storage did not differ from that of 48 hours'. Microtensile bond strength data was not normally distributed according to Kolmogorov-Smirnov test ($p < 0.05$).

Table 8. Mean microtensile bond strengths of test specimens of the experimental primer groups and control group.

| Group | Bond strength 48 hours (MPa) | Bond strength 6 months (MPa) | Bond strength 12 months (MPa) |
|-------|------------------------------|------------------------------|-------------------------------|
| 1 | 18 (6.7) ^{abA} | 24 (11.4) ^B | 23 (11.4) ^{AB} |
| 2 | 21 (7.2) ^{bA} | 22 (5.7) ^A | 22 (7.2) ^A |
| 3 | 31 (9.4) ^{dA} | 24 (9.2) ^B | 23 (6.6) ^B |
| 4 | 30 (9.7) ^{cd} | - | - |

| Group | Bond strength 48 hours (MPa) | Bond strength 6 months (MPa) | Bond strength 12 months (MPa) |
|-------|------------------------------|------------------------------|-------------------------------|
| 5 | 14 (4.3) ^a | - | - |
| 6 | 22 (7.1) ^b | - | - |
| 7 | 20 (7.7) ^b | - | - |
| 8 | 26 (6.9) ^c | - | - |
| 9 | 32 (11.3) ^{dA} | 32 (11.8) ^A | 31 (9.8) ^A |

ANOVA was performed ($p < 0.05$). Superscript letter indicates homogenous subsets (Tukey) ($p < 0.05$). Small letters indicate homogenous subsets by the column 48 hours and capital letters indicate homogenous subsets by each row. Values in parentheses represent SDs.

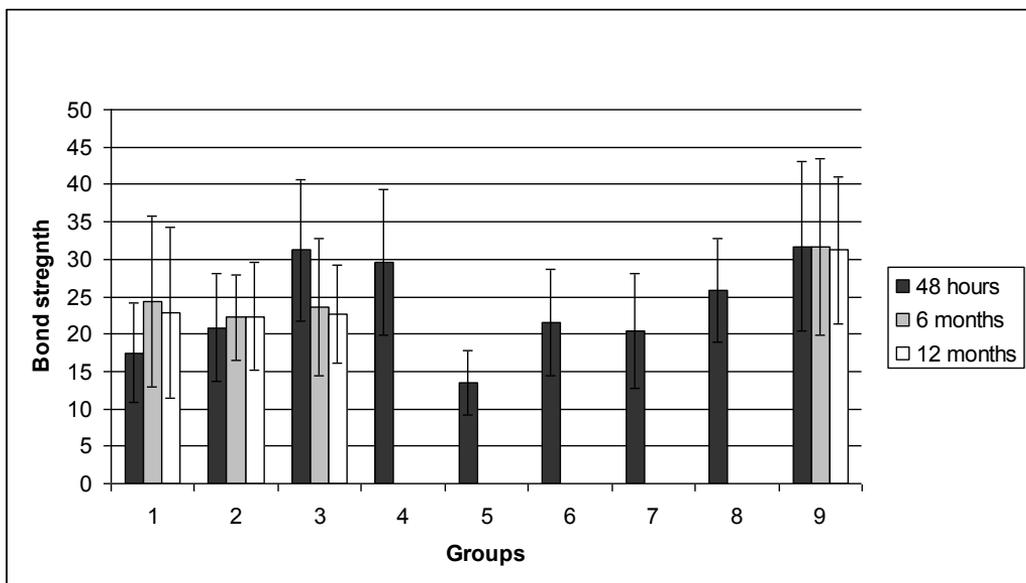


Figure 22. Bond strength values of the test specimens of the microtensile test groups in Studies III and IV. Vertical lines represent SDs.

The results of the fracture surface analysis are seen in the Table 9. The primary type of failure in all groups was a mixed mode of failure. In Groups 1–3 and 9, the amount of adhesive failures tended to increase by the water storing and the amount of cohesive failures tended to decrease. In 48 hours' groups, in Groups 3 and 9, there were more cohesive failures than in other groups.

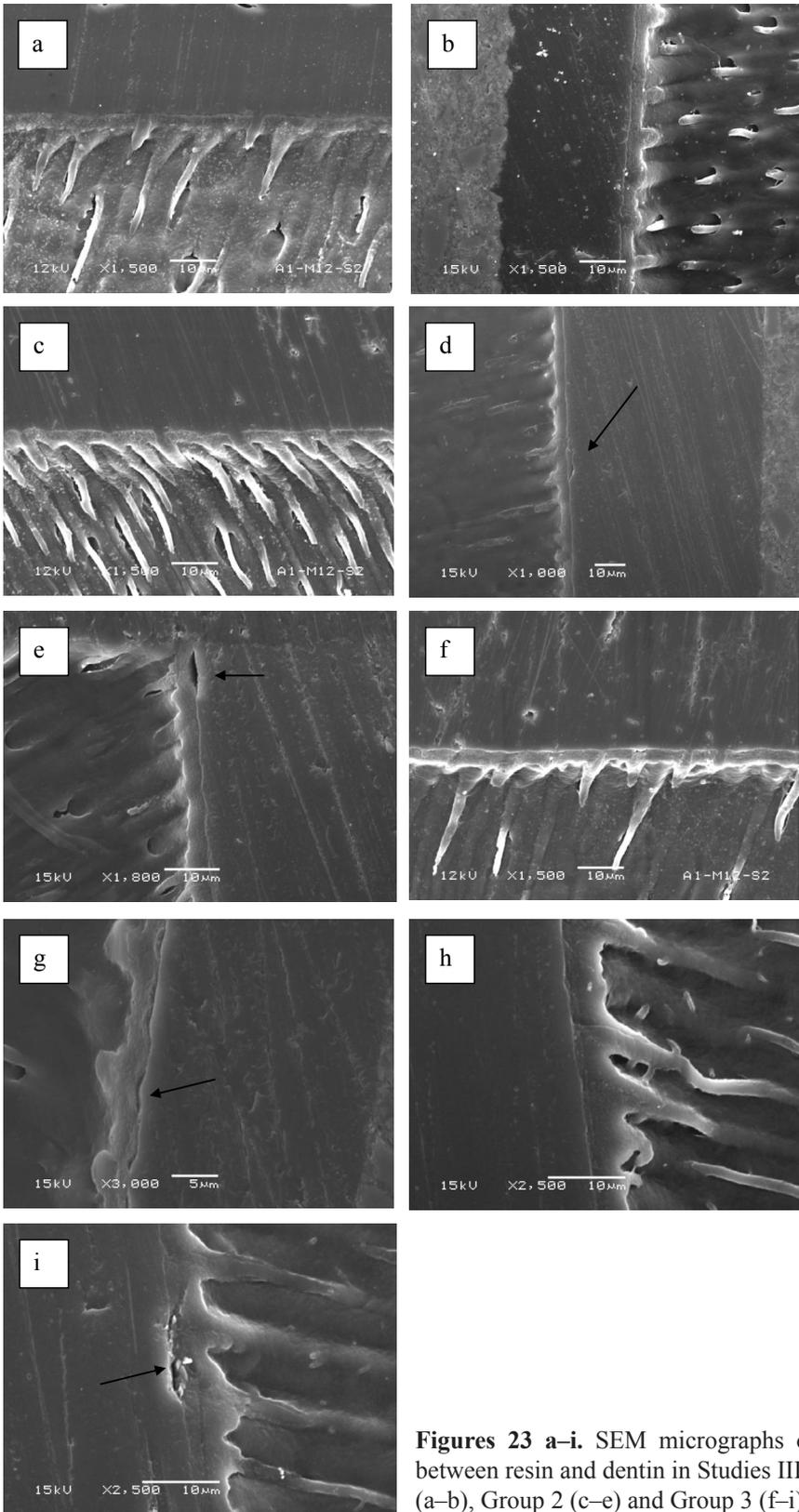
Table 9. Failure modes of the test groups.

| Group | 48 hours | 6 months | 12 months |
|-------|-------------------------------------|------------------------------|-----------------------------|
| 1 | A 88 %, C 4 %, D 1 %, E 6 % | A 97 %, D 3 % | A 78 %, C 5 %, D15 %, E 3 % |
| 2 | A 90 %, C 4 %, D 3 %, E 3 % | A 93 %, D 7 % | A 81 %, D 19 % |
| 3 | A 84 %, B 2 %, C 10 %, D 2 %, E 2 % | A 76 %, B 2 %, D 19 %, E 2 % | A 81 %, B 3 %, D11 %, E 6 % |
| 4 | A 92%, B 4%, C 1%, E 3% | - | - |
| 5 | A 89%, C 2%, D 7%, E 2% | - | - |
| 6 | A 78%, C 4%, D 15%, E 4% | - | - |
| 7 | A 89%, D 6%, E 5% | - | - |
| 8 | A 83%, B 1%, C 2%, D 5%, E 9% | - | - |
| 9 | A 87 %, C 12 %, E 1 % | A 94 %, C 3 %, D 3 % | A 80 %, C 5 %, D 15 % |

A = mixed mode failure, B = cohesive in dentin, C = cohesive in composite, D = adhesive between adhesive resin and dentin, E = adhesive between adhesive resin and composite.

5.6 SEM analysis of interface between resin and dentin (III, IV)

Scanning electron micrographs from Studies III and IV are seen in Figures 23 a–i. Interfaces between resin and dentin were homogenous in the control group both after 48 hours' (23 a) and 12 months' water storing (23 b) and contained no voids or cracks. Figure 23 c is from the experimental Group 2 (solvent-monomer ratio 1:1) after 48 hours' water storing. Interface between resin and dentin was homogenous. Figures 23 d–e are from the experimental Group 2 after 12 months' water storing. Some crack formation and voids were found (arrows) at the interface of resin and dentin. Figures 23 f–g are from the experimental Group 3 (solvent-monomer ratio 5:1) after 48 hours' water storing. Many voids (arrows) at the interface of resin and dentin could be seen (Figure 23 g) but also areas of homogenous interface could be found (Figure 23 f). Figures 23 h and 23 i are from the experimental Group 3 after 12 months' water storing.



Figures 23 a-i. SEM micrographs of the interfaces between resin and dentin in Studies III and IV. Group 9 (a-b), Group 2 (c-e) and Group 3 (f-i).

6. DISCUSSION

6.1 General discussion

The aim of this series of studies was two-folded. Firstly this series of *in vitro* studies aimed to evaluate whether poly(paraphenylene) based RRP as a new material in dentistry has potential to be used as an organic filler in dental resins. It was hypothesized that because of good mechanical properties poly(paraphenylene) based RRP could be used, for instance, as a filler material. To be used in clinical applications, an important feature in a material is good adhesion to other dental materials and to tooth substrate (Marshall et al. 2010). Good adhesion will help to preserve tooth structure, optimize attachment at the dentin or restoration and prevent microleakage. Therefore, the adhesive properties of RRP were evaluated in these studies (Studies I and II).

The second aim of these studies was to evaluate the usability of a new dentin primer system (Studies III and IV). An important feature of dentin bonding agents is the capability to preserve bond strength in long term (Abdalla and Feilzer 2008, De Munck et al. 2003, Osorio et al. 2008). Because of this the long term bond strength of experimental dentin bonding system was tested. The essential property, when talking about the long term bond strength, is the quality of formed interface between resin and dentin (Abdalla and Feilzer 2008, Breschi et al. 2008, De Munck et al. 2003). Also the interfaces between resin and dentin of the tested bonding agents were therefore analyzed.

The laboratory works for these studies were performed in the University of Turku, Institute of Dentistry, Biomaterials research laboratory and in Turku Clinical Biomaterials Centre laboratory. These laboratories are under quality assurance system. To standardize the methods and minimize possible researcher-related variability, the same person fabricated all test specimens and performed the testing with two exceptions. In Study I, the test specimens which were fully of RRP were fabricated by the manufacturer of RRP. In Study IV, another person worked as an assistant when preparing samples for SEM. Another person performed dehydrating, epoxy casting and toluidin dyeing for these SEM specimens.

As a statistical analysis, ANOVA (analysis of variance) was extensively used in these studies. ANOVA is a parametric test method and for the use of this test, the data has some prerequisites. Firstly, the data should be normally distributed, and secondly, variances and standard deviations in different groups should be close to each other. Kolmogorov-Smirnov test was used in present studies to evaluate the normality of data from mechanical test. Kolmogorov-Smirnov test revealed that only flexural modulus data was perfectly normally distributed. Therefore, one should be careful not to overestimate the results

from mechanical studies. However, the requirement for normality is quite strong demand and is rarely perfectly valid.

6.2 Denture base polymer-RRP material combination studies (I)

To test the mechanical properties of denture base polymer-RRP material combination, a three-point bending test and a surface hardness test were chosen as test methods. A three-point bending test according to the International Standardization Organization 1999 (ISO 1567) was used to test the flexural strength and modulus of the test specimens. These test methods were chosen since they provide simple methods to test the basic mechanical properties of materials. However, the three-point bending test has some concluded limitations. Bending induces a stress gradient in the test specimen and a relatively small part of specimen is exposed to high tensile stress (Ritter 1995). Additionally, specimens are sensitive to edge and surface machining processes, which leads to presence of processing flaws facilitating crack initiation (Pick et al. 2010, Ritter 1995, Rodrigues Junior et al. 2008). Bi-axial flexure strength testing has been recommended for dental materials testing to decrease variability in strength data (Ban and Anusavice 1990, Palin et al. 2003). One advantage of a bi-axial test method over a uni-axial three-point bending test is that clinically relevant specimen geometry allows for complete and controlled curing of each disc shaped specimen when light curing method is used. Bar-shaped specimens in a three-point bending test require an overlapped light activation procedure when using diameter shaped light guide (Palin et al. 2003, Rodrigues Junior et al. 2008). In present study, this was not a problem since the denture base polymer specimens were cured in a pressure-curing device with the help of heat. For the surface hardness test, Vickers surface hardness scale was chosen (Darvell 2002). The limitations of Vickers surface hardness testing are related to the microscopic measurement of hardness indentations. These microscopic measurements can be affected by the operator related variation, resolution of the optical system and also by elastic recovery of the material (Shahdad et al. 2007, Wassell et al. 1992). During loading in indentation test, the visco-elastic deformation leads to unknown increase in the depth of indentation and to unknown rate of recovery of the indentation after the indenter is removed leading to uncertainty of indentation size measurement (Shahdad et al. 2007, Wassell et al. 1992). The effect of surface roughness on indentation measurements has been studied and has been presented to be one of the variables which could influence interpretation of indentation results especially at low penetration depth (Chung and Yap 2005). In present study, the surface of test samples was polished with 4000 grit silicon carbide grinding paper to eliminate excessive surface roughness. We also used a relatively high load during indentation to create a deep enough indentation.

To calculate the water sorption and solubility for denture base polymer-RRP material combination, the same test specimens as for mechanical testing were used. The weight changes of the test specimens during the water storing were measured. Weight changes were compared to original dry weights of the test specimens and percentage weight changes were calculated. After this, the test specimens were tested until fracture in three-point bending test and surface microhardness test was performed. Then, fractured specimens were dehydrated in an oven to calculate water sorption and solubility values. Water sorption was calculated by comparing hydrated mass to dehydrated mass. Solubility was calculated by comparing the weight change of untested specimens at 44 days time-point to calculated sorption values after dehydration in oven. These calculation methods are not strictly under the specification of International Standards Organization (ISO 1567). According to ISO standard, sorption and solubility should be calculated per unit volume cubic millimetre instead of per unit mass. However, it was evaluated that the used calculation methods offered basic information about the sorption and solubility properties of tested materials. Because of the used calculation methods, the sorption and solubility results of this study are not directly comparable to other studies.

The manufacturer of RRP Parmax 1240® (presently called PrimoSpire 120) has reported flexural strength of 310 MPa and flexural modulus of 8.3 GPa for Parmax 1240® (PrimoSpire technical data sheet). The results from the present study are in accordance to these reported values. Previous study made by Morgan et al. has concluded the surface hardness of Parmax 1240® to be two times as high as traditional engineering thermoplastics (Morgan et al. 2006). The Vickers surface hardness values for Parmax 1240® from present study showed similar kind of result. Addition of Parmax 1240® fillers increased the flexural modulus and surface microhardness but decreased the flexural strength compared to the unfilled denture base polymer. No previous studies about the effect of the Parmax 1240®-filler on mechanical properties of denture base polymer were found.

To evaluate the capability of RRP to adhere to denture base polymer, a SEM examination was performed. When PMMA is used as a denture base material, polymerized powder-like PMMA is mixed with methyl(methacrylate) liquid (MMA). MMA liquid contains a small amount of dimethacrylate as cross-linking agent. When PMMA powder and MMA monomer liquid are mixed, semi-IPN structure is formed (semi-interpenetrating polymer network) (Vallittu 2009). In Study I, SEM-micrographs revealed differently cross-linked polymer layers around PMMA beads suggesting the existence of IPN-layer which was not seen between RRP fillers and polymer matrix (Fig. 17). Formation of the IPN-layer is based on capability of the monomer of the resin matrix to dissolve the filler particles. In the case of PMMA, the solubility parameter of PMMA and monomers of the resin, predominately MMA, are close to each other and surface of the PMMA bead can be dissolved. Information about the solubility parameter of used Parmax 1240® RRP

is not available. However, Parmax 1240® is dissolvable in dichloromethane (DCM) and the solubility parameter of DCM is close to parameter of MMA. For this reason, it was expected that Parmax 1240® might have had the capability for IPN-bonding with MMA. However, in the case of RRP fillers, MMA was not able though to dissolution of the filler surface and thus no visible IPN-layer was formed.

The results from the SEM examination matched well with the results from mechanical testing. In this study, it was expected that filler particles with higher strength could have increased the strength of the denture base polymer. This would have required adequate adhesion between the reinforcement and surrounding polymer matrix. Because of the low strength values of the filled polymer, it was supposed that there has not been adequate interfacial adhesion between the RRP fillers and denture base polymer. SEM micrographs supported this assumption. This hindered load transfer from the matrix to the reinforcing fillers.

It has been studied that the formation of IPN-layer is dependent of the contact wetting time of monomer and polymer. For instance, in the denture base polymers, the degree of dissolution and swelling of PMMA beads depends on the contact wetting time with MMA in its liquid form, i.e. as a solvent capable of dissolving the PMMA beads (Vallittu et al. 1994a, Vallittu 2009). In heat-cured denture base polymers, the dough time is longer than in autopolymerizing denture base resins. Longer dough time allows PMMA beads to be dissolved almost fully by the monomers. Whereas in the autopolymerizing resins, the polymerization is started quickly and penetration of MMA to PMMA is limited to a few micrometers (Vallittu 2009). In present study, we used autopolymerizing denture base resin and the polymerization was initiated after only a couple of minutes after mixing the resin. Though MMA had only limited time to dissolve and swell the surface of fillers made of Parmax 1240®. This limited time might have hindered the adhesion between RRP filler and denture base polymer.

It has also been shown that the polymerization activation temperature affects the formation of IPN. At higher temperatures the bond strengths are increased. This is due to deeper penetration of the monomers (Vallittu 1995). In this study, we used polymerization temperature of 80°C which is higher than the typical polymerization temperature for autopolymerizing denture base polymers (45–55°C). Different temperatures might have an effect on adhesion of RRP to PMMA based denture base resin.

The rate of diffusion of monomers into polymer is related to the glass transition temperature of the polymer. The swollen secondary IPN-layer is thicker in autopolymerized denture base polymer than in heat-cured polymer, since the glass transition temperature is lower in autopolymerized denture base resin (Vallittu 2009). The glass transition temperature is dependent on the tacticity of the polymers. Polymers can be atactic, syndiotactic or isotactic structures (Figure 24). Nuclear magnetic resonance analyses indicate that the

PMMA beads with high glass transition temperatures are predominantly syndiotactic (Ruyter and Svendsen 1980). Information about the tacticity of Parmax 1240® is not available. However, it has been studied that glass transition temperature of Parmax 1000® [poly(benzoyl-1,4-phenylene)] can be varied 68 celcius degrees depending on the regiochemical placement of lateral benzoyl groups (Wangt and Quirk 1995). Atactic type of Parmax 1240® would probably offer lower glass transition temperature and easier secondary IPN-formation compared to syndiotactic Parmax 1240®. Random distribution of side chains in homopolymers and especially in copolymers will enhance solubility by lowering the symmetry of the polymer chain, thereby decreasing crystallinity and glass transition temperature (Marocco et al. 1993).

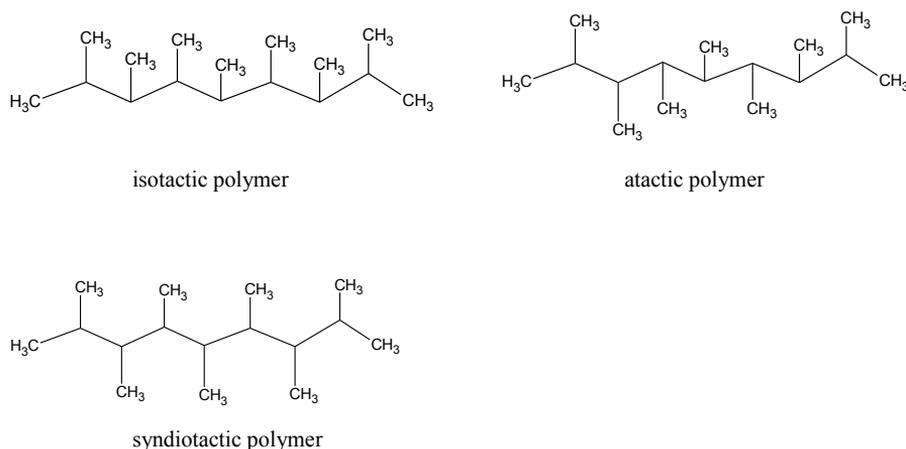


Figure 24. Different tacticities of idealized methacrylated polymers. Tacticity refers to the orientation of sidegroups of polymers. In isotactic type all the sidegroups are in the same side of the polymer chain. In atactic type the sidegroups are randomly divided to both sides of the main chain. In syndiotactic type the sidegroups are divided evenly to both sides of main chain.

In random orientated fiber reinforced composites the length and adhesion of fibers should provide load transfer from polymer matrix to the fibers (Vallittu et al. 1994b). The shortest effective fiber length is called the critical fiber length (Petersen 2005). The critical fiber length in glass fiber denture base resin composites has been concluded to be 6 mm (Karacaer et al. 2003). Deteriorated or initially poor adhesion between fibers and polymer matrix increase the critical fiber length. In this study, the size scale is different since the used fibers are molecular scale fibers. The molecular weight of Parmax 1240® polymer has been reported to be 26.900–30.000 and the length of RRP filler particles in this study measured from SEM-figures is around 50µm (Connolly et al. 1995, Ha et al. 2001). However, since the adhesion between RRP fillers and polymer matrix is not optimal, longer particle length could increase the mechanical properties of RRP-denture base polymer composite. Another thing which must be taken into consideration when talking about critical fiber length is the aspect ratio of fiber. By aspect ratio, the

relationship of length and width of fiber is meant. It has been concluded that as fibers lengthen in relation to their diameters, strengths, modulus and toughness, all of them increase (Petersen 2005).

6.3 BisGMA-TEGDMA-resin-RRP material combination studies (II)

The shear bond strength test method was selected to determine the adhesive capability of BisGMA-TEGDMA-resin to poly(paraphenylene) based RRP with different surface treatments. One limitation of shear bond strength test has concluded to be the non-uniform stress distribution at the bonded interface (Braga et al. 2010, Della Bona and van Noort 1995). It has been shown that stresses close to the loading area are much higher than the nominal shear value (Braga et al. 2010). However, it has also been shown that the choice of testing assembly has great influence on stress distribution (Braga et al. 2010). The notched rod, like the one used in the present study, has been suggested to give larger contact area between the composite and the loading device, and this leads to better stress distribution (Braga et al. 2010). It has been also shown that the elastic modulus of tested materials influence the stress concentration. Generally, a higher modulus mismatch between substrates increases the stress concentration at the interface resulting in lower bond strengths (Braga et al. 2010). Della Bona and van Noort have recommended the use of tensile test instead of shear bond strength test in testing the bond strength of resin composite to ceramic. They have concluded that in shear test, the failure mode is often cohesive in ceramic rather than adhesive interface suggesting that the bond strength exceeds the cohesive strength of the ceramic (Della Bona and van Noort 1995). In present study, however, most of the failures were detected to be adhesive in nature. The detail which we ignored in present study was the limitation of surface treatment to the actual bonding area of BisGMA-TEGDMA resin. ISO standard suggests the application of adhesive only to the real bonding area and not to the entire substrate surface. There is a consensus in current literature that the shear stress is not uniformly distributed across and not necessarily focused at the true interface when surface treatment is done to the entire substrate surface (Van Meerbeek et al. 2010). This omission was performed in present study, since by the used methods, it was impossible, for example, to limit the heat treatment to the 3.6 mm diameter bonding area. The crosshead speed used in present study was 1.0 mm/min. It has been suggested that the crosshead speed seems to have little influence on bond strength result (Braga et al. 2010). Despite of its limitations, the shear bond strength test has concluded to remain as a popular test method to screen new adhesive formulations on their bonding effectiveness (Van Meerbeek et al. 2010). Scanning electron microscope analysis was chosen as the test method to evaluate the influence of resin dissolving time, heat treatment and solvent DCM based primers on the surface of the RRP substrate.

It was assumed that interpenetrating polymer network (IPN) would be one possible adhesion mode between these two materials. IPN formation was expected to happen at the interface of the RRP substrate and used resin. IPN formation would require resin to dissolve the surface of RRP substrate. Poly(paraphenylene) based RRP is a linear polymer and BisGMA-TEGDMA-resin is a cross-linked polymer, and because of this reason, a semi-IPN was assumed to be formed. Resin was led to dissolve/swollen into ready-polymerized RRP substrate and this is why sequential IPN was predicted to form (Sperling and Mishra 1995). The results of this study showed that if resin was led to dissolve on the surface of the RRP substrate long enough adhesion between resin and the RRP substrate was increased. However, SEM micrographs exposed no dissolved layer between RRP and resin. For this reason, it is probable that prolonged time only enables resin to penetrate to the irregularities of the ground surface of RRP and no true IPN formation exists. In general, it has been shown that there is a linear relationship between bond strength and time of action of solvent on the substrate (Vallittu et al. 1994a).

Different kinds of surface treatments were tested in this study to increase the bond strength between the RRP substrate and resin and to modify the substrate surface. The surface of RRP was treated by increased temperature or with dichloromethane based primers. Heat treatment was tested since it has been previously concluded that the polymerization activation temperature affects the bonding of the denture base polymer to denture teeth (Vallittu 2009). Adhesion of denture base polymer to denture teeth is based on IPN formation. At higher temperatures, the bond strengths are increased regardless of the type of denture base polymer (Vallittu 2009). Vallittu et al. have demonstrated that non-linear relationship occurs between temperature increase of dissolving agent and substrate, and bond strength (Vallittu and Ruyter 1997). Similar kind of result was found in present study. 60-second and 180-second heat treatment on the surface of RRP increased bond strength. 10-second heat treatment was not effective.

Three dichloromethane based experimental primers were tested. Primers were expected to lower contact angle of resin on the RRP substrate surface, increase the surface area or offer the possibility for IPN bonding. The first primer contained only DCM, the second contained PMMA dissolved into DCM and the third one contained RRP and BisGMA-TEGDMA-resin dissolved into DCM. The first primer did not increase bond strength and SEM evaluation also revealed that this primer did not have any effect on the ground surface of the RRP substrate. After DCM solvent treatment, the surface of the RRP substrate had similar kind of ground appearance as after no treatment. However, SEM evaluation presented that primers which contained additives (PMMA or RRP/resin) modified the surface of the RRP substrate. After PMMA/DCM-primer, the surface of the RRP substrate had isolated holes, and after RRP/resin/DCM-primer, the surface of the RRP substrate had a number of small holes. PMMA/DCM-primer did not have an effect on bond strength but RRP/resin/DCM-primer increased bond strength. We hypothesize

that the reason for good bond strength result with RRP/resin/DCM-primer are small blister like structures consisted on the surface of the RRP substrate. We hypothesize that this new kind of surface serves large surface area for BisGMA-TEGDMA-resin to penetrate and adhere.

The reason for why RRP/resin/DCM-primer modifies the surface of the RRP substrate is not known and requires further studies. We believe that one hypothesis behind it could be spot-like dissolving of the surface of the RRP substrate. This means that diffusion of DCM solvent into the polymer matrix is easier at the interface of RRP-filler and the RRP substrate. That is to say, RRP fillers act like dust particles and bind solvent to themselves. This leads to the formation of porous surface of RRP. Similar kind of interfacial porosity formation (IPF) has been previously shown in fabrication of fiber reinforced composites. Mattila et al. demonstrated in their study that the exposure of the reinforcing fibres played a significant role in porosity formation (Mattila et al. 2004). SEM micrographs revealed that also PMMA/DCM-primer altered the surface of the RRP substrate and created isolated holes. It can be hypothesized that also PMMA/DCM-primer produces some interfacial porosity formation. Molecular weight (M_w) of used PMMA was 350.000 and M_w of RRP (Parmax 1240®) has been reported to be 26.900–30.000 (Connolly et al. 1995, Ha et al. 2001). It can be feasible that the large difference between M_w induced differences in surface reactions.

6.4 Properties of experimental dentin primer (III, IV)

The aim of the present dentin primer studies was to test a new experimental primer constituent in a current adhesive system. Currently both hydrophilic and hydrophobic monomers are used in dentin adhesive systems. Hydrophilic monomers are claimed to increase water sorption and decrease bond strength especially in one-step self-etch adhesives (Cadenaro et al. 2009a, Carrilho et al. 2005, Carrilho et al. 2008, Carvalho et al. 2003, De Munck et al. 2005, Eliades et al. 2001, Ikeda et al. 2008). Researchers and clinicians have raised many concerns regarding long-term bonding effectiveness of hydrophilic bonding agents (De Munck et al. 2005). For these studies BMEP and EGMP were chosen due to their hydrophilicity. They were hypothesized to penetrate well into acid etched moist dentin. It was also hypothesized that with the help of MPS silane a nanoscale inorganic phase with Si-O-P reaction and nanoscale organic phase would be formed by acrylate polymer backbone. It was hypothesized that by these reactions the hydrophilic monomers would turn more hydrophobic and this way the effect of water would not destroy resin-dentin interface. MPS was used in its unhydrolyzed form. Hydrolysis of MPS was expected to happen rapidly, when the acidic phosphate monomer and residual water at dentin interface come in contact with MPS. This primer design ensures that MPS does not start polycondensation intrinsically but polymerises

at the interface (Matinlinna et al. 2004). In the presence of residual water the BMEP and EGMP might also dissociate into ionized forms and act as self-etching monomers.

Rigid rod polymer Parmax 1240® was tested as a new kind of filler in an adhesive system. Dichloromethane was chosen as a solvent to the experimental primers because it is one of the few solvents capable of dissolving used RRP and it evaporates rapidly at room temperature. The effect of different solvents on dry and wet dentin surface has been demonstrated (Agee et al. 2006, Carvalho et al. 2003, Pashley et al. 2001, Perdigao et al. 1999). It has been concluded that the ability of adhesive systems solvent to maintain the demineralised dentin collagen matrix is crucial for the bonding procedure and the resulting bond strength (Carvalho et al. 2003). Water has been introduced to be the best solvent to maintain the dentin matrix (Pashley et al. 2001). The hydrogen bonding cohesion parameter of DCM ($9.6 \text{ MPa}^{1/2}$) is lower than the parameter of water ($40.4 \text{ MPa}^{1/2}$) (Barton et al. 1991). Thus DCM might not be able to maintain the structure of the demineralised dentin matrix in an expanded configuration during and after resin infiltration and wet-bonding technique is needed (Barton 1991). Another important point concerning DCM is that it has some dose-dependent toxic effects on human body (Mizutani et al. 1988, Morris et al. 1979, Sahu and Lowther 1981, Skrabalak and Babish 1983). If DCM wanted to be used as a primer component, biocompatibility tests should be performed. In present studies DCM was used to enable the use of RRP in primers.

Voco Solobond Plus was chosen for control adhesive system for these studies. Voco Solobond Plus belongs to the fourth generation of bonding agents and can be considered as “golden standard”. This adhesive system contains 35% phosphoric acid etching gel; primer with acetone, water, hydroxymethacrylate fluorides, acidic monomers and maleic acid; and adhesive with acetone, Bis-GMA, TEGDMA, hydroxymethacrylate and camphorquinone (Voco Solobond Plus technical datasheet). This product was chosen as control material because of its solvent system. Voco Solobond Plus is meant to be used by wet-bonding technique. Acetone is not capable of maintaining the dentin collagen network efficiently and therefore “wet bonding” technique is needed (Agee et al. 2006, Pashley et al. 2001). It was hypothesized that experimental solvent DCM would perform similarly and need the wet-bonding technique.

To determine the mechanical properties of tested primers, the interface at dentin and composite resin was subjected to microtensile bond strength test. The problem with bond testing in dentistry is that no broad agreement exists within the scientific community as to the appropriate performance, usage and interpretation of these tests and any attempts at standardization of test methods have been difficult (Armstrong et al. 2010, Scherrer et al. 2010). Sano et al. introduced microtensile test method for dentistry to measure the ultimate tensile strength and modulus of elasticity of mineralized and demineralised dentin in 1994 (Sano et al. 1994a). Microtensile test method permits testing of a small

area providing better stress distribution during loading compared to conventional shear and tensile methods (Phrukkanon et al. 1998, Pashley et al. 1999, Sano et al. 1994b). Microtensile test method has concluded to have many advantages: more adhesive failures and fewer cohesive failures, means and variances can be calculated for a single tooth, permits testing of irregular surfaces.

However, the microtensile test methods have also some limitations, which needs be discussed closer. The drawbacks reported for microtensile test method are, for instance, labor intensity, technical demand and dehydration potential of these smaller specimens (Armstrong et al. 2010, Pashley et al. 1995). One limitation of the present study is in the low number of test specimens in 6 month and 12 month groups. Since test specimens came only from three teeth, the effect of individual tooth is great. Eckert and Platt have tested the hypothesis that there is a correlation between beams taken from the same tooth for microtensile testing that will impact the statistical interpretation of the results. Their results supported the hypothesis and they recommended that analyses of microtensile dentin bond strength studies need to account for correlations between beams to avoid over-stating statistical significance of the study results (Eckert and Platt 2007). Because correlations between beams were not done in present studies, one must be careful not to overestimate the results. Furthermore, the limitation is large variations in bonding area of the test specimens. It has been shown that the smaller bonding area of test specimens leads to higher microtensile bond strength values (Phrukkanon et al. 1998, Sano et al. 1994b). In this study, the size of the test specimens varied from 0.74 mm² to 1.30 mm². This high variation in bonding area likely led to high standard deviations.

In present studies, non-trimmed stick-shaped specimens were used instead of trimmed hour-glass shaped specimens. The non-trimming microtensile bond strength test was used because it enables a single tooth to give more test specimens than the original test method (hour-glass test method) (Pashley et al. 1999). The non-trimming technique also enables the measurement of relatively low bond strengths (Pashley et al. 1999). Hour-glass shaped specimens were not used since trimming is technique sensitive and it induces additional stress to specimens. The use of stick shaped specimens has also been recommended since undesirable stress concentration caused by the notch is not present in them (Neves et al. 2008). However, non-trimmed stick shaped specimens are claimed to have stress concentrations generated by the specimen preparation defects at the sharp corners. This has also reported to lead relatively high incidence of pre-test failures (Armstrong et al. 2010). However, pre-test failures were not experienced as a significant problem in these studies (Studies III and IV) and because of this they were not included into results. Usually, pre-test failures are included in results as null result and this decreases the average bond strengths. However, Eckert and Platt have concluded that the groups with higher proportions of spontaneously debonded beams also have lower microtensile bond strengths for the beams which did not spontaneously debond, so

similar kind of results are obtained whether or not debonded beams are included as zero bond strength (Eckert and Platt 2007).

In these studies, the outer-most layers of enamel and dentin were removed during specimen preparation. This was done to ensure that all the bonding was done to dentin only and no enamel was present in bonding surface. The amount of test specimens used per tooth was higher compared to many other studies. Some studies have shown microtensile bond strength to vary depending whether used inner or outer layer of dentin as test substrate and because of this only small amount of test specimens per tooth are used (Pereira et al. 1999). However, in present study the distance from midpoint of tooth was not found to affect the bond strength (unpublished data). Yesilyurt et al. have had same kind of results (Yesilyurt and Bulucu 2006).

Another limitation concerning these studies is the used flat testing jig and cyanoacrylate glue. Flat test jig increases the influence of technique sensitivity since the position of test specimen onto jig is difficult (Arsmtrong et al. 2010, Poitevin et al. 2008). To minimize the effect of technique sensitivity by flat test jig and glue method, special attention was paid to induce tensile load perpendicularly to the adhesive bond line when attaching the specimens into jig. It has been concluded that crosshead speed varying from 0.01 mm/min to 10.0 mm/min does not affect the microtensile bond strength (Armstrong et al. 2010). The used crosshead speed in present studies was 1.0 mm/min.

In present study, microtensile bond strength measurement was used to judge the reliability of the dentin adhesive system. However, it needs to be also mentioned that a high tensile strength of a dentin adhesive system does not automatically prove that the system works reliable under clinical conditions. For example, glass-ionomer cements have concluded to have very low microtensile bond strengths; however, they succeed clinically very well (Jokstad 2004, Xie et al. 2008). Reason for this is the strong attraction of glass-ionomer to tooth substrate but poor cohesive strength of material itself (Xie et al. 2008). In microtensile studies of glass-ionomers, the failures typically occur cohesively in glass-ionomer (Xie et al. 2008).

Additionally, contact angle measurements were performed to test the functionality of primers. For adhesion to occur the adhesive must wet the substrate. The most common method of observing wetting is measuring the contact angle (Marshall et al. 2010). Contact angle represents the energetic equilibrium between the solid, liquid and gas phases involved. Wetting is categorized from liquid contact angle as non-wetting ($>90^\circ$), wetting ($<90^\circ$) and spreading ($\sim 0^\circ$). Low contact angles are formed by liquids or monomers on clean high energy surfaces. High energy surfaces include solids that are strong, hard, crystalline and have high melting points. Etched enamel is a high energy surface (Marshall et al. 2010, O'Brien 2008). The matters which influence contact angle measuring are, for instance, roughness and cleanness of substrate surface (Marshall et al.

2010). In present study, same kind of substrate surface was used in each group to ensure the comparativeness of test groups.

It has been concluded that durable interface between resin and dentin formed during bonding of composite resin to dentin is a key factor when concerning long term bond strength (Abdalla and Feilzer 2008, Breschi et al. 2008, De Munck et al. 2003). Due to this, the formed interfaces between resin and dentin by tested primers were analyzed by SEM. It has been discussed that biodegradation of the collagen matrix and/or hydrophilic resin components within the resin-dentin interface is related to incomplete penetration of resin into dentin substrate, heterogeneous distribution of resin monomers through the interdiffusion zone, poor polymerization in the presence of water, alterations of the organic matrix during preparatory procedures and hydrolysis of polymeric components or unprotected collagen (Eliades et al. 2001, Hashimoto et al. 2000, Mazzoni et al. 2006, Nunes et al. 2005, Osorio et al. 2008, Sencer et al. 2001, Tay and Pashley 2003, Toledano et al. 2006, Toledano et al. 2007, Yourtee et al. 2001). The limitation of the used SEM method in assessing the interface between resin and dentin is that not all hybrid layer components can be evaluated with this method (Van Meerbeek et al. 1993b). To evaluate all the hybrid layer components such as collagen and monomers transmission electron microscope is needed (Van Meerbeek et al. 1993b). By SEM only the general consistence of resin-dentin interface could be evaluated.

Based on the results of the 48 hours' water storing test specimens' microtensile bond strength and contact angle measurements, it can be concluded that the experimental primers containing dimethacrylate based BMEP monomer or monomethacrylate based EGMP monomer enhanced the resin penetration into the dentin. In 48 hours' water storing, BMEP based primer with high solvent monomer ratio had bond strength as high as the control group. When solvent-monomer ratio was increased from 1:1 to 5:1, both primer groups (EGMP/MPS and BMEP/MPS) revealed clearly increased bond strength values. It was predicted that when a DCM collagen matrix is not fully maintained, there is a higher need for low viscosity primer to infiltrate monomers into denser collagen matrix. Dimethacrylate based phosphate monomer (BMEP) was more efficient than monomethacrylate phosphate (EGMP) in matter of bonding. Because of this, it was decided to concentrate on BMEP based primers in longer term water storing.

Another means of the dentin primer studies was to test if RRP could strengthen the resin-dentin interface. RRP was expected to form a hybrid semi-IPN structure with methacrylate-monomers together with collagen network thus toughening interface and further increasing the bond strength (Figure 25). It has been concluded that the interfibrillar space between collagen fibers after acid-etching is 20 nm. If filler in dentin adhesives is desired to infiltrate collagen network, its size should be smaller than 20 nm (Van Landuyt et al. 2007). The molecular weight of RRP fillers used in this study

is in range of 26.900–30.000, but the limitation of this study is that the actual size of RRP fillers was not determined (Connolly et al. 1995, Ha et al. 2001). It is not known whether RRP fillers penetrated the collagen network or whether fillers remained on top of the collagen network. An increase by RRP fillers in bond strength was observed in 48 hours' groups. Three-way-ANOVA revealed this to be significant, though lower than expected. A low concentration of RRP in the primer could explain the modest increase. A low RRP concentration was used to prevent extensive phase separation during solvent evaporation. Additional limitation of this study also is that it is not known exactly which proportion of RRP coiled out of primers. In this study, polyphenylene based RRP was first dissolved in DCM and after this mixed with other components of the primers. RRP is not fully soluble in EGMP, BMEP and MPS which means that part of it coiled out of primers after mixing. However, also nanoscale RRP clusters presumably reinforce the resin-dentin interface.

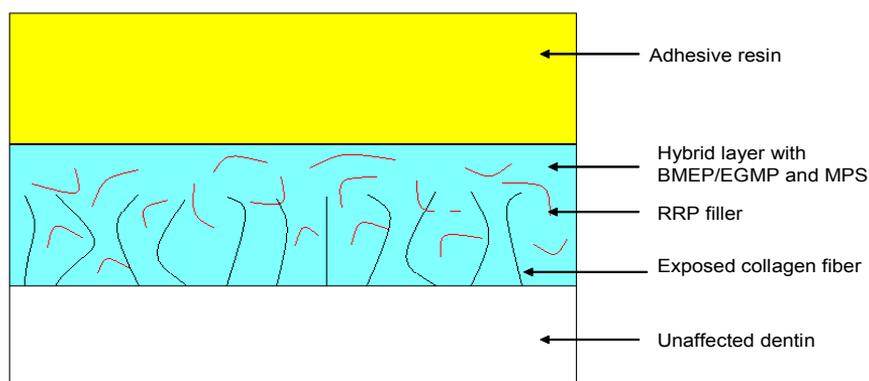


Figure 25. Schematic presentation of the hypothetical structure of resin-dentin interface by experimental primers.

In longer term water storing, high solvent monomer ratio decreased the bond strength of experimental primers. It has been studied that retained solvent in hybrid layer can lead to poor resin polymerisation and increased permeability (Cho and Dickens 2004, Torkabadi et al. 2008, Yiu et al. 2005). It is important that solvents are eliminated before the polymerisation of adhesives so that the interaction between monomers and between them and the dentin matrix may occur more efficiently increasing the optimal conversion and mechanical properties (Garcia et al. 2009, Garcia et al. 2010, Malacarne-Zanon et al. 2009). Ikeda et al. have concluded that complete solvent evaporation can not be done by air-drying and that retained solvent decreases the ultimate tensile strength of adhesives (Ikeda et al. 2005). Fontes et al. have proposed the use of tetrahydrofuran as an alternative solvent to enhance solvent evaporation (Fontes et al. 2009). Cadenaro et al. have studied that the concentration of retained ethanol increased significantly with increasing ethanol concentration in comonomer blend (Cadenaro et al. 2009b). It is probable that in high

solvent-monomer ratio group part of the solvent did not evaporate at bonding stage and this led to poor chemical stability of the interface between resin and dentin in this group. Another explanation for the decreased bond strength in DCM rich primer is interfacial cracks in the interface between resin and dentin. Cho et al. concluded in their acetone based study that acetone rich primers created interfaces with cracks (Cho and Dickens 2004). The SEM figures taken in the present study confirmed the void formation in between dentin and adhesive resin in high solvent-monomer ratio group. Voids could already been detected after 48 hours' water storing. This void formation possibly led to decreased bond strength in this group. In low solvent-monomer group only a few voids were found and this might explain the better stability of the bond strength in this group.

After one year of water storage, the bond strength of control group and experimental BMEP groups with low solvent-monomer ratio did not decrease. The experimental BMEP group with high solvent-monomer ratio was not capable to withstand the degrading effect of water. One of the major facts which must be discussed is the water storing manner which was used in these studies. In these studies (Studies III and IV), teeth were stored in water as complete and sectioned not until the end of water storing period. It has been previously shown that if teeth are stored with indirect water storing resin bonded to enamel protects the resin-dentin bond against degradation (Abdalla and Feilzer 2008, De Munck et al. 2003, Osorio et al. 2008). However, when using one-step self-etch adhesives, a bonded enamel margin may be suspected of having a protective role against the degradation of resin-dentin interface because of lower bond strength to enamel (Torkabadi et al. 2008). Another fact which might have helped to preserve bond strength in long-term is the used hydrophobic resin layer in top of hydrophilic primer (Reis et al. 2008). It is probable that hydrophobic resin layer increases the hydrophobicity of the adhesive layer rendering them less permeable to water movement and less susceptible to water degradation (Reis et al. 2008).

6.5 Suggestions for future research

Studies I and II gave interesting and promising results which confirmed the good mechanical properties of RRP and showed that it might be possible to adhere RRP to other dental polymers and this way also to the tooth substrate. However, the exact reason behind the adhesive capability needs to be solved in future. Also, long-lasting water-storing studies and fatigue testing are needed. It has been concluded that extrapolation of clinical recommendations from work of purely in vitro nature, such as the current study, must always be made with caution (Van Meerbeek et al. 2010). Therefore in future, also clinical studies are needed. However, poly(paraphenylene) based RRP has not been used previously in human body, and because of this, also biocompatibility tests should be performed.

The hypothesis in Studies III and IV assumed that hydrophilic BMEP and EGMP monomers would turn more hydrophobic by reacting with MPS silane. In future, it would be interesting to investigate whether reactions between monomers and MPS silane really occur. This could be done for example by measuring the conversion degrees of these monomers in different combinations. In Studies III and IV the fracture modes were detected only by visual examination under light microscope. This kind of examination did not give information about the exact nature of de-bonding mode. In future, it would be beneficial to examine which part of resin-dentin interface is the weakest part. This kind of examination would give valuable information about which part of resin-dentin interface needs to be improved.

7. CONCLUSIONS

With the limitations of this study the main findings and conclusions were:

1. RRP fillers have an effect on the mechanical properties of denture base polymer. Flexural strength was lowered and flexural modulus and surface microhardness increased. Water sorption was reduced by adding RRP fillers to denture base polymer. MMA is not able to dissolve the RRP filler surface and no visually seen IPN-layer is formed between RRP filler and denture base polymer. The first and second hypotheses were partly accepted and the third hypothesis was rejected.
2. A method to attach BisGMA-TEGDMA-resin to RRP substrate was developed. The use of DCM based primer with RRP/resin additives played a significant role in the formation of adhesion. By the use of RRP/resin/DCM-primer, new kind of adhesive surface was created. The hypothesis was accepted. The information about the reasons behind the formation of this new surface remains to be determined in future studies.
3. Rigid rod polymer containing primers could provide similar resin infiltration to dentin collagen-layer as control primer as determined by contact angle measurements and SEM micrographs. RRP increased bond strength values. RRP-primer with BMEP-monomer and high solvent concentration provided comparable bonding properties to commercial control in short-term water storing. The null-hypothesis had to be rejected.
4. High solvent-monomer concentration of experimental primers decreased bond strength in longer-term water storing. The reason for decreased bond strength by high solvent-monomer concentration primer was non-homogenous interface between resin and dentin. In low solvent-monomer concentration groups, the long-term water storing did not decrease the bond strength despite of hydrophilic monomers used. The null-hypotheses had to be rejected.

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