A Study Investigating the Mechanical Testing of a Novel Dental Restorative Material and its Biocompatibility

By

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ABSTRACT

Dental composite materials are evolving continuously with novel Resin Based Composites being at the forefront of dental restorations. The characteristics of these materials need to be such that they are able to withstand both mechanical (masticatory) stress and any chemical activity.

The current study investigates the strength and biocompatibility of three Resin Based Composites (RBCs); Ormocer Admira (VOCO, Cuxhaven, Germany), dimethacrylate Filtek^{TM}Z250 (St Paul, MN, US) and a novel RBC namely X-tra Fil (VOCO, Cuxhaven, Germany). These materials were tested using bi-axial flexure, vickers hardness, water sorption and water solubility tests, but a one-way ANOVA showed no significant difference between their mechanical properties. Cytotoxicity tests were also performed by culturing RBC specimen discs both directly and indirectly with ATCC mouse 3T3 fibroblasts and undifferentiated pulpal fibroblast cells (OD21 cells). These determined that all three materials were cytotoxic to both the cell types, however a one-way ANOVA test showed that there was no significant difference between the materials. This suggests that all the materials exerted a similar cytotoxic effect.

Therefore, the current study indicated that the mechanical and cytotoxic properties of X-tra fil are not an improvement but are similar to those materials already available on the market. However, this study provides a good origin for further research into the properties of these materials.

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Dedicated to.....

My Amaa who has been a constant guide and support throughout my life and studies.
My Aboo who has encouraged me to do my best in everything that I have undertaken.

My Bari Ammi whose prayers are forever protecting me
and
My Mamo whose invaluable advice is engraved in my mind & heart.
(You are dearly missed)

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CHAPTER 1 Introduction

1.1 Clinical Perspectives

1.1.1 Anatomy of the Tooth

Oral health is necessary to maintain total health and is a reflection of, and a main contributor to a healthy immune system. With this in mind, it is known that the orofacial complex is the first line of defence and needs to be maintained (Oral Health, 2005). The oral cavity is where the digestive system originates and the teeth primarily act as a shield against infection and need to be restored accordingly (Van Noort et al., 2002).

The upper and lower jaw in humans work together to act as the protective layer for the oral cavity and subsequently, for the rest of the body (Brand & Isselhard 2003). The teeth themselves are divided into sections and named according to their function with each tooth having a root and crown portion where the crown is covered with enamel and the root is embedded into the alveolar processes and is covered with cementum (Figure 1.1) (Van Noort et al., 2002). The crown itself is the visible part of the tooth which is seen above the gum line and is covered with a tough enamel coat comprising the anatomic crown. Enamel is the hardest substance in the human body and composed of 95% mineral with most of the mineral made up by a compound called hydroxyapatite, enamel is incapable of remodelling or repairing (Brand & Isselhard 2003).

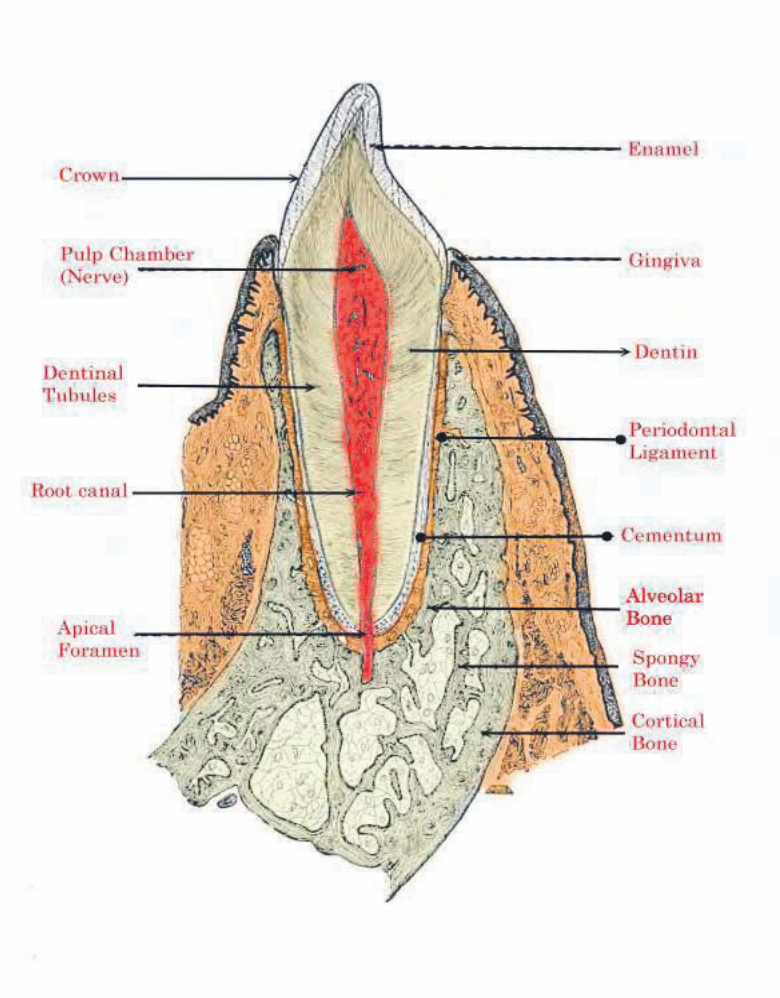


Figure 1.1 Illustration of the tooth anatomy, showing the crown and root sections and giving a description of the other tissues involved in its function. (http://www.doctorspiller.com/tooth_anatomy.htm)

Cementum covers the root of the tooth whilst dentine makes up the bulk of the tooth and although it is a tissue that is harder than bone, it is porous due to its tubular architecture. The porosity contributes to the fact that if dentine becomes exposed, the tooth often becomes sensitive to stimuli, namely, temperature variations, air and touch. (Brand & Isselhard 2003).

1.1.2 The Dentine-Pulp complex

The development, structure and function of the dentine and pulp are closely related. Even though the anatomical structures of mature dentine and pulp are different, they still function together as the "dentine-pulp complex" (Orchardson & Cadden 2001). Together, the dentine-pulp complex shows a range of responses to caries of the dental tissues, which represents a summation of injury, defence and repair mechanisms (Smith 2002). Dentine also contains the mineral hydroxyapatite but to a lesser degree than enamel. There are a number of different layers of dentine with the initial layer (mantle layer) being formed by newly differentiated odontoblast cells derived from the dental papillae (Orchardson & Cadden 2001). The inner part of the dentine contains tubules which contain a liquid derived from the pulpal extracellular fluid (Orchardson & Cadden 2001). The tubules themselves contain extensions of cells from the pulp of the tooth including odontoblast processes and afferent nerve terminals. The dentine is supplied with nutrients by the pulpal blood vessels however no blood vessels are present in the dentine itself (Turner et al., 1989). Nerves of the dentine-pulp complex detect sensitivity of the tooth and also play a role in the regulation of secondary and tertiary dentine deposition (Holland 1994, Turner et al., 1989).

It is during the actual development of the tooth that the primary dentine layer is produced. This dentine continues to be deposited by the odontoblasts at a slow pace, throughout the lifespan of the tooth and this post developmental dentine is termed, secondary dentine. In some cases, dentine can become damaged and the body reacts to this by initiating further dentinogenic mechanisms (Holland 1994). In areas of the tooth damaged due to occlusion or disease, there is a layer of dentine rapidly produced to replace that which is damaged, i.e. deposited in response to

relevant stimuli. This newly deposited dentine is referred to as tertiary dentine. Results from recent studies have shown that there are two types of tertiary dentine, reactionary and reparative, produced as a result of different levels of pulpal stimulation (Smith et al., 1995). Reactionary dentine is laid down by primary odontoblasts in response to a mild stimulus, whilst reparative dentine is produced by secondary odontoblast-like cells derived from pulp cells, in response to a more intense stimulus, which resulted in the death of the primary odontoblasts (Smith et al., 2002). It has been demonstrated in previous studies that the extracellular matrix around the dentine contains significant amounts of TGF-beta 1, a growth factor previously shown to influence odontoblast differentiation and secretory behavior (Holland 1994). Activation of tertiary dentinogenesis therefore most probably results from solubilization and release of growth factors archived within dentine which subsequently signal appropriate cellular events (Smith et al., 1995).

Inflammatory responses are triggered by the tooth becoming injured or when disease present. There are two extremes to this response, as mild acute injuries can result in repair (Smith et al., 1995) whilst more severe or chronic injury or disease can result in death of the pulp. Pulp death can represent a serious clinical problem, as the dentine can no longer be repaired and the teeth may become discoloured (non-vital) and can fracture under masticatory stress (Orchardson & Cadden 2001). However, this structural weakness is more likely to be due to the amount of tooth matter lost as opposed to the intrinsic weakness of the non-vital dentine. As a result, the vitality of the tooth influences the restorative strategies applied to the damaged tooth (Holland 1994, Orchardson & Cadden 2001).

Dental caries is one of the most prevalent chronic diseases in the world and results from dental erosion due to bacterial acids derived from the microbial plaque which consequently cause cavities (Ardakani et al., 2004). Early stage incipient caries are limited to a demineralized surface without a cavity and can be treated with fluoride and antimicrobial treatment. However, a carious lesion which reaches the cavitation stage usually represents a more serious clinical problem and is therefore treated using restorative techniques (Ardakani et al., 2004).

1.2 Development of Dental Restorative Materials

1.2.1 Resin-Based Composite Materials

One of the first dental restorative material (dental amalgam) was introduced in the early nineteenth century in France where the process of restoration involved heating the alloy to 100^{o}C and pouring it directly into the prepared cavity (Greener 1979). Over the years, dental amalgam has been a controversial restorative material with one of the main issues surrounding its use being the fact that it contains mercury, which is known to be toxic. Nevertheless, amalgam materials have been the most widely used direct restoratives in dentistry (Osborne & Swift 2004). Restorative techniques have now however developed, alongside newer dental materials. These resin-based composites (RBCs) are constantly assessed to ensure their efficiency and more importantly their safety for use in humans.

Commercially available RBCs for use as direct filling materials were introduced in the 1960s following the pioneering work of R.L. Bowen, the Associate Director of the American Dental

Association Research Unit at the National Bureau of Standards (Palin et al., 2003). A novel RBC material composed of 25wt% (weight percent) of polymerisable resin and 75wt% quartz or alumino-silicate glass filler was introduced and was set to bring the existing resin-based technology into a new era (Palin et al., 2003). As a result, developments in present-day composites have produced excellent anterior restorations and can be used in selected situations for occlusal surfaces. Newer RBCs showed promise for extended applications as amalgam use amongst practitioners continues to decrease (Bayne et al., 1994). More recently, studies on posterior usage of RBC introduced packable RBCs as an alternative to amalgam and these are now used extensively in the restoration of posterior teeth due to the aesthetic demands of the general public (Bala et al., 2003). Based on the filler load, these materials were expected to exhibit superior physical and mechanical properties additional to improvements in handling (Tagtekin et al, 2003). However early attempts to place RBCs in posterior teeth had only limited success because of insufficient material properties (Manhart et al., 2000). The wear of RBCs in the oral environment is complex and diverse with fundamental wear mechanisms such as adhesion, abrasion, attrition, fatigue and corrosion operating independently or in combination to exacerbate the wear process in the oral cavity (Palin et al., 2005). Inadequate resistance to this wear process, in the body of the posterior restorations, results in a loss of anatomic form under masticatory abrasion, attrition fracture within the margins and marginal leakage due to polymerisation shrinkage (Manhart et al., 1999). Therefore, with these wear mechanisms in mind, it has been stated previously that no current dental restorative material meets all these requirements enabling it to be considered ‘ideal’ (de Souza Costa et al., 2003).

Over recent years, studies have been conducted analyzing the wear of RBCs over varying periods of time and improvements have been made in the composition of these materials to make them more resilient to stress. Whilst the mechanical properties and abrasion resistance of RBCs have improved, the placement of posterior resin based restoration remains very technique sensitive (Bala et al., 2003). However, despite these improvements there continues to be a problem in stress-bearing situations. When studied more intensely it was found that the problem was that smaller particles have higher surface areas and tendencies to agglomerate (Yap et al., 2004). This problem is partly circumvented by matching filler sizes to improve packing efficiencies, by using pre-cured particles of highly filled composite (Bayne et al., 1994). Dental RBCs are essentially comprised of a resin matrix (organic phase), filler matrix coupling agent (interface), filler particles (dispersed phase) and other minor additives including polymerization initiators, stabilizers and colouring pigments (Yap et al., 2004) and are essentially required to have long-term durability in the oral cavity (Kanchanavasita et al., 1997). Manufacturers have increased the range of shades of light activated resin composites to meet the increasing aesthetic requirements of both patients and practitioners (Shortall 2005). The aesthetic success of tooth-coloured restorative dental materials such as RBCs, is influenced by several factors including translucency and opacity which are viewed as being vital components as they are indicators of the quality and quantity of light reflection on curing (Azzopardi et al., 2009). Ideally, translucency of the aesthetic restorative materials should not change after curing, however this is not always the case (Woo et al., 2008).

Direct restorations are placed immediately into a prepared cavity in a single visit. The types of restorative materials that are advocated include dental amalgam, glass-ionomers, resin ionomers

and some RBC materials. Indirect restorations are prepared from impressions taken from the patient and cemented into the prepared cavity at a later date. The types of restorative materials advocated include inlays, onlays, veneers, crowns and bridges fabricated using gold, base metals alloys, ceramics or RBCs (Craig et al., 2001).

Glass-ionomers are cements which are popular with dentists as core materials because of their adhesive properties and ease of handling. However, conventional glass-ionomers are only suitable where there is already substantial tooth substance remaining to support the material and where it can withstand any resistance on the natural tooth. RBC materials are rapidly becoming the primary restorative material of choice for replacing tooth structure and the low percentage of biological problems reported for RBCs is testimony to their relative biocompatibility (Osborne et al., 2004). As a result, new RBCs termed “packable” or “condensable” RBCs are being promoted as amalgam alternatives (Bala et al., 2003).

Indirect materials can also be utilized in the restoration of teeth. The placement of an indirect restoration requires the preparation of a cavity with undercut-free cavity walls to allow a path of withdrawal and insertion of the completed restoration (Craig et al., 2001). The preservation of remaining tooth structure is important because the restoration relies on the strength and integrity of the remaining prepared tooth substance for retention and conversely the restoration is a source of strength for the remaining tooth structure (McCabe & Walls 1998). However, the less enamel and dentine present the greater the risk of mechanical and biological failure. Ultimately, the choice of restoration is determined by the damage to the tooth and the resultant dental tissue left to support the subsequent restoration.

1.2.2 Composite Material Chemistry

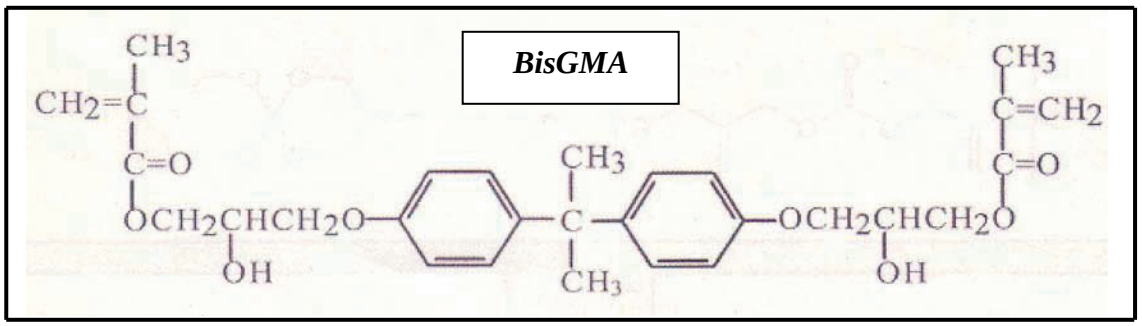
RBCs are three-dimensional combinations of at least two chemically different materials with a distinct interface (Yap et al., 2004, Guiraldo et al., 2008). Their properties depend on several factors, related to the polymer matrix, the filler particles and the coupling between filler and matrix (Asmussen & Peutzfeldt 1998). RBCs generally consist of a monomeric matrix resin, a silanated inorganic filler, a polymerisation initiator system, inhibitors for storage stability and pigmentation for shading (Eick et al., 2002). More specifically RBCs are composed of an organic matrix, load particles (glass, quartz and/or melted silica) and a bonding agent, usually an organic silane with a dual characteristic enabling chemical bonding with the load particle and co-polymerisation with the monomers of the organic matrix (Guiraldo et al., 2008). RBCs containing high filler levels have superior physical, chemical and mechanical properties, but clinically it is the RBCs containing the smaller filler particles that are the easiest to use (Bayne et al., 1994). RBCs are commonly chosen for their use clinically according to their durability and resistance to fractures that are small/medium in size and also according to how successful they are at withstanding moderate chewing forces (Van Noort et al., 2002). More recently, to resolve any disadvantages that RBCs may have had, such as wear resistance or lack of proximal contacts, packable RBCs have been introduced and contain a filler distribution that gives the material different handling properties (Bala et al., 2005). Improvements in the physical properties of the materials together with their positive clinical performances encourage the use of posterior RBCs as a viable alternative to amalgam (Manhart et al., 1999). Notably, RBCs are less stable in fluids especially in saliva where their degradation rate is increased however this

depends on the chemical nature of the monomers, amounts of dimers and oligomers and the degree of crosslinking in the polymerised matrix (Özcan et al., 2005).

The organic matrix of conventional RBCs used in clinical practice, is based on methacrylate chemistry, in particular that of dimethacrylates (Palin & Fleming 2003). Free-radical polymerisation of dimethacrylate monomers by ultraviolet irradiation along with molecules packing more closely together leads to bulk contraction. In this process the RBC gelates when the curing contraction overtakes the flow of the curing monomer (Palin et al., 2003). Early systems for bonding composites to enamel were based on acid conditioning (etching) and micromechanical interlocking with bonding agents (unfilled BIS-GMA) (Bayne et al., 1994). The initial dimethyl methacrylate monomer is produced by the reaction between bisphenol-A and glycidylmethacrylate (2,2-bis[4-(2-hydroxy-3methacrylyoxypropoxy)phenyl] propane) whose initial synthesis in 1956 initiated a new era of dental RBCs (Bowen 1962). BisGMA (Figure 1.2a) is a highly rigid and viscous material (Palin et al., 2003) with the monomer systems of most RBCs to date being based on Bis-GMA or Bis-GMA derivatives (Asmussen & Peutzfeldt 1998). With respect to restorative materials used in dentistry, the complex environment in which they are placed contains saliva which is comprised of a variety of inorganic and organic species. In consequence the uptake of fluid and the solubility of these materials are of considerable clinical significance (Kanchanavasita et al., 1997). Whilst BisGMA is hydrophobic and is not particularly successful in spreading over wet surfaces due to the fact that dried enamel does not contain moisture, the bonding of BisGMA is generally appropriate (Bayne et al., 1994).

In recent years, the high viscosity of Bis-GMA lead to its incorporation with lower molecular weight dimethycrylate monomers producing a material with appropriate viscosity where fillers can be added (Asmussen & Peutzfeldt 1998). BisGMA was identified as being unsuitable for use clinically and as a result the addition of a co-monomer triethyleneglycol dimethacrylate (TEGDMA) (Figure 1.2b) was required. Subsequently TEGDMA was used to reduce the viscosity of the initial liquid and therefore improve the rheological properties of the composite (Palin et al., 2003, Kim et al., 2006). However, the main problem with using TEGDMA as a diluent was that following irradiation (curing) a relatively large amount of volumetric shrinkage occurred which increases the likelihood of mechanical failure following clinical placement resulting in pulpal infection (Palin et al., 2003, Bayne et al., 1994). Other RBCs utilise urethane dimethacrylate 1,6-bis(methacrylyloxy-2-ethoxycarbonylamino)-2,4,4-tri-methylhexane, (UDMA) (Figure 1.2c) used alone or in combination with Bis-GMA and TEGDMA (Asmussen & Peutzfeldt 1998, Palin et al., 2003, Chung et al., 2002). The advantages of UDMA have been reported to be lower viscosity and a greater flexibility of the urethane linkage, which may improve the toughness of the RBCs based on this monomer (Asmussen & Peutzfeldt 1998). It has therefore been proposed that the incorporation of UDMA, as opposed to TEGDMA, improves the mechanical properties of the RBC materials (Asmussen & Peutzfeldt 1998, Palin et al., 2003). Studies have also suggested that by selecting specific combinations of these resins, it may be possible to produce RBCs that are tailored to specific clinical applications (Park et al., 1999). As a result, the range of indications where RBCs are used has significantly increased due to enhancement of their physical and mechanical properties (Keulemans et al., 2009).

a



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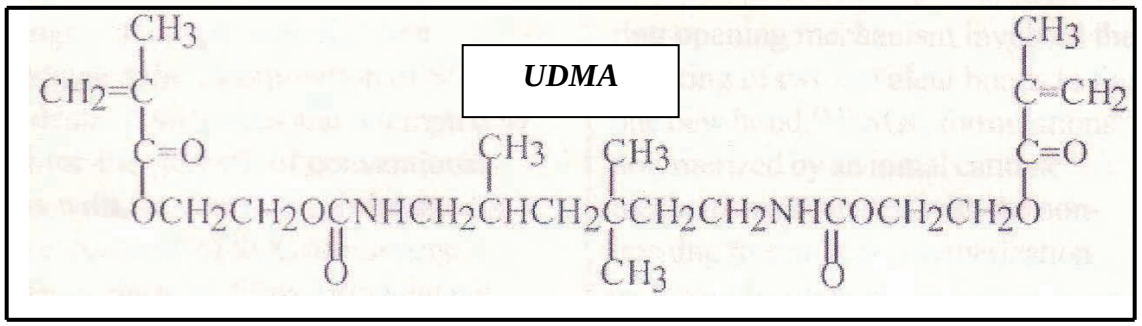

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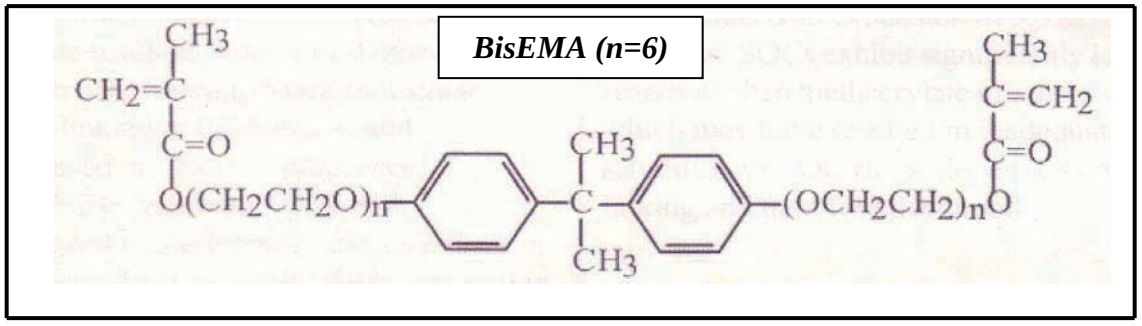


Figure 1.2 Schematic representations of the chemical structure of the resins a) BisGMA, b) TEGDMA, c) UDMA and d) BisEMA, utilised in RBC materials.

RBC material chemistry is continuously developing with new materials generally exhibiting superior physical and mechanical properties in addition to improvements in their handling characteristics (Sharkey et al., 2001). Recently a new type of inorganic-organic hybrid dental material, known as Ormocers, was developed was and this was first applied as a dental restorative material in 1998 (Taher 2002). Ormocers are hybrid polymers that incorporate a metal oxide backbone (mainly polysiloxane) with organic crosslinking (e.g. acrylate or styryl polymer). The synthesis of this composite was developed using special substitutes to create a complex structure, formed by only one polymerisable double bond and alkoxy group, responsible for the formation of the Si-O-Si structure (Cunha et al., 2003). After incorporation of filler particles, the Ormocer composites can be manipulated similar to hybrid composites (Bala et al., 2005). Due to the different matrix system in Ormocer resins and the incorporation of different filler molecules (up to 67% volume) these composites have been shown to have a higher surface roughness, but a higher hardness and wear resistance as compared to conventional hybrid RBCs (Tagtekin et al., 2004). In addition, the inclusion of fillers can cause the material to have a low water absorption and solubility. Due to the variation of their chemical composition, the properties of the materials can be tailored accordingly to their clinical application (Figure 1.3) (Poppal 2004).

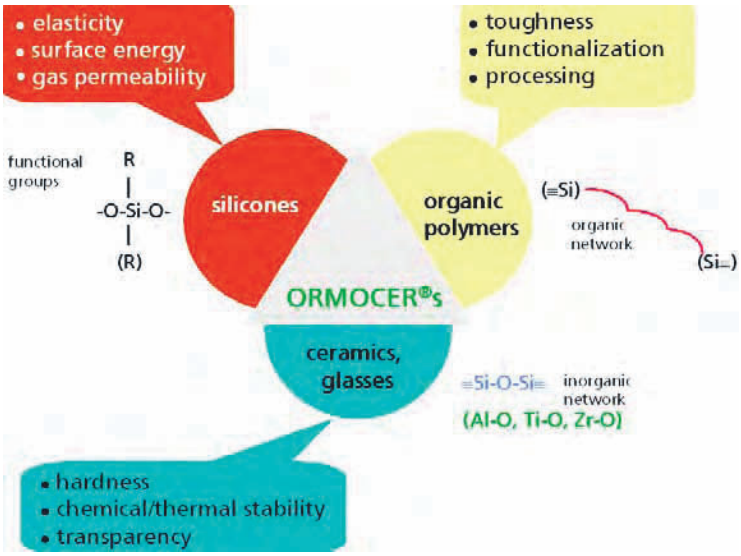


Figure 1.3 Illustration of an Ormocer material, showing how each component alters the resultant properties and therefore the function and clinical application. (http://www.isc.fraunhofer.de/alteseiten/ormocere_e/index_o0.html)

A newly developed Ormocer is Admira (VOCO, Cuxhaven, Germany) and the chemical technology applied in this material is somewhat different from conventional RBCs as it has been shown to exhibit a higher surface hardness in comparison to comparable materials (Tagtekin et al., 2004, Yap et al., 2004). The relative proportion of its structural elements are what determines the material properties of an Ormocer and these are regulated by i) the choice of base materials, ii) how the inorganic polycondensation reaction is conducted, and iii) control of the linking reactions which lead to the organic network (Poppal 2004*)*. Ormocers consist of an inorganic-organic network matrix derived through polymerization while their filler particles are imbedded in this cross-linked inorganic and organic matrix. Whilst the average particle size is 0.7 µm, which is comparable with most mini-fill RBCs (Yap et al., 2004), Ormocers are comprised of matrices which shrink less and were originally developed for electronic applications reliant on an

inorganic alkoxysilane network which is chemically attached to traditional methacrylate groups (Bouillaguet et al., 2002). Multifunctional urethane and thioether(meth)acrylate alkoxysilanes acting as sol-gel precursors have now been developed for synthesis of inorganic-organic copolymer Ormocer composites for use as dental restorative materials (Manhart et al., 2000). The toxicological potential of this material is also considered lower than that of conventional composite restoratives since the acrylates and methacrylates are silane bound and covalently linked to the inorganic network. To improve the handling of this material, dimethacrylate is also incorporated (Cunha et al., 2003).

1.3 Polymerisation of Light-Activated RBCs

The polymerisation of the monomers in a RBC material are initiated by free radicals and there are several different types of catalysts, including both thermal and chemical, which are capable of promoting this polymerisation reaction (Kim et al., 2004). Currently, the approach most commonly used clinically is that of photochemical catalysis with different RBCs utilising different photochemical systems that are activated by different wavelengths of light. In general however light curing units have a minimum light intensity of 300 mW/cm^2 (Bayne et al., 1994).

Visible light-curing units, or LCUs, are therefore an integral part of modern adhesive dentistry. They are not only used to cure RBCs but also resin-modified glass ionomers, preventive pit-and-fissure sealants, as well as materials which bond orthodontic brackets to teeth (Dunn & Bush 2002). Along with the strength of the material, the luting technique is also important for the clinical success of a restoration (Pazin et al., 2008). As the light source can significantly affect

the polymerisation process, combined with this increased use of bonded composite resins in dentistry, the development of advanced photochemical technology designed to improve the resin polymerization process has been stimulated (de Araújo et al., 2008, Pazin et al., 2008). The aims of photopolymerization have been stated as ensuring that there is uniform conversion in the depth of the restoration along with the shortest possible irradiation time and subsequently a low shrinkage stress (Shortall 2005).

1.3.1 Light curing techniques

Until recently, light emitted from a conventional quart-tungsten halogen light bulb (QTH) was used to cure composite resins and bonding agents with these still being the most commonly used lights today (Guiraldo et al., 2008, Bagis et al., 2008, Arrais et al., 2007). However, certain factors can compromise the performance of halogen light curing units (LCUs), such as fluctuations in the line voltage, the condition of the bulb and filter, as well as bulb overheating within the unit. These problems can subsequently reduce the efficiency and lifetime of the halogen lamps leading to poorly polymerized composite resins with impaired mechanical properties (Arrais et al., 2007). Advances in the field of light-curing have been remarkable, mainly following the development of blue light-emitting diodes (LED) for the photoactivation of resin composites (Camilotti et al., 2008). These LEDs have been available since the mid-1990s and now use a new semiconductor material system where the gallium nitride involved forms the basis for the blue emission and is also responsible for the high efficiency of devices that use it. These together are both characteristics that are essential when used in the dental curing

application (de Araújo et al., 2008, Tolosa et al., 2005). Notably, LED LCUs also consume little power in operating and do not require filters to produce blue light (de Araújo et al., 2008).

Visible light activated RBCs were introduced in the 1970's and research indicated that whilst the ultraviolet energy has limited penetration within the dental structures it also has limited penetration within the composite itself, which is subsequently a disadvantage of this method (Filipov & Vladimirov 2006). This limitation has prompted the development of composites which contain camphorquinone (CPQ) (Figure 1.4) which is a catalyst for the polymerising reaction enabling it to proceed in the stimulated state (Tanoue et al., 2007, Filipov & Vladimirov et al., 2006). The use of Gallium nitride LEDs that produce a narrow wavelength peak ~470 nm, which is approximately the absorption peak of CQP, results in more effective polymerisation (de Araújo et al., 2008, Tolosa et al., 2005). Additional or alternative initiators responding to different wavelengths are also being introduced in some composites (Bennett & Watts., 2003). The mechanical and physical properties of resin composites light-cured by these LED systems have also been reported, such as compressive and flexural strength, hardness, degree of conversion and depth of cure and the use of visible light curing (VLC) for polymerisation of dental materials has now become an essential part of a contemporary dental practice (Wiggins et al., 2004).

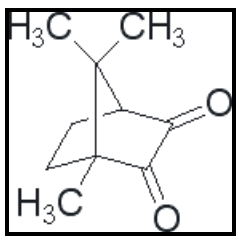


Figure 1.4 The chemical structure of camphorquinone (CPQ).

1.3.2 Chemistry of Light Activation

Light irradiation of RBCs based on bisphenol-A-dyglycidyl-ethe-dimethacrylate (BisGMA) chemistry involves the free-radical polymerisation of the monomeric constituents leading to bulk contraction of the material (Davidson et al., 1984). Clinically this may be manifested as cuspal movement on polymerisation (Abbas et al., 2003). The LEDs commonly produce a narrow spectrum of blue light in the 400 – 500nm range (with a peak wavelength of ~460 nm). This is the useful energy range for activating the diketone-type photoinitiator CPQ molecule (Figure 1.4) most commonly used to initiate the photopolymerization of dental monomers (Guiraldo et al., 2008, Camilotti et al., 2008, Wiggins et al., 2004).

The polymerisation process generates a polymer network through the substitution of the carbon double link s (C=C) by simple covalent links (C-C) (de Araújo et al., 2008). The basic composite insertion and polymerization protocol usually recommends the curing of increments not thicker than 2 mm to guarantee an effective polymerization with the light guide being as close as possible to the composite surface to ensure the light is not dissipated (de Araújo et al., 2008). The properties of light-activated composites vary in accordance with the type of light-curing unit used for polymerisation, and the type of laboratory light-polymerising unit also affects the post-curing properties of indirect composites (Tanoue et al., 2007).

1.3.3 Degree of Conversion of RBCs

The degree of conversion (DC) of the composite material is an important aspect related to the durability of the restorations as it is directly related to the physical and mechanical properties of the material (de Araújo et al., 2008). Indeed, the DC in light cured RBCs varies within the bulk of the specimen as the conversion process is dependant on light energy for activation (Rueggeberg & Craig., 1988). The DC depends upon the factors such as monomer structure, amount and type of filler particles, composite shade, light curing time and curing depth. The curing depth in turn is dependant on the intensity of the radiation emitted from the light curing unit (Cook 1982). The reduction of the C=C bond rate represents the DC and this has been shown to maintain a direct relationship with the composite resin microhardness, therefore a hardness test can be used to indirectly evaluate DC (de Araújo et al., 2008).

Absorption and scattering of the light by filler particles can regulate attenuation of light in the material, therefore the light transmission within the RBC and the source of light used are important factors involved in achieving DC (DeWald& Ferracane 1987, McCabe & Carrick 1989). Indeed optical scattering has been widely studied due to its important effects on the colour and translucency of dental materials (Campbell et al., 1986). Research subsequently indicates that cure depth may also be related to the translucency (shade) of the RBC (Ferracane et al., 1986). In relation to this, it was originally theorized (Clewell 1941) that optical scattering is dependant upon the wavelength of the illumination. When the size of the scattering particle is much greater than the wavelength of the illumination, it is predicted that the scattering will be inversely proportional to the size of the particle and that the wavelength will have no effect (Clewell 1941, Campbell et al., 1986). Nowadays, commercial composites contain about 50%

volume filler with this component expected to have a great effect on the optical properties of the composite (Campbell et al., 1986).

Limited cure depth has been a major clinical problem as the presence of unpolymerised or partially polymerised material in the restoration can lead to reduction of mechanical properties and/or pulpal tissue toxicity (Kawaguchi et al., 1994). Subsequently, the strength and hardness of the composite restoration can be compromised due to unreacted components being leached from the restoration. Combined this increases the likelihood of restoration failure leading to tissue irritation and secondary caries development (DeWald & Ferracane 1987).

1.4 Characterisation Techniques

1.4.1 Determination of Relevant RBC Mechanical Properties

Filler content, size and particle distribution have all been shown to influence the physical and mechanical properties of the RBCs. It is known that filler volume friction and load level of the

RBCs correlate with the material strength and elastic modulus, as well as the fracture toughness (Manhart et al., 2000). RBC restorations placed in the posterior region of the mouth are subjected to compressive loading during the masticatory cycle and as a result previous studies have investigated the compressive strength of posterior composite restorations under laboratory conditions, to assess their wear potential (Palin et al., 2003). Evaluation of strength related properties of experimental and commercially available RBCs have previously been investigated diametral tensile compressive and flexural strength tests. The degree of conversion in RBC materials containing Bis-GMA and TEGDMA has been found to decrease with an increasing content of Bis-GMA. Despite the increasing content of Bis-GMA it has been found previously that this does not result in reductions in strength and hardness (Asmussen & Peutzfeldt 1998). Material properties, such as fracture resistance, elasticity, and marginal degradation of materials under stress have usually been evaluated by the determination of the material parameters flexural strength, flexural modulus and fracture toughness (Manhart et al., 2000). Bi-axial flexure strength testing is known to be advantageous over uni-axial diametral tensile and compressive testing methods but is employed less frequently for the assessment of dental composites (Palin et al., 2003). The bi-axial flexure test has been used frequently to determine the fracture characteristics of brittle materials. The measurement of brittle materials under bi-axial rather than uni-axial flexure conditions is often considered more reliable because the maximum tensile stresses occur within a central loading area allowing for slightly warped specimens to be tested producing results unaffected by the edge condition of the specimen (Ban et al., 1992). There were initially three bi-axial flexure test designs: ball-on-ring, piston-on-three-bal, and ring-on ring. It was found that only the ball-on-ring loading configuration was satisfactory as uncertainties exist about the fracture stresses for the other two cases (Shetty et al., 1980). It was

concluded that the stresses which developed on the support side were not significantly affected by the loading condition, while the stresses on the loaded side were dependant on the loading condition (Ban et al., 1992).

As well as the bi-axial flexure strength, the chemical effects of solvent or mixtures may soften the resin in the RBC, in which a solvent penetration to a depth of just a few micrometres is sufficient to alter the frictional coefficient, which will therefore influence the wear behaviour (Tagtekin et al., 2004). A second problem is that test pieces are prepared with a flat surface, whereas teeth and restorations have complex morphology which result in differential stresses at various sites on the restoration surfaces (Tagtekin et al., 2004). According to Watts (1996) these methacrylates act as either open chains or residual free monomers, weakening the mechanical/physical properties of the dental material. In this situation, free monomers may be leachable in saliva, causing secondary caries, increasing the water sorption and consequently, interfering with the colour stability of the dental material (Watts, 1996). Consequently, a number of free monomers in the base of a RBC restoration (in direct contact with dentine substrate) seem to play an important role in the cytopathological effects of a restorative RBC by causing damage to the pulpal tissue (de Souza Costa et al., 2003).

Currently polymerization contraction ranging from 1.7 – 5.7% is the most adverse property of the available RBC materials (Lutz et al., 1991) as it is a major cause of its clinical failure. Notably the shrinkage stress on polymerisation may compromise the quality of the bond at the tooth–restoration interface and can lead to micro-leakage of bacteria, leading to pulpal inflammation, necrosis and secondary caries (Chung et al., 2002, Bhamra & Fleming, 2008). All RBCs shrink during light irradiation and it is important to minimize the effects of composite

shrinkage by incrementally placing materials at 1-2mm of depth in any increment for optimum polymerization with most conventional RBCs have a limited depth of cure of normally no more than 2–4 mm (Klaff 2001, Palin et al., 2003). However, even with meticulous clinical technique, there may not be ideal moisture control in the proximal box during bonding procedures and these margins are more susceptible to later debonding and so are at higher risk for microleakage or secondary caries (Bayne et al., 1994).

The durability of marginal adaptation is negatively influenced by three factors:

- Residual internal stresses generated by the polymerization shrinkage challenge the adhesive bond to the cavity walls and margins unless they are relieved by structural changes within the RBC restoration or the adjacent enamel and dentin,
- Chemical degradation debonds the tooth restoration interface,
- The differing physical properties of the dental hard tissues and the bonded materials have the potential to become destructive during mechanical and thermal stressing. (Lutz et al., 1991).

Although RBCs possess many advantages, including their ability in aqueous environments to absorb water, their release of unreacted monomers and the ingress of water can, in time, lead to the deterioration of the physical/mechanical properties. These problems are mainly due to a hydrolytic breakdown of the bond between silane and filler particles, filler-matrix debonding or even hydrolytic degradation of the fillers (Siderou et al., 2003). Notably whilst polymerization shrinkage can be reduced through limiting the degree of monomer conversion this will subsequently have adverse effects on the physical and mechanical properties of restorations.

Therefore maximum monomer conversion is always desired to ensure optimum properties and biocompatibility (Santos et al., 2004).

1.4.2 Determination of RBC Biocompatibility and Cytotoxicity

Cell culture studies are frequently used to assess the potential cytotoxicity of RBCs, their eluents, or individual components (such as monomers). Variable levels of cytotoxicity have been demonstrated for several RBC materials and their components. However, few studies have evaluated the relationship between cytotxicity and the structures of resin monomers (Issa et al., 2004). RBCs have been shown to exert a significant cytotoxic effect in cell culture and this has been proven to be caused by residual uncured monomer or oligomer (Ferracane & Condon 1990). RBCs are used with increased frequency as posterior restorative materials because of demand for both aesthetic restorations and worries about possible adverse effects of dental amalgam. However, pulpal sensitivity problems are more likely to occur with RBC materials due to gap formation secondary to polymerization shrinkage which occurs for many traditional materials (Bouillaguet et al., 2002). Most dental materials have to contact or interact with body tissue and fluids, so material selection must take into consideration not only mechanical and physical properties but also biological compatibility of a material (de Souza Costa et al., 2003). Although novel experimental RBC systems have exhibited promising mechanical properties, the slow development of flexural strength coupled with cytotoxicity and mutagenic concerns of oxirane-based resin blends for dental RBC application has prompted research into the development of an alternative class of ring-opening monomers (Palin et al., 2005). If a material has a high cytotoxicity initially, but gradually improves with aging, then this material might be

viewed more favourably than a material which continues to be cytotoxic following aging (Wataha et al., 2003). It has long been discovered that RBC materials can result in pulp inflammation (Nalçaci et al., 2004). Although the physical properties of RBCs are constantly being improved, *in vivo* studies have shown that their use is occasionally associated with necrosis and irritation of the pulp as well as the periodontium (Nalçaci et al., 2004). Adequate polymerization is a crucial factor in maximizing the physical properties and clinical performance of composite resin restorative materials and cytotoxicity is generally a result of residual uncured monomer or oligomer (Nalçaci et al., 2004, Wataha et al., 1992). Even in fully set RBC materials, substantial amounts of short-chain polymers remain unbound, with the result that there is possible elution of leachable toxic components towards the pulp (Nalçaci et al., 2004). There is also a correlation between the amount of uncured leachable resin in the RBC and the magnitude of the cytotoxic effect (Mantellini et al., 2003).

In previous studies a ring of inhibition of growth around the alloy as well as densitometric and visual intensity of the stained monolayer were used for quantification of the toxic effect. The value of any of these *in vitro* screening tests is dependant upon its repeatability, and an interpretable and translational measure of cytotoxicity. Repeatability can be difficult to obtain because of the many potential variables that can bias results between tests (Wataha et al., 1992). The rate of cell division is itself a tightly regulated process that is ultimately associated with growth, differentiation and tissue turnover. However, when cytotoxic stimuli are intense, cells may escape from the cell cycle and undergo a programmed cell death called apoptosis (Mantellini et al., 2003).

1.5 Aims of the Present Investigation

The aims of the present study were to analyse a commercially available dimethacrylate RBC (Filtek^{TM}Z250, 3M ESPE, St Paul, MN, US), an Ormocer (Admira, VOCO, Cuxhaven, Germany) and a novel RBC, namely X-tra Fil (VOCO, Cuxhaven, Germany). These dental materials were tested for bi-axial flexure, to determine the strength, and water sorption, water solubility and Vickers hardness measurements were determined following short-term (0.1, 0.5, 1, 4, 24 and 48 h) and medium-term (1, 4, 12 and 26w) water immersion at 37±1^{o}C. RBC materials are usually only cured to a depth of 2mm with concerns arising about the efficiency of a deeper cure, however, the novel composite X-tra Fil was tested to a depth of 4mm (as proposed by the manufacturer). X-tra Fil (t) was cured to a depth of 0-2mm and X-tra Fil (b) was cured to a depth of 2-4mm. It has been suggested that components released from RBC materials can be irritants to the pulpal tissues and be detrimental to cell viability, therefore the current study assessed the cytotoxic effects of each material in direct contact with ATCC Mouse Fibroblasts (3T3s) and Undifferentiated Pulpal Fibroblast cell lines (OD21s). The influence of substances leached from the RBC materials when immersed for short-term periods in growth medium was also determined.

CHAPTER 2 Methods and Materials

2.1 Biomaterials and Composite Material Testing

2.1.1 Materials

The materials tested in the current study included a conventional hybrid RBC (Filtek^{TM}Z250, 3M ESPE Dental Products Division, St Paul, MN, US; shade A3: batch 20050211) which contained BisGMA, UDMA and Bis-EMA resins with small amounts of TEGDMA and 61 vol.% zirconia/silica filler. The filler particle size in Filtek^{TM}Z250 was 0.01 to 3.5 µm, with an average particle size of 0.6 µm. The Ormocer (organically modified ceramic) used was Admira (Voco, GmbH, Cuxhaven, Germany; shade A3; batch 20050329) and contained as reported by the manufacturer BisGMA, UDMA and TEGDMA with 60.2% by volume of bariumboroaluminiumsilicate filler. Admira is a mixture of radiopaque glass ceramic with an average particle size of 0.7 µm and micro-fillers from pyrogenic SiO with particle sizes of approzimately 0.04 µm (as reported by the manufacturer). The recently marketed X-tra fil (Voco, GmbH, Cuxhaven, Germany; shade A3; batch 20050329) which contains a resin mixture of Bis-GMA, UDMA and TEGDMA with 70.1% by volume bariumboroaluminiumsilicate filler (as reported by the manufacturer), was also examined.

Preparation of Composite Material Samples

The uncured paste of Filtek^{TM} Z250 and Admira was weighed out and packed into a black, circular, nitrocellulose mould, 11mm diameter and 2mm depth (Figure 2.1).

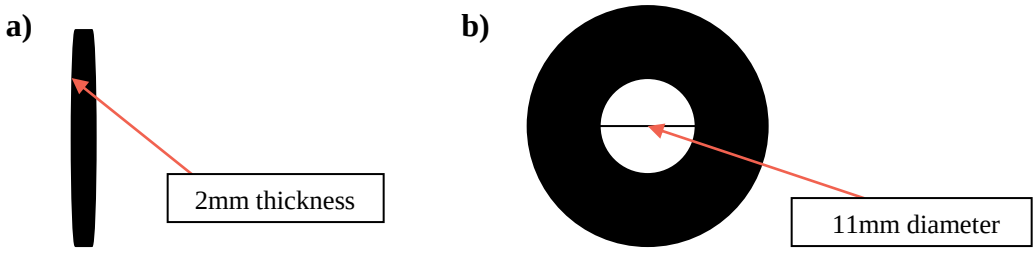


Figure 2.1 Diagram illustrating the nitrocellulose mould used. a) side view showing the 2mm thickness and b) top view demonstrating the 11mm diameter of the mould.

The mould was lined with DVA Very Special Separator, to ensure that the disc would be able to be removed from the mould once it had been cured. Cellulose acetate strips were placed on each surface of the mould in order to prevent oxygen inhibition. The mould was placed on a black Nylotron base and a 1Kg, steel load was placed on the top surface for 30 seconds for pressure, to ensure consistent packing. The specimens were then irradiated with a conventional halogen XL2500 curing-light (3M ESPE Dental Products, St Paul, MN, US) according to the time periods specified by manufacturer's instructions for each material, i.e. 20 seconds for both Filtek^{TM} Z250 (3M ESPE) and Admira (VOCO) (Figure 2.2). The light-intensity of the curing-unit was measured prior to the fabrication of each specimen set (to be between 660 and 680 mW/cm^2) using a Model 100 curing radiometer (Demetron Research Corp., Danbury, CT). A 13mm LCU tip diameter was employed to ensure adequate irradiation of the entire specimen. Following irradiation, the specimen was removed from the mould and any excess material was removed using Silicon Carbide abrasive paper (500 grit, Struers, Copenhagen, Denmark). Each disc was labelled, ensuring that the surface that was directly exposed to the light was clearly marked.

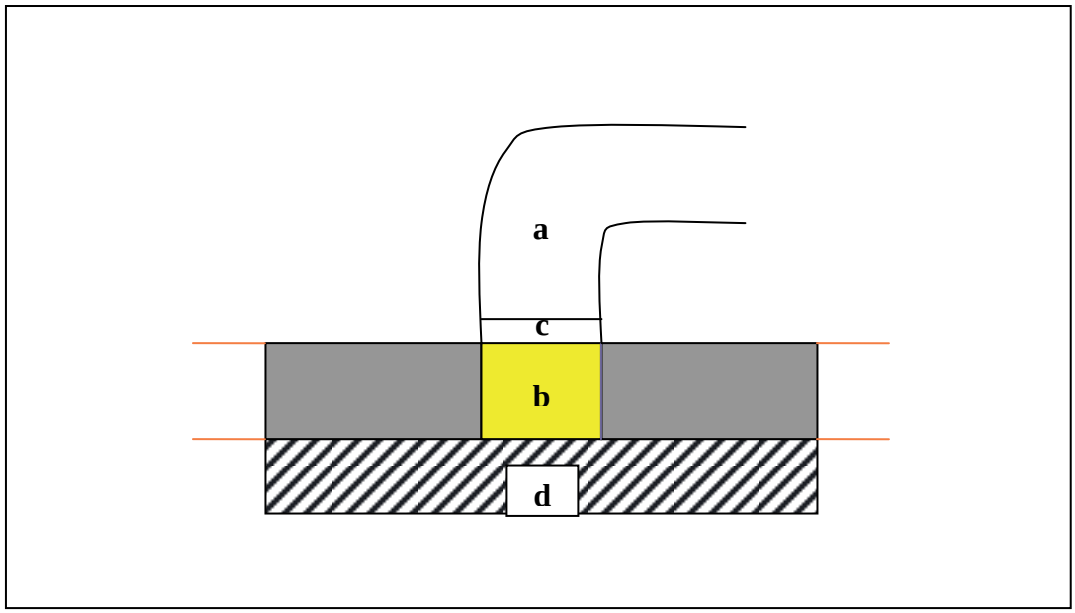


Figure 2.2 A schematic diagram of the curing method used to produce each specimen disc. The curing tip was positioned above the specimen surface (a), using a ring-spacer of thickness 2mm and 11mm diameter (c). The specimen (b) was placed within a black nitrocellulose mould with acetate strips covering each surface and light-irradiated above a black Nylotron base (d).

To investigate the manufacturers' claim that X-tra fil could be cured to a depth of 4mm, two black nitrocellulose moulds were employed. The reason for manufacturing two separate specimen discs i.e X-tra fil (t) (0-2 mm) and X-tra fil (b) (2-4 mm) was so that the two types could be compared with regards to their strength, water solubility, water sorption, hardness and biocompatibility. This analysis will provide an indication as to whether X-tra-fil can be fully cured to a depth of 4mm. Prior to irradiation, two moulds were placed on top of each other, separated by an acetate strip, and specimens were cured in accordance with the procedure outlined above for 40s which was the time recommended by the manufacturers (VOCO). The cured discs were then labelled as outlined above. The specimens were divided into two groups of 30 specimen discs X-tra fil (t) (0-2 mm curing depth) and X-tra fil (b) (2-4 mm curing depth).

2.1.2 Bi-axial Flexure Strength Determination

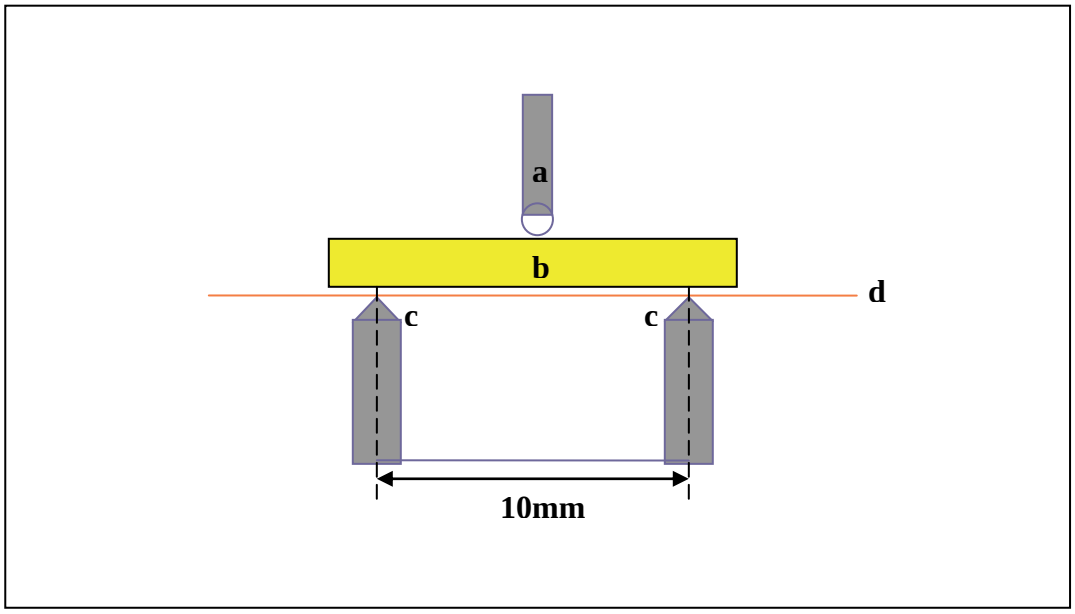
Thirty specimens of each RBC group were investigated and were prepared according to the method previously described (Section 2.1.1) and incubated for 24 hours at 37^{o}C. The bi-axial flexure strength of each specimen, was determined using a universal tensile testing instrument, Model 5544 (Instron Ltd, Buckinghamshire, England). Each of the specimens was tested by imposing a central load using a 4mm ball indenter on a knife-edge support, at a crosshead speed of 1mm/min. The specimens were loaded uniformly and this was assisted by the use of a thin, square sheet of rubber which was placed between the knife edge support and the sample itself (Figure 2.3). The specimen number, load at fracture number of fracture fragments and disc thickness at the point of fracture was recorded for each specimen.

The bi-axial flexure strength for each disc was calculated in accordance with Equation 2.1

$$\sigma_{\max} = \frac{P}{h^2}\{(1+\nu)[0.485 * \ln(\frac{a}{h}) + 0.52] + 0.48\} \qquad \textbf{Equation 2.1}$$

where σ_{max} was the maximum tensile stress, *P* the measured load to fracture, *a* the radius of the knife-edged support, υ the Poisson's ratio for the material (a value of 0.225 was substituted for the RBCs and Ormocer (Palin et al., 2003) and *h* was the specimen thickness measured with a micrometer screw gauge (Moore and Wright, Sheffield, England) accurate to 10 μm.

a)



b)

Figure 2.3
a) A schematic diagram illustrating the ball-on-ring bi-axial flexure test. A 4mm ball indenter (a) imposes an increasing load on the cured surface of an 11mm diameter, 2mm thick disc-shaped specimen (b) which was placed centrally on the 10mm circular knife edge support (c). A thin rubber sheet was placed between the knife-edge support and the specimen to accommodate for slight distortions in the peripheral thickness of the specimen (d).

b)A photograph illustrating the 4mm ball indenter set up which was used to exert pressure on the disc-shaped specimen surface (cured side uppermost).

The bi-axial flexure for each specimen was calculated and the mean and standard deviation of these results for each material was then determined. From the bi-axial flexure strength values, the variability of each data set was calculated using the Weibull Distribution (Weibull 1951). The Weibull equation assumes the most critical flaw in a specimen is responsible for specimen failure and is based on the 'weakest' link hypothesis, derived to assess the probability with which failure occurs within a material when a given load is applied (Weibull, 1951). The basic form of the Weibull distribution is calculated in accordance with Equation 2.2

$$P_f = 1 - \exp\left[-\left(\frac{\sigma}{\sigma_0}\right)^m\right] \qquad \textbf{Equation 2.2}$$

where σ_0 and m are constants. m is known as the Weibull modulus characterising the 'brittleness' of a material. A higher value of m indicates a close grouping of the flexure stress data and σ_0 is the normalising constant or the characteristic stress (MPa) which is calculated at 63.21% failure probability. The 95% confidence limits for the groups were calculated and differences were considered to be significant when the confidence intervals did not overlap.

2.1.3 Water Sorption and Water Solubility Determination

In accordance with the ISO specification for water sorption and solubility of polymer-based filling, restorative and luting materials (ISO 4049; 2000), disc-specimens (11.0 ± 0.1mm diameter and 1.0 ± 0.1mm thickness) were prepared according to the procedure outlined above and assessed following short- and medium-term water immersion periods. For the medium- term

immersion periods (1, 2, 4, 12 and 24 weeks) each specimen of Filtek^{TM}Z250, Admira and X-tra fil (t) (0-1mm) and X-tra fil (b) (3-4mm) was weighed and transferred to a lightproof desiccator containing dehydrated silica gel (Fischer Scientific, Leicester, UK) maintained at 37 ± 1^{o}C for 22 hours followed by 23 ± 1^{o}C for 2 hours. The X-tra fil (b) (3-4mm) was the bottom 1mm which was representative of the material that was irradiated through the top 3mm of material, whilst Filtek^{TM}Z250, Admira and X-tra fil (t) (0-1mm) was the top 1mm where the top of the specimen was directly exposed to the light. The specimens were then reweighed and the conditioning cycle repeated until the mass loss of each specimen (m_1) was not more than 0.001g. After the conditioning cycle, the diameter and thickness of each specimen was measured using a micrometer screw gauge accurate to 10μm to calculate the specimen volume (V) in mm^3. It was not possible to achieve the preconditioning with the short-term (0.5, 1, 4, 24 and 48 hours) immersion samples due to time constraints (Palin et al., 2005). Five specimens for each short- and medium-term immersion period investigated were suspended in 1.5ml of high purity double distilled water in each well of a 24 well plate (Costar® Corning Inc., NY, USA) and subsequently stored in a lightproof container maintained at 37 ± 1^{o}C. In an attempt to avoid variations in the pH level of the solute, the distilled water was replaced every seven days. Following each short- and medium-term immersion period each specimen was removed and the excess water eliminated using absorbent tissue. In addition, the sample was waived in air for 10 seconds and reweighed (m_2). The immersed specimens were subjected to the aforementioned conditioning cycle until the mass loss of each specimen (m3) was not more than 0.001g. The water sorption (WS) and the water solubility (SL) of each of the five disc-shaped specimens for the short- and medium-term immersion periods were calculated in accordance with Equations 2.3 and 2.4 (Palin et al., 2005)

$$WS = \frac{m_2 - m_3}{V}$$ **Equation 2.3**

$$SL = \frac{m_1 - m_3}{V}$$ **Equation 2.4**

Diffusion Coefficient

Established diffusion theory previously used for estimating the water uptake behaviour of RBCs (Sideridou et al., 2004, Kanchanavasita et al., 1997, Kalachandra et al., 1987) was employed as the method of calculating the diffusion coefficient utilized. Fick's Law (Ferracane et al., 2006) predicts for the initial stages of water sorption (when $M_t/M_e \leq 0.6$) that

$$\frac{M_t}{M_e} = 2\left(\frac{Dt}{\pi l^2}\right)^{\frac{1}{2}}$$ **Equation 2.5**

where *Mt* was the mass uptake (g) at time *t* (s), *Me* was the mass uptake (g) at equilibrium, *l* was the specimen thickness (m) such that *D* is the diffusion coefficient (m2s-1) calculated from the gradient of M_t/M_e against $t^{1/2}$.

2.1.4 Vickers Hardness Testing

The specimens used for the water sorption and water solubility test were used to determine how the ageing in distilled water over short- and medium-term immersion periods alters the surface hardness. This was performed using the Duramin-1 Vickers Hardness tester (Struers, Glasgow, Scotland) (Figure 2.4).

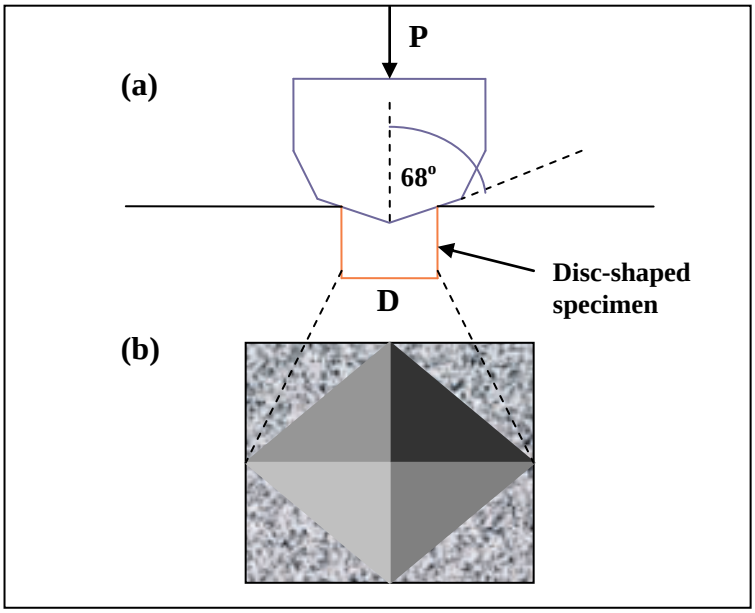


Figure 2.4 A schematic diagram illustrating (a) the diamond crosshead indenter with an indentation angle of 68° and (b) the indent pattern at the surface of the disc-shaped specimen produced as a result of an applied load (P) from the Vickers Hardness tester. The average diagonal distance (D) can subsequently be used to calculate the VHN (kgf/mm^2).

The machine is fitted with a diamond pyramid indenter head which, under a predetermined load, applied a downward force to the surface of the sample disc, leaving an indentation. The size of the indentation was then measured according to each diagonal distance of the pyramid shaped indent. The size of the diagonal distance produced by the indenter head was measured using a micrometer screw gauge which was built into the microscope and attached to the Duramin-1 Vickers Hardness tester. The gauge provided the average value for the diagonal distance (D) produced by each indent in micrometers (µm). For each specimen disc, five measurements were taken on each side. After gathering the diagonal distance values, the Duramin-1 Vickers Hardness tester calculated the Vickers Hardness number for each separate indentation on the discs. Subsequently the mean was calculated for data set at each immersion time period. Five

hardness measurements were taken across the top and bottom surfaces immediately after immersion using a 500g load for 15s and a mean Vickers hardness (VH) was calculated in accordance with Equation 2.6.

$$VH = \frac{1.854P}{D^2} \qquad \textbf{Equation 2.6}$$

where P was the predetermined load applied (g) and D the average diagonal distance (μm) where the angle of the indentation of the diamond pyramid head tip of the Vickers indenter Duramin-1 (Struers, Copenhagen, Denmark), namely 68º.

2.1.5 Statistical Analysis

Multiple comparisons of the bi-axial flexure strength, water sorption and water solubility and diffusion coefficients and Vickers hardness for the top and bottom surfaces group means were made utilising one-way analysis of variance (ANOVA) and Tukey's multiple range test at a significance level of $P<0.05$ (using SPSS® version 11.5 for Windows®).

2.2 Cell Culture Studies

2.2.1 Culture of ATCC Mouse 3T3 Fibroblasts and Undifferentiated Pulpal Fibroblast Cell Lines (OD21 cells).

2.2.1.1 Preparation of Basic Growth Medium (Complete Medium)

The preparation of all media was carried out under sterile conditions in the laminar flow hood to prevent contamination. Complete medium was prepared prior to the culture of ATCC Mouse 3T3 Fibroblasts (3T3 cells) and Undifferentiated Pulpal Fibroblast cell lines (OD21 cells). The components of the complete medium were Dulbecco's Modified Eagle Medium (DMEM) without glutamine, 4.5g/l glucose, 1.5g/l sodium bicarbonate (Labtech International, UK), supplemented with 10% Foetal Calf Serum (FCS) (Labtech International, UK),100 units/ml of Penicillin (Sigma, UK), 100 µg/ml of Streptomycin (Sigma, UK) and 25mM HEPES buffer (Sigma, UK) at pH 7.4; Dulbecco's Modified Eagle Medium (DMEM) with 0.297g/500ml L-Glutamine (filter sterilised), 4.5g/l glucose, 1.5g/l sodium bicarbonate (Labtech International, UK), supplemented with 10% Foetal Calf Serum (FCS) (Labtech International, UK), 100 units/ml of Penicillin (Sigma, UK), 100 µg/ml of Streptomycin (Sigma, UK), respectively. HEPES buffer was synthesized by dissolving 2.383g of HEPES powder (Sigma, UK) in 10ml of distilled water. The pH of this resulting solution was then adjusted accordingly using 1M Hydrochloric Acid (Sigma, UK) and 1M Sodium Hydroxide (Sigma, UK) solutions, until a stable pH of 7.4 was achieved. The resultant buffer was then filter sterilised prior to addition to the complete medium for 3T3 cells.

2.2.1.2 Preparation of Maintenance Medium

Both the 3T3 and OD21 cells have a reasonably high rate of proliferation with both cell types achieving maximum confluence density of 40,000 and 30,000 cells in a 75cm^2 and 25cm^2 flask respectively, over a period of approximately three days. However, in the present study, a slower rate of proliferation was needed to ensure that the cells were confluent on the day they were needed for sub-culture. The components of the maintenance medium for 3T3 and OD21 cells were DMEM without glutamine, 4.5g/l glucose, supplemented with 2% FCS, 100 units/ml of Penicillin, 100µg/ml of Streptomycin and 25mM HEPES buffer at pH 7.4; DMEM with 0.297g/500ml L-Glutamine (filter sterilised), 4.5g/l glucose, 1.5g/l sodium bicarbonate, supplemented with 2% FCS, 100 units/ml of Penicillin and 100µg/ml of Streptomycin, respectively.

2.2.1.3 Preparation of Trypsin-EDTA Solution

Trypsin-EDTA solution was used to sub-culture the cells once they had reached their optimum confluency. This solution was used because it causes cell detachment from the surface of the culture flasks without causing irreversible damaging, however over exposure can lead to cell death. Therefore care was taken to ensure that the cells were not exposed to the Trypsin-EDTA for longer than 5 minutes or at least until all the cells were seen, using a light inverted microscope, to have detached from the surface of the flask. The components of Trypsin-EDTA solution were 1:250 Trypsin powder (sigma, UK) containing 1:500 Nα-benzoyl-L-arginine ethyl ester (BAEE) units/mg solid trypsin activity and 3 Nα-benzoyl-L-tyrosine ethyl ester (BTEE) units/mg solid chymotrypsin activity. From this mixture 10mg/ml was dissolved into Hank's Balanced Salt Solution with EDTA (HBSS + E) and the pH of the resulting solution was

adjusted accordingly by the addition of 1M NaOH (filter sterilized) until a stable pH of 7.4 was achieved.

2.2.1.4 Retrieval of Cells from Liquid Nitrogen

The required amount of complete medium was pre-equilibrated in a humidified incubator at 37^{o}C and 5%CO_2 until needed. The frozen vial of cells was removed carefully from liquid nitrogen and the cap loosened slightly in the laminar flow hood. The vial was placed immediately in the water bath (42^{o}C) where the thawing process takes place rapidly, ideally in 1-2 minutes to try and maintain cell viability. Care was taken not to overheat the cells. Once thawed, the vial was sterilized by rinsing it with 70% ethanol and the 500µl of cell suspension was transferred into the flask already containing the equilibrated complete medium. The newly seeded flask was placed in a humidified incubator at 37^{o}C and 5%CO_2 for 24 hours to allow the cells to attach. Subsequently, the medium was replaced to remove all traces of Dimethyl Sulphoxide (DMSO) (Sigma, UK) because this can cause toxicity and cell death. DMSO was present in the cryogenic solution that the cells were frozen in. the cryogenic solution consists of 70% DMEM, 20% FCS and 10% DMSO.

2.2.1.5 Sub-Culture of Cell Lines.

Once confluent, the cells were sub-cultured to separate flasks. In the present study, one flask containing growing cultures was sub-cultured into three new flasks. The required amount of complete medium for both the 3T3 and OD21 cells was prepared and equilibrated in a humidified incubator at 37^{o}C and 5%CO_2 (15ml of complete medium for each 75cm^2 flask). The flask containing the growing cultures was removed from incubation and the medium disposed of

in “Chloros” bleach (Chloros, UK) in the laminar flow hood. The culture surface was washed briefly with 5ml HBSS (without EDTA) and aspirated. 5ml of Trypsin-EDTA solution was then added and the flask incubated for approximately 5 minutes at 37^{o}C and 5%CO_2 . Flasks were observed frequently under the light inverted microscope (Keyence, UK) to determine whether the cells had detached from the flask surface. Once the cells were seen to have detached successfully, the flask was tapped gently to disassociate the cells. All cell clumps were eliminated for 3T3 and OD21 cells by gently pipetting the cell suspension. During this stage, care was taken not to lyse the cells due to vigorous pipetting. The monocellular suspension was transferred into a sterile 10ml tube using the relevant pipette and 5ml of the equilibrating complete medium was added to stop the action of the Trypsin-EDTA solution. The serum in the medium stops trypsin activity as it contains inhibitors. The cell suspension for 3T3 and OD21 cells were centrifuged (Jouan B4i) at 1000rpm for 2 minutes and 800 rpm for 5 minutes, respectively, to pellet the cells. The supernatant was carefully aspirated with care taken not to disrupt the cell pellet. Cells were resuspended in 3ml of complete medium and gently swirled to ensure a homogenous suspension. This cell suspension was then divided equally between three newly labeled flasks and 14ml of complete medium added to each flask. The newly seeded flasks were then placed in a humidified incubator at 37^{o}C and 5%CO_2 and checked under the light inverted microscope every 24 hours until the cells were seen to be confluent, i.e. a monolayer of cells seen on the flask surface. At this point the cells were further sub-cultured.

2.3 Determination of Cytotoxicity of Composite Materials

2.3.1 Direct Contact Technique

Thirty specimens of each composite material were made as previously described (Section 2.1.1) along with 6 Teflon discs were used as a control for each immersion time period. For the cytotoxicity assays (including both direct contact and the media immersion techniques), X-tra fil specimens were cured to a depth of 2mm in contrast to the mechanical testing studies reported which utilized X-tra fil cured to a depth of 4mm. Teflon was used as a control as previous work indicates it does not affect cell growth (Wataha et al., 1992). Samples were placed in a humidified incubator at 37^{o}C and 5%CO_2 for 24 hours. After incubation, the specimens were sterilized in the laminar flow hood by wiping each one with 70% ethanol and then placing them into each well of a 24 well plate (Thermo Fisher Scientific, UK) (Figure 2.5). Five plates were set-up for each of the time periods of 24 hours, 48 hours, 72 hours, 1 week and 2 weeks. 25,000 cells of both 3T3 and OD21 cells were seeded in each well of the 24 well plates (see sub-culturing method 2.2.1.5) with complete medium added ensuring that the volume in each well was 1.5ml. Plates were incubated at 37^{o}C and 5%CO_2 for the relevant time periods after which the cell numbers were counted using the Haemocytometer method (Section 2.3.1) or the cell density was determined using the Neutral Red Assay (Section 2.3.2).

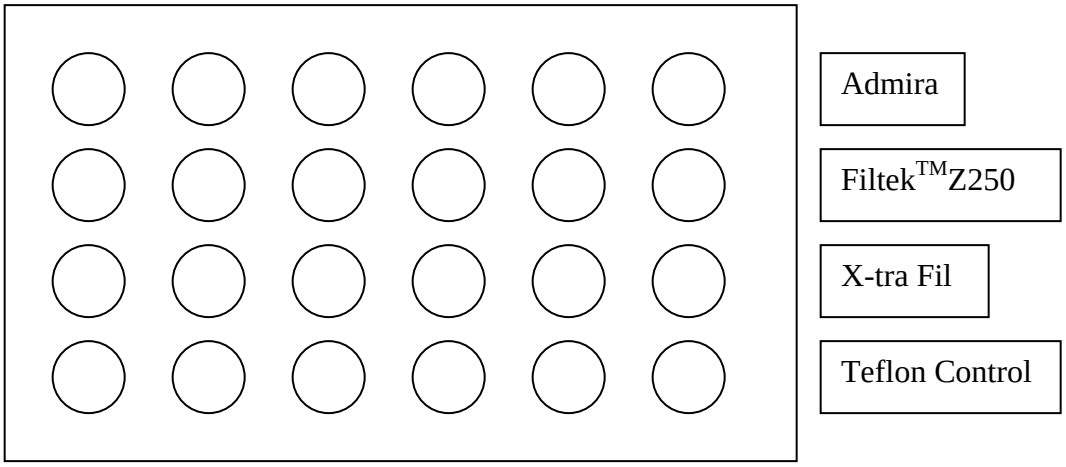


Figure 2.5 Schematic diagram illustrating the organisation of each 24 well plate which were set up for each of the time periods of 24h, 48h, 72h, 1week and 2weeks, for the Ormocer Admira (VOCO), the dimethacrylate RBC Filtek™Z250, the novel RBC X-tra Fil and the Teflon Control. This enabled 6 replicates of each material for each of the time periods.

2.3.2 Media Immersion Technique

Thirty specimens of each composite material were fabricated using the method previously described (see 2.1.1). These specimen discs were then subjected to the conditioning cycle until their mass was constant (1×10^{-3}g) (m_1). The samples were sterilised in the laminar flow hood by wiping them with 70% ethanol and then placing in each well of a 24 well plate (Figure 2.6) ready for immersion in medium. Each well was filled with 1.5 ml of growth medium and placed at 37^{o}C and 5%CO_2 for time periods of 24hours, 48hours, 72hours, 1week, and 2weeks. Twenty-four hours prior to the conclusion of each time period, each well of a new 24 well plate was seeded with the relevant cell type at a density of 25,000 cells (see sub-culturing method 2.2.1.5). This approach was used to allow the attachment of the cells before the medium was replaced with that from the specimen disc immersions. After 24 hours, the growth medium in the new plate was aspirated and replaced with the immersion growth medium containing substances

leached from each composite material. The plate was then placed in a humidified incubator at 37^{o}C and 5%CO_2 for 72hours. Following 72 hours of incubation, the cells in 3 of the 6 wells for each composite material were subjected to counts using the Haemocytometer method and the final 3 wells were analysed using the Neutral red assay method (Figure 2.6). Following each immersion time period, the composite specimens were removed from each well of the 24 well plate and blotted with absorbent tissue to remove any excess medium. The sample was then waived in the air (23 +- 1^{o}C) for 10 seconds and weighed again (m_2). This value was recorded and the sample was again subjected to the conditioning cycle, until the mass loss was no more than 1 x 10^{-3}g (m_3).

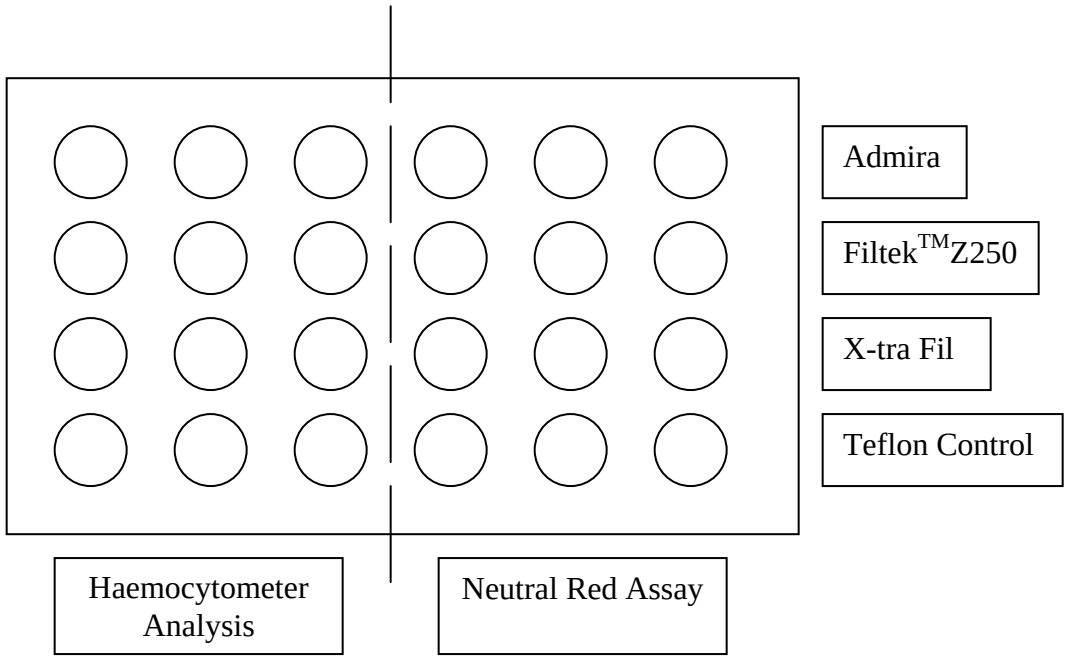


Figure 2.6 Schematic diagram illustrating the organisation of each 24 well plate set up for each of the media immersion time periods of 24h, 48h, 72h, 1week and 2weeks, for the Ormocer Admira (VOCO), the dimethacrylate RBC Filtek™Z250, the novel RBC namely X-tra Fil and the Teflon Control. The samples subjected to cell counting using the Haemocytometer technique and the samples subjected to the Neutral Red Assay are shown.

2.4 Cell Viability

2.4.1 Determination of Cell Density using the Haemocytometer Technique

Once the cells present in the 75cm^2 flasks were confluent they were prepared for cell counting using the haemocytometer. The required amount of complete medium was placed in the humidified incubator at 37^{o}C and 5%CO_2. The medium present in the flask was then discarded and 5ml of Trypsin-EDTA was added. This was then placed at 37^{o}C for approximately 5 minutes to allow the cells to detach from the flask surface. Once detached, the cell suspension was transferred from the flask into a sterile 10ml tube using a pipette and 5ml of the fresh growth medium was added. The 3T3 and OD21 cell suspension was centrifuged at 1000rpm for 5minutes and 800rpm for 3 minutes, respectively, to give a cell pellet. The supernatant was then carefully aspirated taking care not to dislodge the cell pellet. 1ml of fresh growth medium was added and the suspension was swirled to ensure that any cell clumps were removed and a monocellular cell suspension was produced. In order to perform cell counts over a 5 day period, five 35 cm^3 sterile culture dishes were seeded with 100,000 cells for each cell type. These dishes were incubated for 1hour to allow the cells to attach, after which 1ml of complete medium was added.

Cell counts were taken everyday for five days. Each day one sample was removed from incubation and the culture medium transferred into an eppendorf. 500µl of Trypsin-EDTA solution was added and the dish then placed at 37^{o}C and 5%CO_2 for 5 minutes. After incubation, the cell suspension was added to the eppendorf containing the culture medium and was vortexed to destroy any cell clumps ensuring a monocellular suspension. From this cell suspension, 100µl was transferred into a fresh eppendorf. To ensure that only live cells were counted, 30µl of 1M Trypan Blue Dye (Sigma, UK) was added. This stains dead cells which can therefore be

excluded from the cell counts. This mixture was incubated for 10 minutes at room temperature and the cell suspension counted using a haemocytometer. A glass coverslip was placed over the haemocytometer and 10µl of the cell suspension pipetted into each side of the groove. The haemocytometer was then placed under a light inverted microscope and the 5x5 grid identified (Figure 2.7). The total cells present in this grid were counted and cells stained blue were also recorded. This procedure was repeated 10 times. The results were recorded and the numbers of blue stained cells were subtracted from the total number of cells counted. Eventually, once all the cell counts had been recorded, a graph was plotted of percentage cell viability of control against time.

Figure 2.7 Illustration of the haemocytometer grid as seen under the light inverted microscope. The cell count for each day was taken from the central square. Key for size of each shaded area is shown.

2.4.2 Neutral Red Absorption Assay

For this assay, 24 well plates were used and 25,000 cells were seeded per well (see sub-culturing method 2.2.1.5). Plates were incubated in a humidified incubator at 37^{o}C and 5%CO_2 for 24 hours to allow the cells to adhere. After 24 hours, the first plate was removed from incubation. The components of the 0.4% aqueous neutral red stock solution were 0.4g of neutral red (Sigma) in 100ml of distilled water. From this stock solution the working neutral red solution was prepared by adding 12.5µl/ml of the neutral red stock solution to 12mls of growth medium for each cell type. The culture medium from each well was aspirated and replaced with 800µl of the filter sterilised working neutral red solution. The plate was then incubated for 3 hours at 37^{o}C and 5%CO_2. Following incubation, the neutral red media was discarded and each well was rapidly washed with the "wash solution". This consisted of 40% formaldehyde, 10% w/v Calcium Chloride (10g $CaCl_2$ in 60ml distilled water plus 40ml of 10% buffered formalin). Following washing, 800µl of "dye extraction solution" was added to each well and the plate was left at room temperature for 20minutes. The components of the dye extraction solution were 1% Glacial Acetic (Sigma) acid and 50% ethanol (Sigma) (99ml of 50% ethanol plus 1ml of glacial acetic acid). The plate was then placed on a plate shaker for 1minute and the absorbance of each well was read at 630nm on a Bio-Tek plate reader (Vermont, USA). The values obtained were expressed as a percentage of the Teflon control.

CHAPTER 3 Results

3.1 Bi-axial Flexure Strength Analysis

The mean bi-axial flexure strength of the groups of 30 disc-shaped specimens of Filtek™ Z250, Admira, X-tra fil (t) (0-2mm) and X-tra fil (b) (2-4mm) were 142 ± 20MPa, 131 ± 17MPa, 156 ± 14MPa and 148 ± 18MPa, respectively (Table 3.1). A one-way analysis of variance (ANOVA) and paired Tukey test comparisons at the 95% significance level revealed that there was no significant difference ($P>0.05$) between the mean bi-axial flexure strengths of the Filtek™ Z250, Admira, X-tra fil (t) and X-tra fil (b) investigated in the current study (Table 3.1).

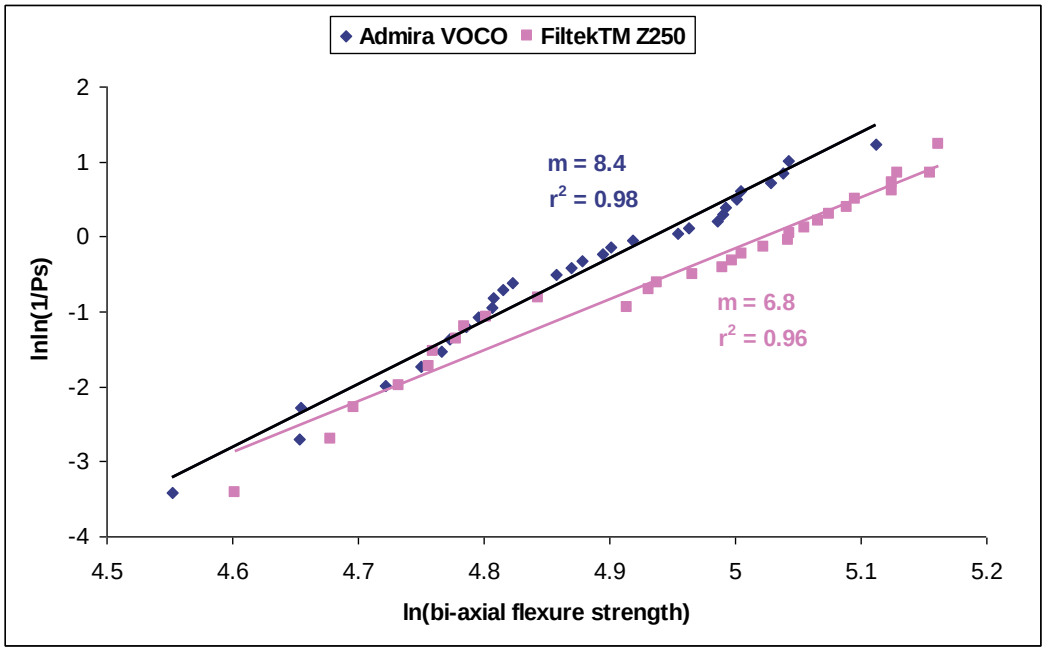
The bi-axial flexural strength data was ranked in ascending order and a Weibull analysis was performed and recorded. The 95% confidence intervals of the bi-axial flexural strength data was also recorded (Table 3.1). The reliability of the strength distribution was increased for the X-tra fil (t) compared with the Filtek™ Z250, Admira, X-tra fil (b) groups as the confidence intervals failed to overlap significantly. In addition the Filtek™ Z250 group was identified as having reduced reliability of the flexure strength data compared with the Admira and X-tra fil (b) groups where the confidence intervals overlapped (Table 3.1). In addition, the characteristic stress (σ_0) was identified to decrease for all materials except X-tra fil(t) where the value remained constant (Table 3.1)

	Admira	Filtek Z250	X-tra fil (t)	X-tra fil (b)
Mean Strength (MPa)	131 (17)	142 (20)	156 (14)	148 (18)
Characteristic Stress (MPa)	123	139	156	146
Weibull Modulus	8.4 (1.2)	6.8 (1.2)	12.3 (1.2)	8.6 (1.2)
Confidence Intervals	7.9-8.9	6.3-7.3	11.2–13.4	7.9-9.3
R^2-value	0.98	0.96	0.95	0.96

Table 3.1 The mean bi-axial flexure strength and reliability of Filtek™ Z250, Admira and X-tra fil (t: 0-2mm) and X-tra fil (b: 2-4mm) following 24 hour immersion in a lightproof water-bath maintained at 37 ± 1°C. Standard deviations are displayed in parentheses.

The r^2 value provides an indication of the data correlation coefficient which is calculated following the utilisation of a least squares analysis on the plots of $\ln\ln(1/P_s)$ against the $\ln\sigma$. An r^2-value equal to 1 would represent a perfect alignment along the line of best fit (Figure 3.1). However, an r^2-value of 0.95 is generally accepted to be a good agreement of the flexure strength with the regression line (Lipson and Sheth, 1973). A varied number of fracture fragments for each disc shaped specimen were produced following the bi-axial flexure strength testing (Table 3.2).

(a)



(b)

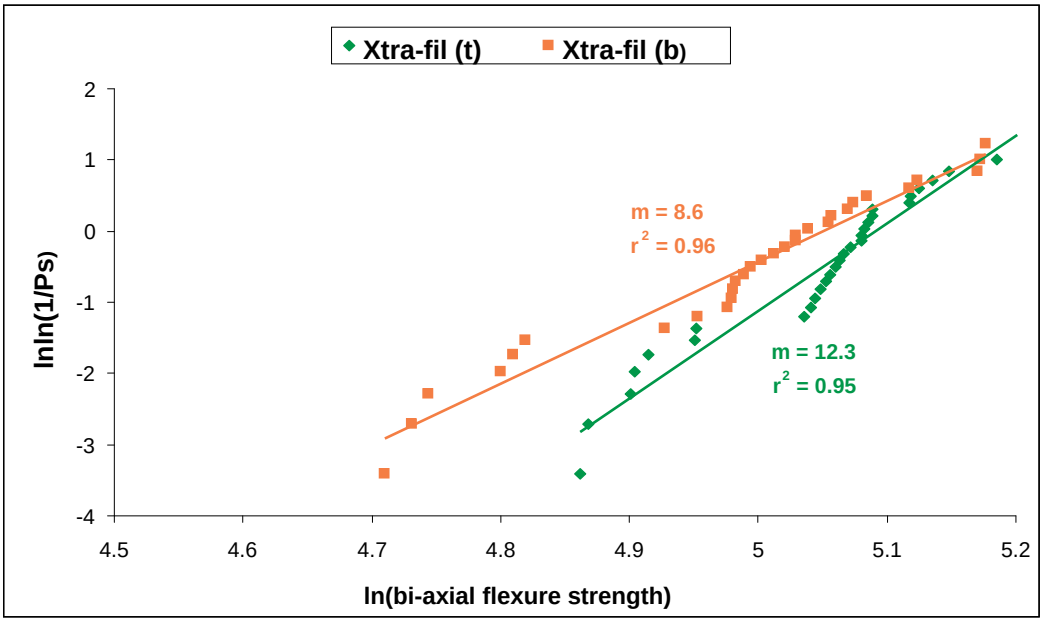


Figure 3.1 Weibull plots illustrating the bi-axial flexure strength distributions, Weibull moduli (m) and r^2 values for (a) Admira VOCO and Filtek^{TM} Z250 and (b) X-tra fil(t) (0-2mm curing depth) and X-tra fil (b) (2-4mm curing depth). The r^2 value for each of the composite materials was greater than 0.95 suggesting that the flexural strength data for all the materials was representative of a uni-modal distribution of a single type of defect.

Bi-axial flexure fracture fragments

A varied number of fracture fragments for each disc shaped specimen were produced following the bi-axial flexure strength testing (Table 3.2; Figure 3.2).

Both Table 3.2 and Figure 3.2 show that X-tra fil (t) had fracture fragments of either 3 or 4 while Admira and Filtek^{TM}Z250 had a range from 2 to 7. This suggests that X-tra fil cured to a depth of 2mm has properties making it more resilient to fracture, however in this study there was no significant evidence to prove this statement and further research would need to be completed before any conclusions made

Number of Fragments	Admira (VOCO)	Filtek^{TM} Z250	X-tra fil(t)	X-tra fil(b)
2	2	1	-	4
3	3	6	24	18
4	5	13	6	8
5	14	9	-	-
6	4	1	-	-
7	2	-	-	-

Table 3.2 Following bi-axial flexure strength testing the number of fracture fragments produced for each restoratove material was recorded. In contrast, the novel RBC namely X-tra fil seems to have a higher number of disc shaped specimens producing a lower number of fracture fragments suggesting that it possesses properties more resilient to loads applied to it.

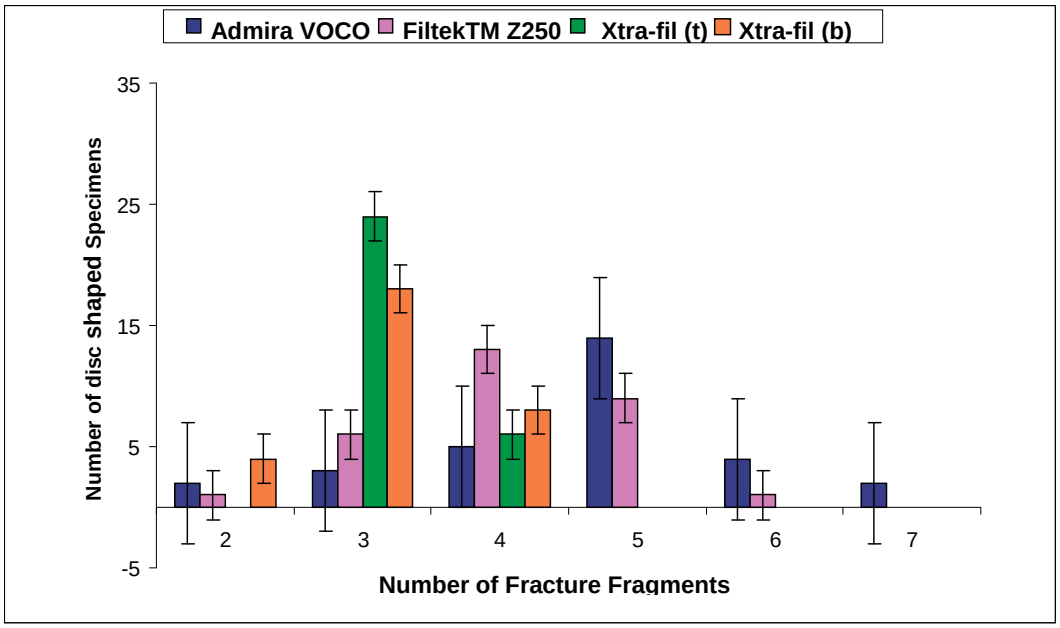


Figure 3.2 The combined plot for the number of fracture fragments produced for 30 disc shaped specimens of each composite material. In contrast the specimens of Admira VOCO and Filtek™ Z250 have a wider range of numbers of fracture fragments, with specimens producing fracture fragment numbers ranging from 2 through to 7. X-tra fil exhibited less varied fracture fragment numbers ranging from 2 through to 4. This suggests that X-tra fil may possess properties that can withstand larger loads applied to it.

3.2 Effect of Post-Irradiation Water Immersion

3.2.1 Water Sorption

The mean water sorption for both short-term (0.5, 1, 3, 24 and 48 hours) and medium-term (1, 2, 4, 12 and 24 weeks) immersion periods were recorded (Tables 3.3 & 3.4). In line with the specification standard for polymer-based filling, restorative and luting materials (ISO 4049: 2000) the water sorption values obtained for Filtek™ Z250 (12.3 ± 1.8μg/mm^3) and Admira (16.0 ± 1.5μg/mm^3) were significantly increased ($P<0.05$) compared with X-tra fil (t) (5.4 ± 0.7μg/mm^3) and X-tra fil (b) (6.8 ± 0.6μg/mm^3) but lower than the specification standard at 1 week of ≤40μg/mm^3, respectively.

Short-Term immersion periods (hours)	Material	Water Sorption (μg/mm^3)	Water Solubility (μg/mm^3)
0.5	Admira	1.1 (0.5)	1.7 (0.2)
	Filtek™ Z250	0.7 (0.6)	1.9 (0.4)
	X-tra fil (t)	0.7 (0.5)	0.4 (0.2)
	X-tra fil (b)	0.9 (0.3)	0.5 (0.2)
1	Admira	2.8 (0.4)	2.4 (0.2)
	Filtek™ Z250	2.5 (0.7)	2.2 (0.4)
	X-tra fil (t)	1.0 (0.6)	0.5 (0.2)
	X-tra fil (b)	1.2 (0.7)	0.7 (0.3)
4	Admira	4.9 (0.5)	2.5 (0.2)
	Filtek™ Z250	5.2 (0.4)	2.3 (0.9)
	X-tra fil (t)	1.8 (1.0)	0.9 (0.7)
	X-tra fil (b)	3.7 (0.6)	1.4 (0.5)
24	Admira	9.6 (0.4)	3.3 (0.3)
	Filtek™ Z250	10.3 (0.9)	2.4 (0.5)
	X-tra fil (t)	4.0 (0.5)	0.8 (0.2)
	X-tra fil (b)	4.7 (0.6)	1.6 (0.3)

48	Admira	13.1 (0.9)	4.2 (0.3)
	Filtek™ Z250	13.1 (0.6)	2.5 (0.2)
	X-tra fil (t)	5.1 (0.6)	0.9 (0.6)
	X-tra fil (b)	6.2 (0.2)	1.9 (0.4)

Table 3.3 The mean water sorption and solubility of Filtek™ Z250, Admira, X-tra fil (t) (0-2mm curing depth) and X-tra fil (b) (2-4mm curing depth). following short-term immersion periods of 0.1, 0.5, 1, 4, 24 and 48 hours immersion in a lightproof waterbath maintained at 37 ± 1°C. Standard deviations are displayed in parentheses.

3.2.2 Water Solubility

The mean water solubility values were recorded for each of the materials following short-term (0.5, 1, 4, 24 and 48 hours) and medium-term (1, 2, 4, 12 and 24 weeks) immersion periods (Tables 3.3 & 3.4). In line with the specification standard for polymer-based filling, restorative and luting materials (ISO 4049: 2000) the water sorption values obtained for Filtek™ Z250 (2.7 ± 1.6μg/mm^3) and Admira (4.3 ± 0.2μg/mm^3) were significantly increased ($P<0.05$) compared with X-tra fil (t) (0.8 ± 0.2μg/mm^3) and X-tra fil (b) (2.4 ± 1.1μg/mm^3) but lower than the specification standard at 1 week of ≤7.5μg/mm^3, respectively.

Medium-Term immersion periods (weeks)	Material	Water Sorption (μg/mm^3)	Water Solubility (μg/mm^3)
1	Admira	16.0 (1.5)	4. (0.2)
	Filtek™ Z250	12.3 (1.8)	2.7 (1.6)
	X-tra fil (t)	5.4 (0.7)	0.8 (0.2)
	X-tra fil (b)	6.8 (0.6)	2.4 (1.1)
2	Admira	18.0 (1.8)	5.2 (1.0)
	Filtek™ Z250	18.9 (1.7)	3.1 (1.1)
	X-tra fil (t)	8.1 (0.5)	0.9 (0.2)
	X-tra fil (b)	8.3 (0.5)	2.6 (1.0)
4	Admira	25.8 (2.5)	7.0 (0.9)
	Filtek™ Z250	27.5 (2.1)	4.3 (1.4)
	X-tra fil (t)	10.0 (1.3)	0.9 (0.3)
	X-tra fil (b)	11.7 (0.7)	2.7 (1.3)
12	Admira	29.8 (2.9)	7.2 (0.8)
	Filtek™ Z250	28.4 (2.3)	7.3 (1.5)
	X-tra fil (t)	14.9 (3.8)	1.8 (0.5)
	X-tra fil (b)	13.2 (2.2)	2.7 (1.2)

24	Admira	30.6 (2.7)	7.4 (0.9)
	Filtek™ Z250	28.6 (1.9)	7.7 (1.7)
	X-tra fil (t)	16.2 (3.1)	2.4 (0.9)
	X-tra fil (b)	15.6 (5.1)	2.8 (1.0)

Table 3.4 The mean water sorption and solubility of Filtek™ Z250, Admira and X-tra fil (t) (0-2mm curing depth) and X-tra fil (b) (2-4mm curing depth) following medium-term immersion periods of 1, 2, 4, 12 and 24 weeks immersion in a lightproof waterbath maintained at 37 ± 1°C. Standard deviations are displayed in parentheses.

Diffusion Coefficient

The diffusion coefficient (m^2s^{-1}) calculated from the gradient of M_t/M_e against $t^{1/2}$plot (Figures 3.3 and 3.4) and highlight that Filtek™ Z250 and Admira have the largest diffusion coefficients ($3.27x10^{-13}$ and $4.04x10^{-13}ms^{-1}$, respectively) compared with X-tra fil (t) and X-tra fil (b) specimens ($1.89x10^{-13}$ and $2.65x10^{-13}ms^{-1}$, respectively). The higher the diffusion coefficient, the greater the rate of uptake of water by each of the RBC materials i.e. the higher the water sorption of each RBC. From the graphs plotted (Figures 3.4 and 3.5), it can be seen that some of the points for all materials have deviated from the line of best fit as well as having a low r^2-value which means that the coefficients observed may not be accurate. This would be rectified by repeating the whole process again and by using a larger number of samples.

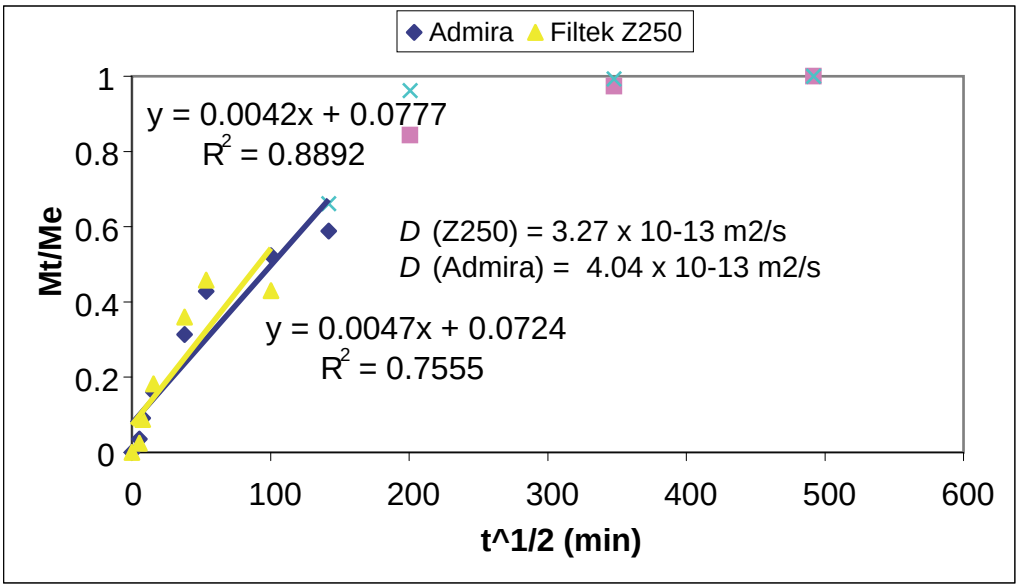


Figure 3.3 Graphical representation of M_t / M_e against $t^{1/2}$ exhibiting the rate of water sorption and associated diffusion coefficients (D) for Admira and Filtek™ Z250

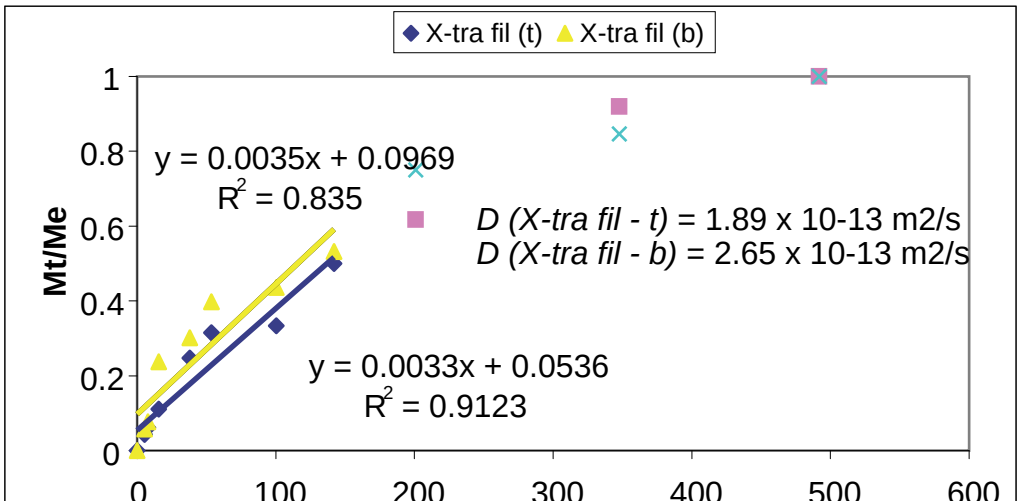


Figure 3.4 Graphical representation of M_t / M_e against $t^{1/2}$ exhibiting the rate of water sorption and associated diffusion coefficients (D) for X-tra fil (t) and X-tra fil (b).

3.3 Vickers Hardness

3.3.1 Effect of Water Immersion on Hardness

The Vickers hardness number (VHN) for the top and bottom surfaces of each specimen of each material was recorded following immersion for short-term (0.5, 1, 4, 24 and 48 hours) immersion periods (Table 3.5) and following medium-term (1, 2, 4, 12 and 24 weeks) immersion periods (Table 3.6).

Short -Term immersion periods (hours)	Material	Vickers Hardness (Top Surface) (kgf/mm^2)	Vickers Hardness (Bottom Surface) (kgf/mm^2)
0.5	Admira	62.2(0.9)	58.8 (1.6)
	Filtek™ Z250	98.4 (1.6)	91.5 (2.1)
	X-tra fil (t)	107.6 (6.7)	98.8 (2.7)
	X-tra fil (b)	100.1 (2.2)	84.6 (3.6)
1	Admira	64.3 (5.2)	57.2 (3.7)
	Filtek™ Z250	86.8 (5.8)	81.5 (5.1)
	X-tra fil (t)	112.3 (4.4)	106.8 (2.4)
	X-tra fil (b)	102.3 (3.1)	90.4 (6.4)

4	Admira	55.7 (2)	53.6 (1.3)
	Filtek™ Z250	89.6 (6.2)	84.1 (4.2)
	X-tra fil (t)	109.1 (2.7)	106 (2.3)
	X-tra fil (b)	96.5 (4.2)	84.5 (1.9)
24	Admira	59.7 (3.4)	54.2 (0.9)
	Filtek™ Z250	90.4 (5.8)	86.4 (7)
	X-tra fil (t)	106.7 (3.3)	104.8 (2.5)
	X-tra fil (b)	102.1 (4.9)	92.5 (6.7)
48	Admira	57.1 (4.6)	54.2 (2.2)
	Filtek™ Z250	92.4 (2.1)	83.7 (3.2)
	X-tra fil (t)	108.1 (5.5)	103.9 (4.1)
	X-tra fil (b)	92.1 (5.2)	83.8 (2)

Table 3.5 The mean top and bottom surface Vickers Hardness values for Filtek™ Z250, Admira and X-tra fil (t) (0-2mm curing depth) and X-tra fil (b) (2-4mm curing depth) following short-term immersion periods of 0.5, 1, 4, 24 and 48 hours in a lightproof waterbath maintained at 37 ± 1°C. Standard deviations are displayed in parentheses.

Medium -Term immersion periods (weeks)	Material	Vickers Hardness (Top Surface) (kgf/mm^2)	Vickers Hardness (Bottom Surface) (kgf/mm^2)
1	Admira	61.5(2.2)	61.4 (2.1)
	Filtek™ Z250	87.8 (1.6)	82.7 (3.7)
	X-tra fil (t)	100.7 (3)	95.2 (4.2)
	X-tra fil (b)	95.2 (3.8)	83.9 (4)
2	Admira	64.3 (5.2)	69.7 (8.6)
	Filtek™ Z250	86.8 (5.8)	81.5 (5.1)
	X-tra fil (t)	112.3 (4.4)	106.8 (2.4)
	X-tra fil (b)	100.8 (2.1)	88.0 (4.8)
4	Admira	74.9 (6.5)	69.7 (8.6)
	Filtek™ Z250	90.3 (2.3)	84.2 (2.2)
	X-tra fil (t)	99.8 (3.3)	97.8 (2.5)
	X-tra fil (b)	100.8 (2.1)	88.0 (4.8)
12	Admira	72.8 (2.1)	70.3 (1.7)
	Filtek™ Z250	96.4 (4.7)	91.2 (3.2)
	X-tra fil (t)	90.8 (4.6)	91.8 (4.6)
	X-tra fil (b)	83.9 (3.3)	77.7 (7.1)
24	Admira	65.0 (2.6)	61.2 (3)
	Filtek™ Z250	87.9 (8.7)	83.4 (6.7)
	X-tra fil (t)	94.1 (4)	87.8 (3)
	X-tra fil (b)	No value	No value

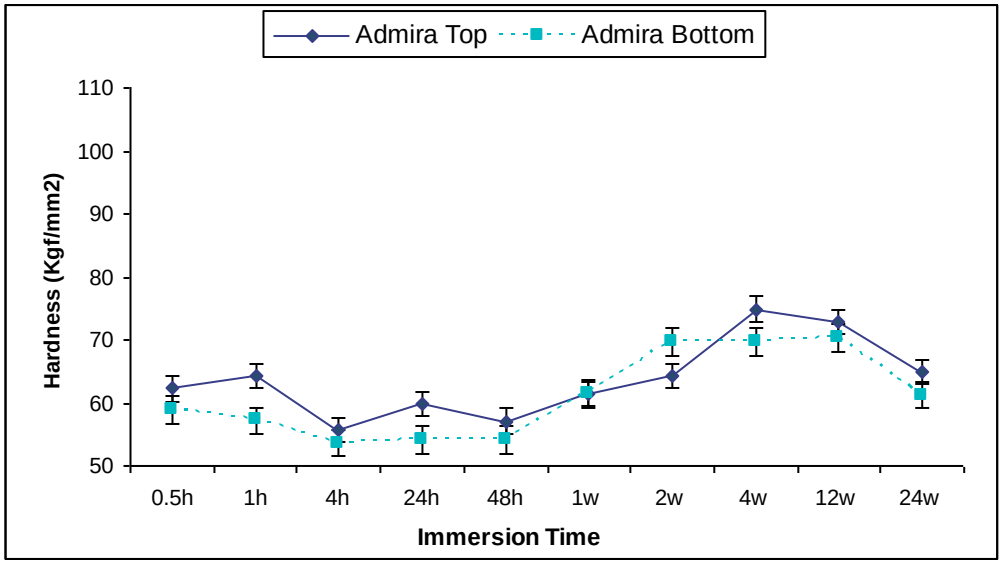
Table 3.6 The mean top and bottom surface Vickers Hardness values for Filtek™ Z250, Admira and X-tra fil (t) (0-2mm curing depth) and X-tra fil (b) (2-4mm curing depth) following medium-term immersion periods of 1, 2, 4, 12 and 24 weeks in a lightproof waterbath maintained at 37 ± 1°C. Standard deviations are displayed in parentheses.

Figure 3.5 illustrates the mean VHN data for the top and bottom surface of Admira and Filtek^{TM}Z250 following both short-term and medium-term immersion periods. Figure 3.6 illustrates the mean VHN for X-tra fil(t) and X-tra fil(b) for both the top and bottom surfaces following short and medium-term immersion periods.

No values were recorded for X-tra-fil (b) immersed for 24 weeks, due to the lack of material available but also due to time restraints within the project, however the hardness of X-tra fil cured to a depth of 4mm could be investigated in future studies. In addition to this, it would be of interest to examine the hardness values for specimens of Admira and Filtek^{TM}Z250 cured to a depth of 4mm and immersed for short and medium term periods.

A one-way ANOVA and paired Tukey tests revealed no significant difference was found between the top and bottom surfaces when analysed individually for Admira, Filtek™ Z250 , X-tra fil (t) or X-tra fil (b) ($P>0.05$).

(a) Admira (VOCO)



(b) Filtek™Z250

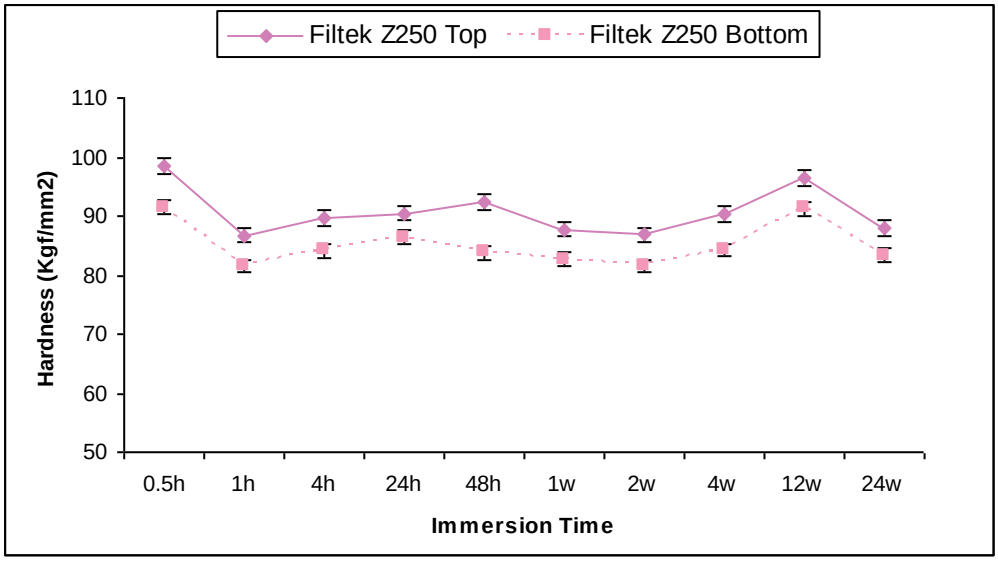
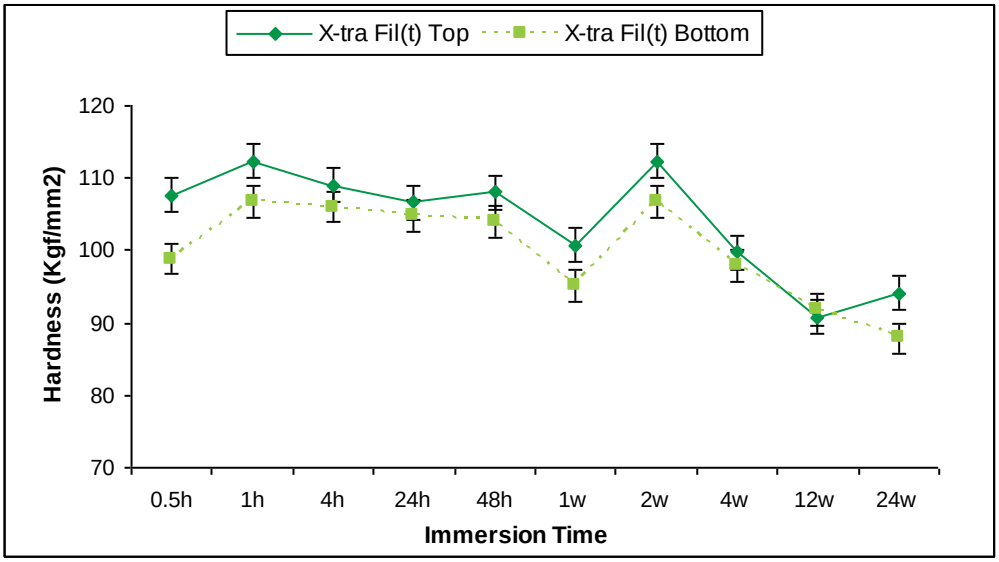


Figure 3.5 The mean Vickers hardness (VHN) of the top and bottom surfaces of (a) the Ormocer Admira (VOCO) and (b) the dimethacrylate RBC Filtek™Z250 following short-term immersion periods of 0.5, 1, 4, 24 and 48 hours and medium-term immersion periods of 1, 4, 12 and 24 weeks in deionised water contained in a lightproof waterbath maintained at 37 ± 1°C.

(a) X-tra fil(t) (0-2mm curing depth)



(b) X-tra fil(b) (2-4mm curing depth)

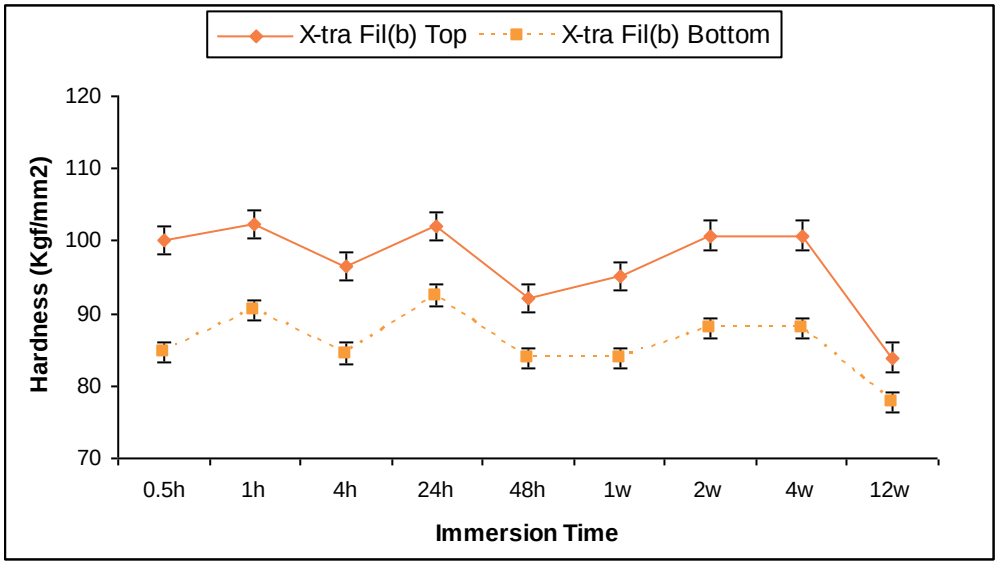


Figure 3.6 The mean Vickers hardness (VHN) of the top and bottom surfaces of specimens of the novel resin based composite (RBC) (a) X-tra fil(t) (0-2mm curing depth) and (b) X-tra fil(b) (2-4mm curing depth) following short-term immersion periods of 0.5, 1, 4, 24 and 48 hours and medium-term immersion periods of 1, 4, 12 and 24 weeks in deionised water contained in a lightproof waterbath maintained at 37 ± 1°C.

3.4 Biocompatibility and Cytotoxicity Analysis

3.4.1 Determination of Cell Viability by Direct Contact with Composite Materials

ATCC Mouse 3T3 Fibroblasts (3T3s)

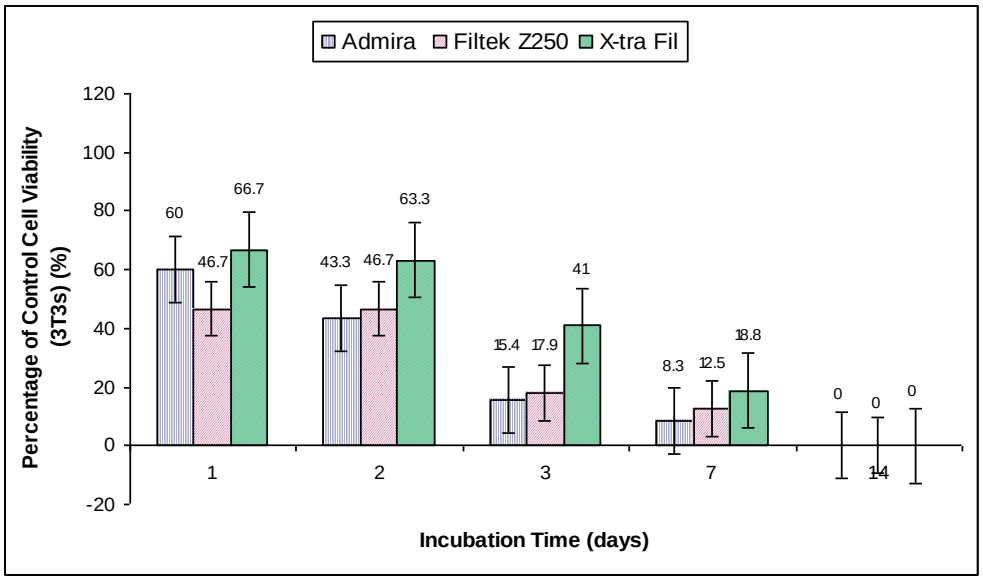
The percentage of the control cell viability was determined for 3T3 cells in direct contact with the Ormocer Admira, dimethacrylate RBC Filtek^{TM}Z250 and the novel RBC X-tra fil, over incubation time periods of 1, 2, 3, 7 and 14 days, using the haemocytometer and neutral red assay technique (Table 3.7).

Incubation Time (Days)	Material	Cell Viability using Haemocytometer (%)	Cell Viability using Neutral Red Assay (%)
1	Admira	60(1.5)	92.9(0.01)
	Filtek Z250	46.7(1.2)	100(0)
	X-tra fil	66.7(1.0)	91(0)
2	Admira	43.3(1.5)	86.8(0)
	Filtek Z250	46.7(1.2)	95.7(0.01)
	X-tra fil	63.3(0.8)	83.6(0)
3	Admira	15.4(1.1)	82(0)
	Filtek Z250	17.9(1.3)	96.9(0)
	X-tra fil	41(0.8)	82(0)
7	Admira	8.3(0.5)	80.3(0.01)
	Filtek Z250	12.5(0.9)	87.7(0.01)
	X-tra fil	18(1.2)	80.4(0.01)
14	Admira	0(0.4)	46(0)
	Filtek Z250	0(0.5)	55.9(0)
	X-tra fil	0(0.8)	47.2(0)

Table 3.7 The percentage of control mean cell viability of ATCC Mouse 3T3 Fibroblasts when in direct contact with 6 specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek^{TM}Z250 and the novel RBC namely X-tra fil over incubation time periods of 1, 2, 3, 7, and 14 days and using the Haemocytometer technique and the Neutral Red Assay. The standard deviations are displayed in parentheses.

When displayed graphically, the haemocytometer and neutral red assay results show different patterns in cell viability (Figures 3.7a and 3.7b). Both Figures 3.7a and 3.7b demonstrated an overall decrease in percentage of control cell viability when in direct contact with all three composite materials. Whilst both Filtek^{TM}Z250 and X-tra fil exhibited marginally higher cell viability over time, this difference was not found to be significantly different. Figures 3.8 and 3.9 provide digital images of cells following direct contact with each material following 24 hours and 1 week incubation in culture. A one-way ANOVA and paired Tukey test however revealed that there was no significant difference between the percentage cell viability of 3T3 cells when in contact with the three composite materials ($P>0.05$), indicating that X-tra fil exhibits similar biocompatible to the other commercially available RBC materials.

a) Analysis of 3T3 cell viability in Direct Contact with Composite Materials (Haemocytometer analysis)



b) Analysis of 3T3 cell viability in Direct Contact with Composite Materials (Neutral Red Assay)

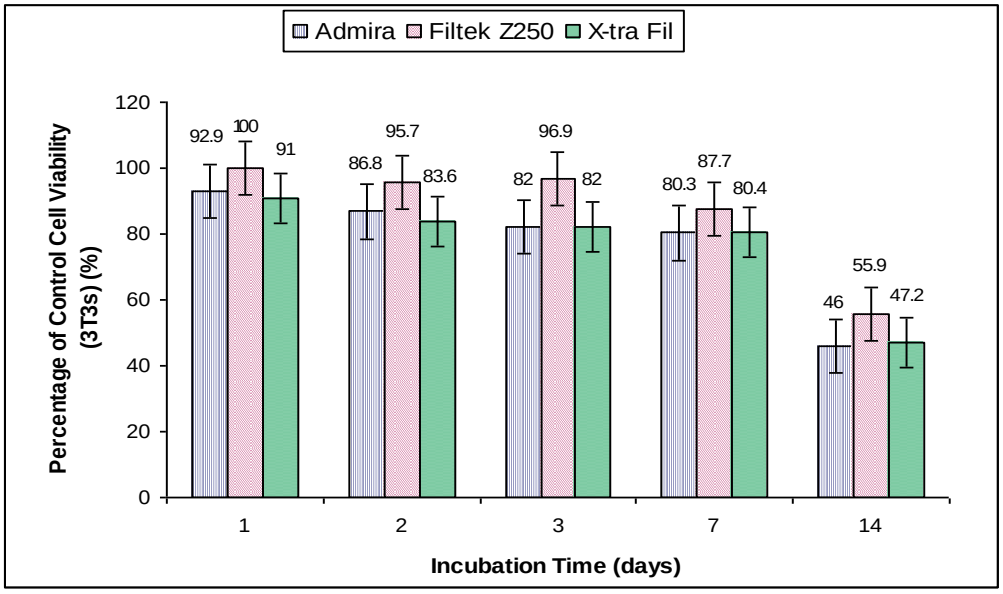
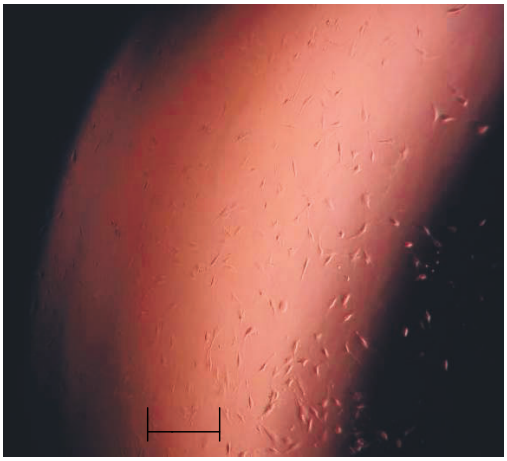
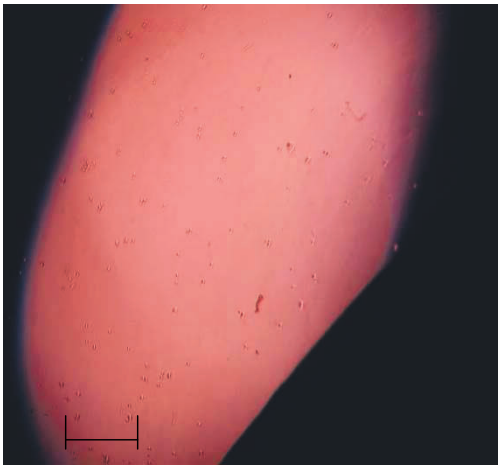


Figure 3.7 The percentage of control cell viability of ATCC Mouse 3T3 Fibroblasts when in direct contact with 6 specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek™Z250 and the novel RBC namely X-tra fil over incubation time periods of 1, 2, 3, 7, and 14 days and using a) the Haemocytometer technique and b) the Neutral Red Assay

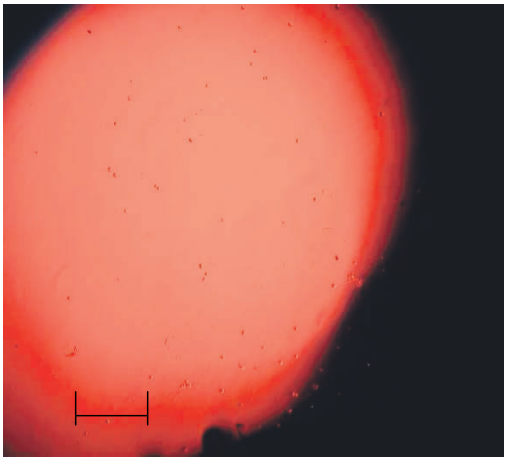
(a) 3T3 teflon control – 24hours



(b) Admira – 24hours



(c) Filtek^{TM}Z250 – 24hours



(d) X-tra fil – 24hours

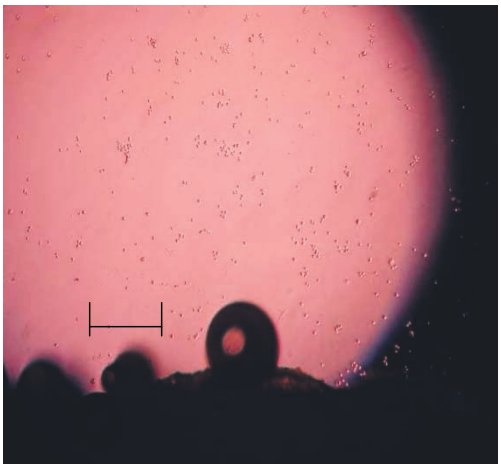
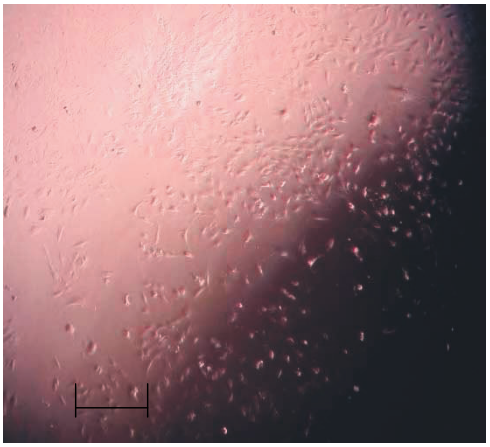
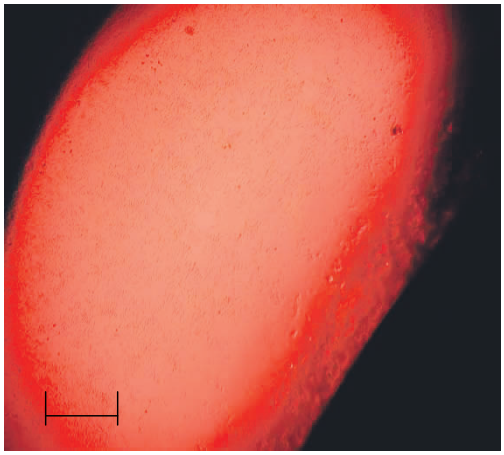


Figure 3.8 Digital images showing the 3T3 cell density and morphology following 24hours incubation at 37±1^{o}C in direct contact with disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek^{TM}Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

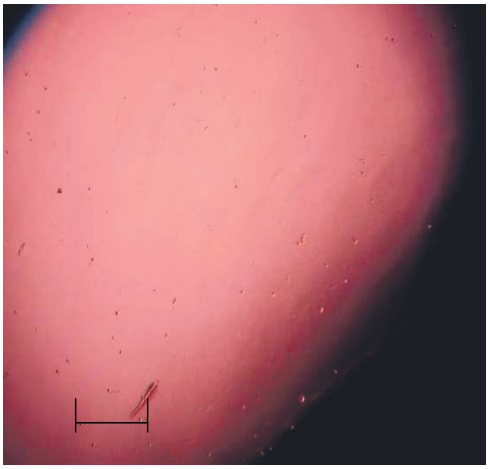
(a) 3T3 teflon control – 1week



(b) Admira – 1week



(c) Filtek™Z250 – 1week



(d) X-tra fil – 1week

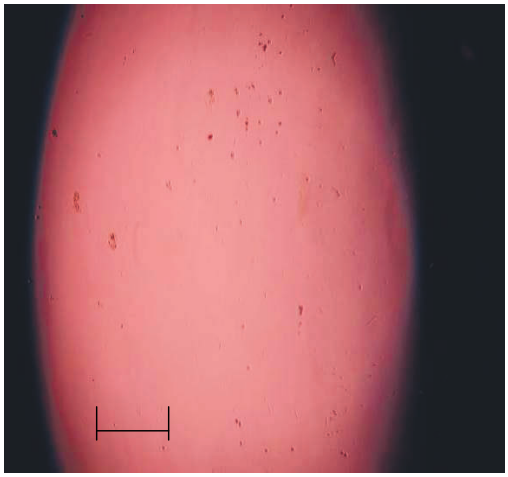


Figure 3.9 Digital images showing the 3T3 cell density and morphology following 1week incubation at 37±1°C in direct contact with disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek™Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

Undifferentiated Pulpal Fibroblast Cell Lines (OD21 cells)

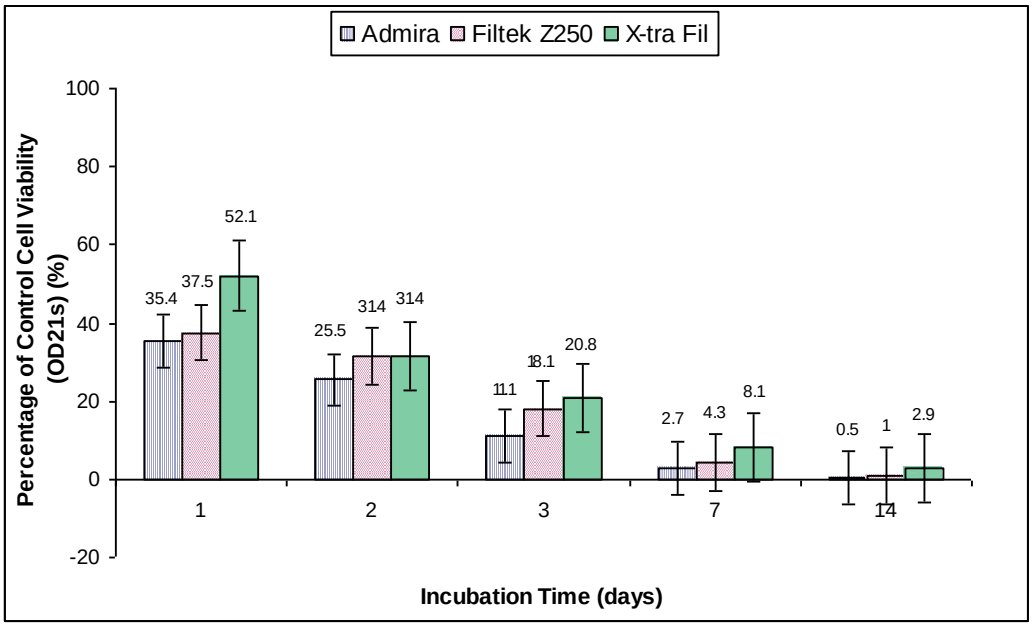
The percentage of the control cell viability determined for OD21 cells in direct contact with the Ormocer Admira, dimethacrylate RBC Filtek^{TM}Z250 and the novel RBC X-tra fil, over incubation time periods of 1, 2, 3, 7 and 14 days, using the haemocytometer and neutral red assay technique (Table 3.8).

Incubation Time (Days)	Material	Cell Viability using Haemocytometer (%)	Cell Viability using Neutral Red Assay (%)
1	Admira	35.4(1.5)	198.9(0.04)
	Filtek Z250	37.5(1.3)	204.3(0.05)
	X-tra fil	52.1(2.0)	153.6(0.01)
2	Admira	25.5(1.0)	164.6(0.05)
	Filtek Z250	31.4(1.0)	136.5(0.07)
	X-tra fil	31.4(1.2)	119(0.05)
3	Admira	11.1(1.0)	49.4(0)
	Filtek Z250	18.1(1.3)	45.3(0)
	X-tra fil	20.8(0.5)	44.3(0)
7	Admira	2.7(0.4)	28.1(0)
	Filtek Z250	4.3(0.8)	28.1(0)
	X-tra fil	8.1(1.6)	28.9(0)
14	Admira	0.5(0.5)	19.6(0)
	Filtek Z250	1(0.8)	19.7(0)
	X-tra fil	2.9(1.6)	20(0)

Table 3.8 The percentage of control mean cell viability of Undifferentiated Pulpal Fibroblast (OD21) cells when in direct contact with 6 specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek^{TM}Z250 and the novel RBC namely X-tra fil over incubation time periods of 1, 2, 3, 7, and 14 days and using the Haemocytometer technique and the Neutral Red Assay. Standard deviations are displayed in parentheses.

The haemocytometer and neutral red assay results show different patterns in cell viability with the neutral red assay resulting in much higher cell viability values than the cell counts with the haemocytometer (Figures 3.10a and 3.10b). Both Figures 3.10a and 3.10b show an overall decrease in percentage of control cell viability when in direct contact with all three composite materials. Data from cell count analysis indicated that X-tra fil appeared slightly less cytotoxic than Admira and Filtek^{TM}Z250 when cells were cultured in direct contact with the materials. However, a one-way ANOVA and paired Tukey test analysis revealed that there was no significant difference between the percentage cell viability values obtained for OD21 cells when cultured in contact with the three composite materials ($P>0.05$). Figures 3.11 and 3.12 provide digital images of OD21 cell culture following direct contact with each material after 24 hours and 1 week incubation at $37\pm1^{o}C$.

a) OD21 cells in Direct Contact with Composite Materials (Haemocytometer analysis)



b) OD21 cells in Direct Contact with Composite Materials (Neutral Red Assay)

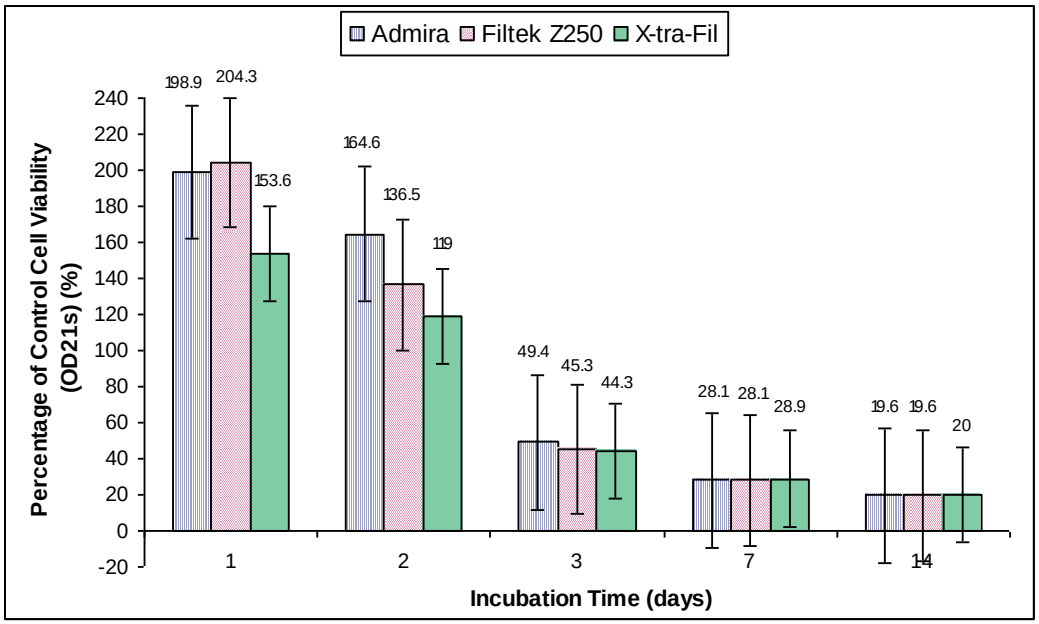
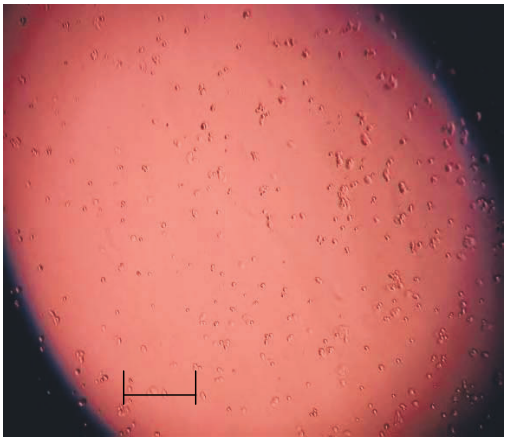
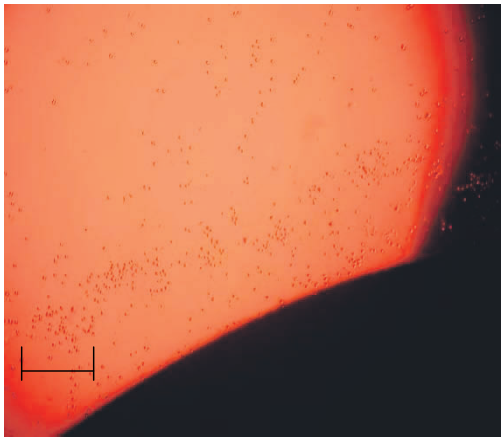


Figure 3.10 The percentage of control cell viability of Undifferentiated Pulpal Fibroblast (OD21) cells in direct contact with specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek™Z250 and the novel RBC namely X-tra fil over incubation time periods of 1, 2, 3, 7, and 14 days and using a) the Haemocytometer technique and b) the Neutral Red Assay.

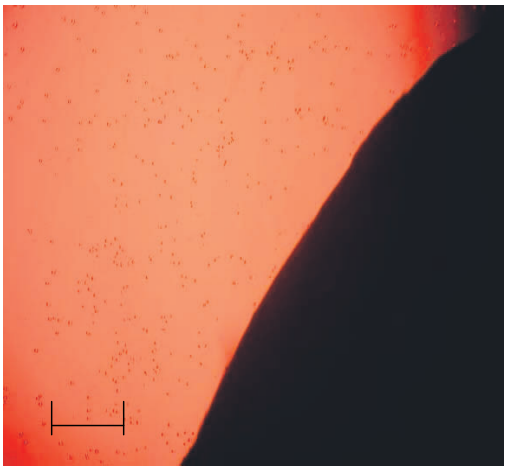
(a) Teflon control – 24hours



(b) Admira – 24hours



(c) Filtek™Z250 – 24hours



(d) X-tra fil – 24hours

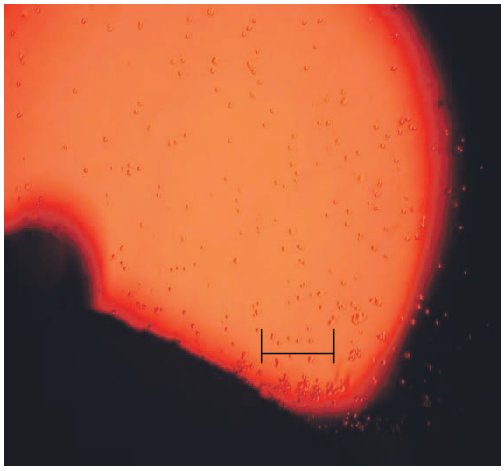
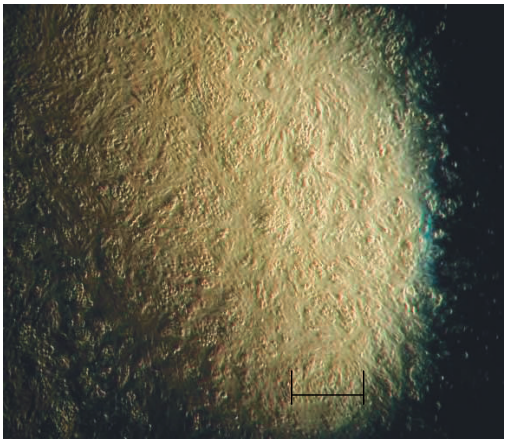
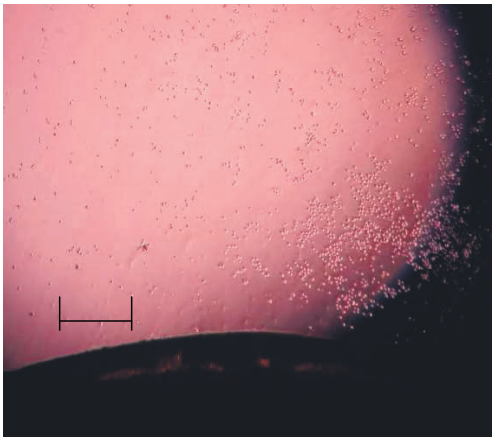


Figure 3.11 Digital images showing the OD21 cell density and morphology following 24hours incubation at 37±1°C in direct contact with disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek™Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

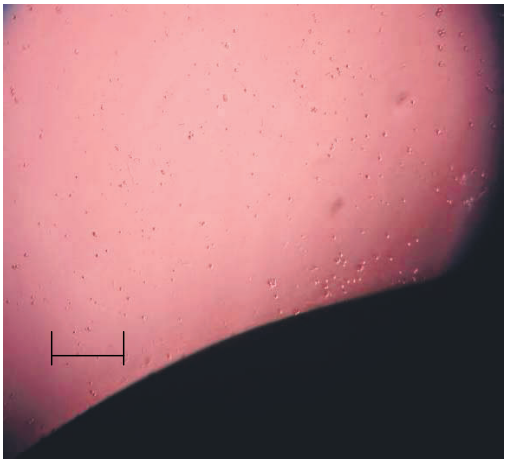
(a) Teflon control – 1week



(b) Admira – 1week



(c) Filtek^{TM}Z250 – 1week



(d) X-tra fil – 1week

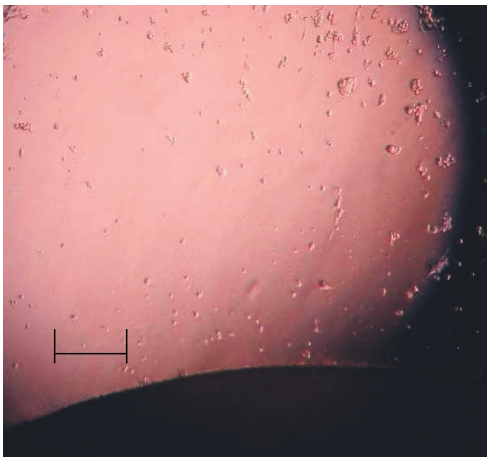


Figure 3.12 Digital images showing the OD21 cell density and morphology following 1week incubation at 37±1^{o}C in direct contact with disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek^{TM}Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

3.4.2 Determination of Cell Viability in Growth Medium containing Composite Material Eluents (Media Immersions).

ATCC Mouse 3T3 Fibroblasts

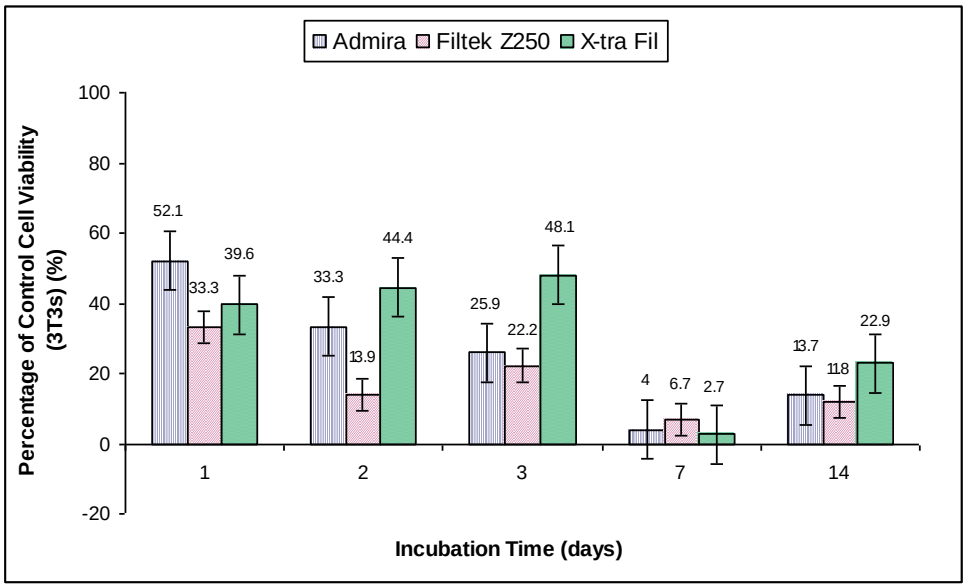
The percentage of the control cell viability determined for 3T3 cells cultured in medium containing substances leached (eluents) from the Ormocer Admira, dimethacrylate RBC Filtek^{TM}Z250 and the novel RBC X-tra fil respectively, immersed/incubated for time periods of 1, 2, 3, 7 and 14 days, using the haemocytometer and neutral red assays (Table 3.9).

Incubation Time (Days)	Material	Cell Viability using Haemocytometer (%)	Cell Viability using Neutral Red Assay (%)
1	Admira	52.1(2.7)	86.3(0)
	Filtek Z250	33.3(2.0)	91.3(0)
	X-tra fil	39.6(1.7)	88.5(0)
2	Admira	33.3(1.1)	85.3(0)
	Filtek Z250	13.9(0.8)	94.1(0.01)
	X-tra fil	44.4(1.4)	79.9(0)
3	Admira	25.9(0.8)	70.1(0)
	Filtek Z250	22.2(0.9)	76.4(0.08)
	X-tra fil	48.1(1.0)	66.7(0)
7	Admira	4(0.5)	62.1(0)
	Filtek Z250	6.7(1.0)	71.6(0)
	X-tra fil	2.7(0.5)	71.6(0)
14	Admira	13.7(3.0)	61.9(0.01)
	Filtek Z250	11.8(1.3)	63.5(0)
	X-tra fil	22.9(1.5)	55.3(0)

Table 3.9 The percentage of control cell viability of ATCC Mouse 3T3 Fibroblasts when cultured in medium containing substances leached from specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek^{TM}Z250 and the novel RBC namely X-tra fil over immersion/incubation time periods of 1, 2, 3, 7, and 14 days and using the Haemocytometer technique and the Neutral Red Assay. Standard deviations are displayed in parentheses.

The haemocytometer cell count and neutral red assay data demonstrated different patterns in cell viability with the neutral red assay resulting in much higher cell viability values than the cell counts obtained using the haemocytometer analysis (Figures 3.13a and 3.13b). However potentially due to variability within the results, one-way ANOVA and paired Tukey test revealed that there was no significant difference between the percentage cell viability within 3T3 cultures when grown in medium containing substances leached from the three composite materials ($P>0.05$).

a) 3T3 cell cultures in medium containing eluents from the Composite Materials (Haemocytometer)



b) 3T3 cell cultures in medium containing eluents from the Composite Materials (Neutral Red Assay)

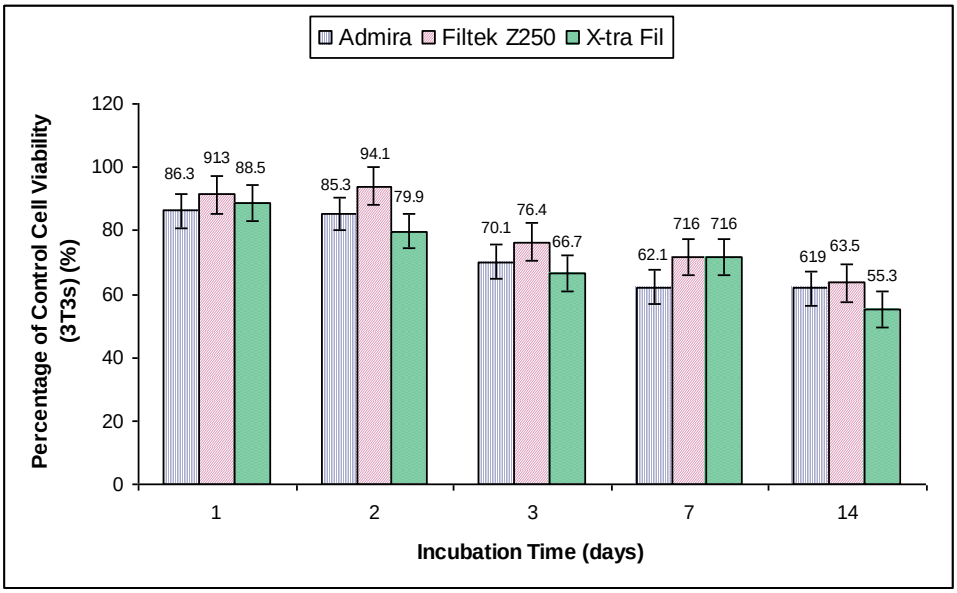
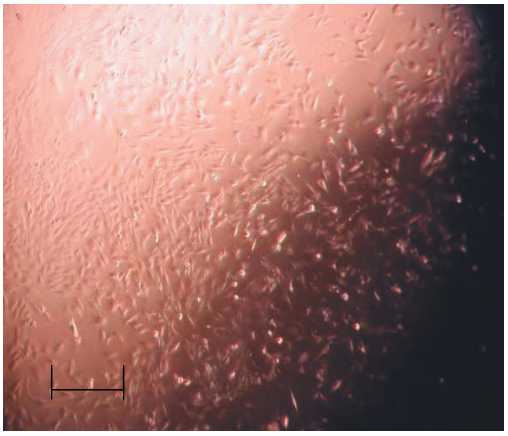
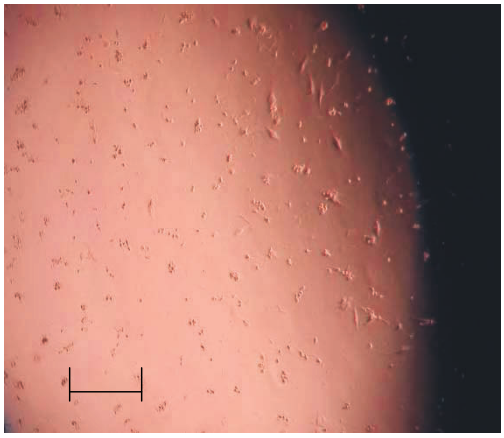


Figure 3.13 The percentage of control cell viability of ATCC Mouse 3T3 Fibroblasts when cultured in medium containing substances leached from specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek^{TM}Z250 and the novel RBC namely X-tra fil over immersion/incubation time periods of 1, 2, 3, 7, and 14 days and using a) the Haemocytometer technique and b) the Neutral Red Assay.

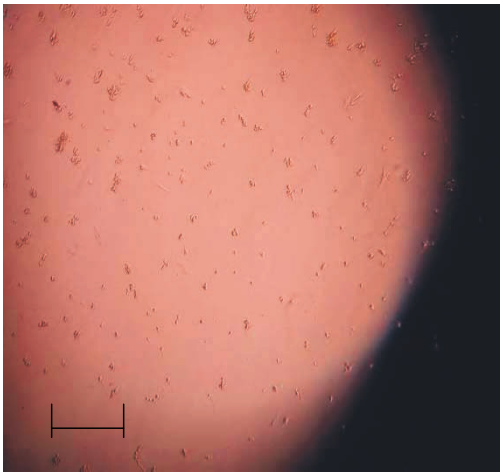
(a) Teflon control – 24hours



(b) Admira – 24hours



(c) Filtek^{TM}Z250 – 24hours



(d) X-tra fil – 24hours

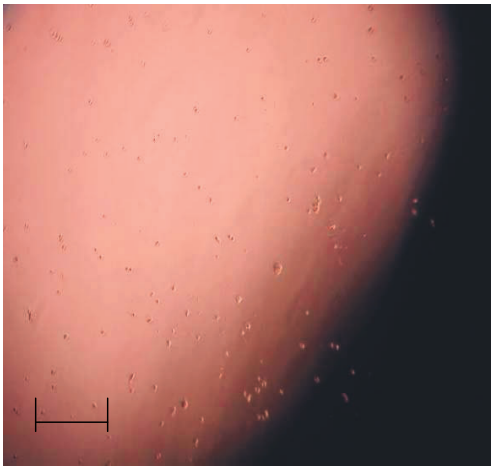
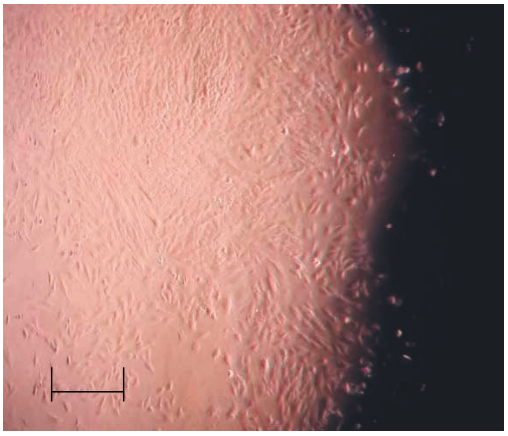
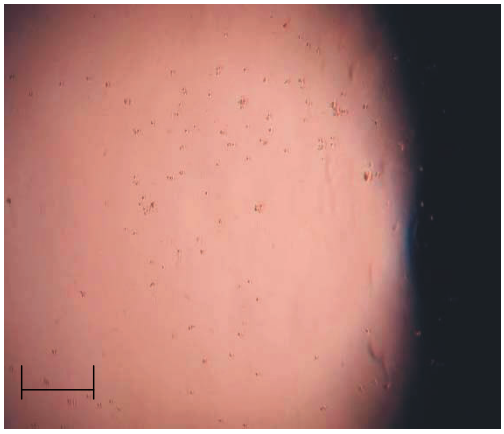


Figure 3.14 Digital images showing the 3T3 cell density and morphology following 24hours incubation at 37±1^{o}C in medium containing leached substances from disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek^{TM}Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

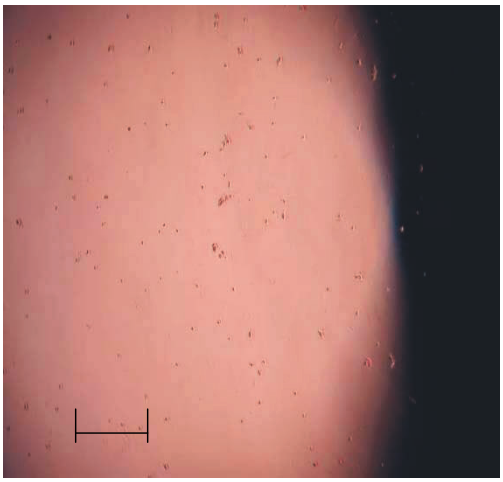
(a) Teflon control – 1week



(b) Admira – 1week



(c) Filtek^{TM}Z250 – 1week



(d) X-tra fil – 1week

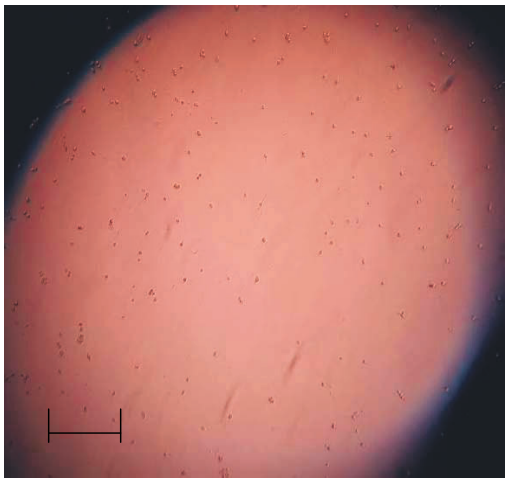


Figure 3.15 Digital images showing the 3T3 cell density and morphology following 1week incubation at 37±1^{o}C in medium containing leached substances from disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek^{TM}Z250 and (d) the novel RBC (X-tra fil). Bar = 100 µm

Undifferentiated Pulpal Fibroblast (OD21) Cells

The percentage of the control cell viability determined for OD21 cells cultured in medium containing substances leached from the Ormocer Admira, dimethacrylate RBC Filtek^{TM}Z250 and the novel RBC X-tra fil, immersed/incubated for time periods of 1, 2, 3, 7 and 14 days, using the haemocytometer and neutral red assay technique (Table 3.10).

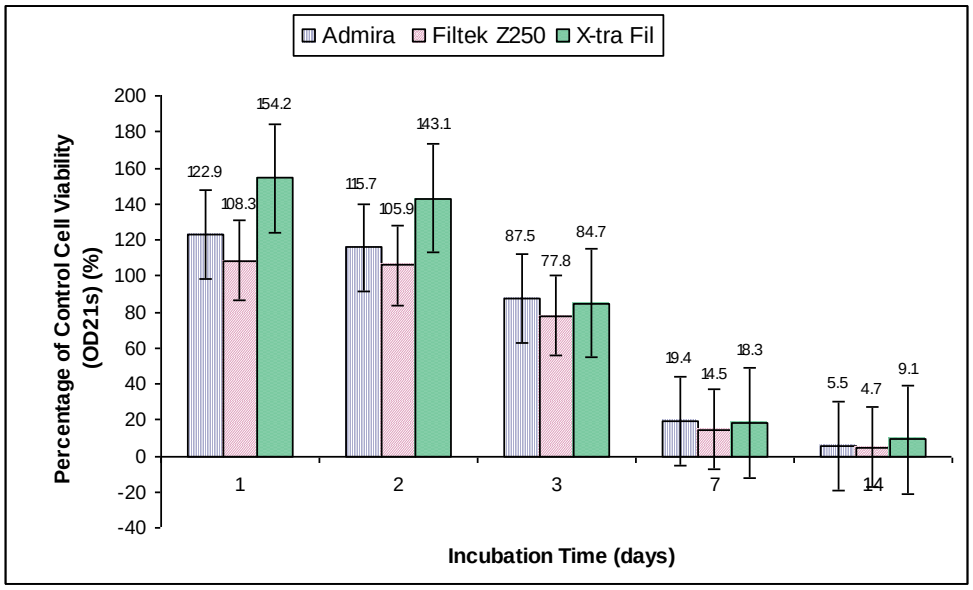
Incubation Time (Days)	Material	Cell Viability using Haemocytometer (%)	Cell Viability using Neutral Red Assay (%)
1	Admira	122.9(2.6)	52.8(0)
	Filtek Z250	108.3(1.9)	54.7(0.01)
	X-tra fil	154.2(2.6)	66.7(0.01)
2	Admira	115.7(1.0)	78.6(0)
	Filtek Z250	105.9(2.8)	56.8(0)
	X-tra fil	143.1(2.8)	83.3(0.02)
3	Admira	87.5(2.1)	101.8(0.03)
	Filtek Z250	77.8(2.5)	106.9(0.03)
	X-tra fil	84.7(1.3)	87.8(0.02)
7	Admira	19.4(2.8)	76.3(0.02)
	Filtek Z250	14.5(1.6)	75.2(0.06)
	X-tra fil	18.3(2.6)	70.6(0.03)
14	Admira	5.5(3.0)	61.7(0.06)
	Filtek Z250	4.7(1.3)	57.6(0.03)
	X-tra fil	9.1(1.3)	60.7(0.04)

Table 3.10 The percentage of control cell viability of Undifferentiated Pulpal Fibroblast cells when cultured in medium containing substances leached from 6 specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek^{TM}Z250 and the novel RBC namely X-tra fil over immersion/incubation time periods of 1, 2, 3, 7, and 14 days and using the Haemocytometer technique and the Neutral Red Assay. Standard deviations are displayed in parentheses.

The haemocytometer and neutral red assay results demonstrated different patterns in cell viability with the cell counts using the haemocytometer resulting in much higher cell viability values (Figures 3.16a and 3.16b). The haemocytometer results suggest that fewer cytotoxic substances are leached from X-tra fil as there appeared to be a higher cell viability with the medium containing the X-tra fil eluents. However, a one-way ANOVA and paired Tukey test revealed that there was no significant difference between the percentage cell viability of OD21 cells when cultured in medium containing substances leached from the three composite materials ($P>0.05$). Figures 3.17 and 3.18 show digital images of OD21 cell viability in medium containing leached substances from each disc shaped specimens of each material following incubation at 37^{o}C for 24hours and 1week.

The cultures contained medium consisting of substances leached from the RBC materials over increasing time periods in this medium resulted in all cultures displaying an initial increase in cell density. Following culture of cells with medium containing substances leached from Admira for 1 week, the cell density decreased rapidly as the cells became infected due to the culture medium. Whilst culture of cells in medium containing substances leached from X-tra fil over a period of 1 week displayed a stable cell density and a gradual increase in cell number (Figures 3.17 and 3.18) the values obtained exhibited no significant difference.

a) OD21 cells cultured in medium containing eluents from the Composite Materials (Haemocytometer analysis)



b) OD21 cells cultured in medium containing eluents from the Composite Materials (Neutral Red Assay)

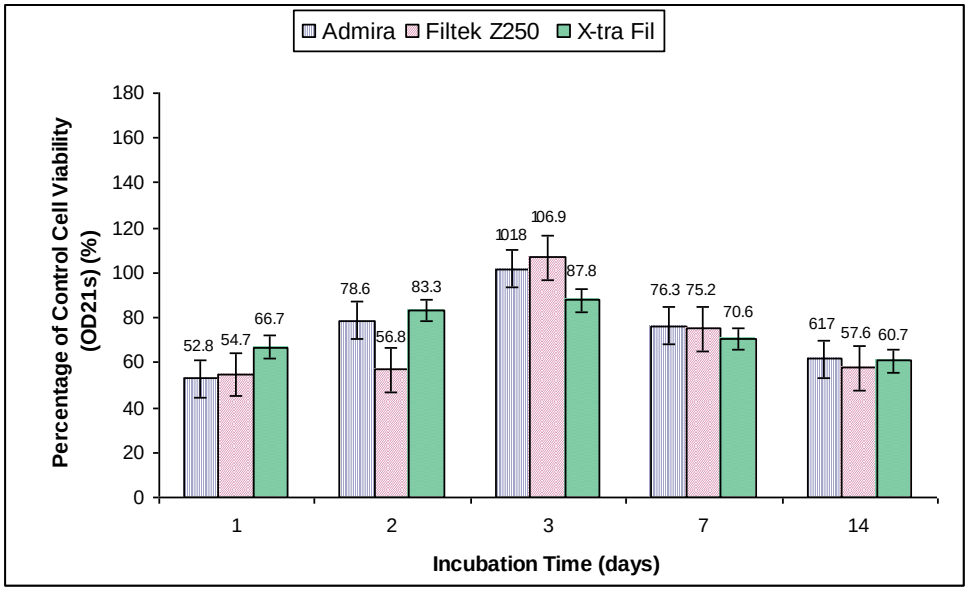
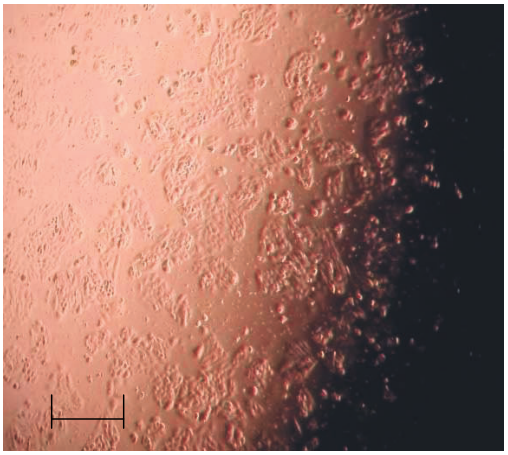
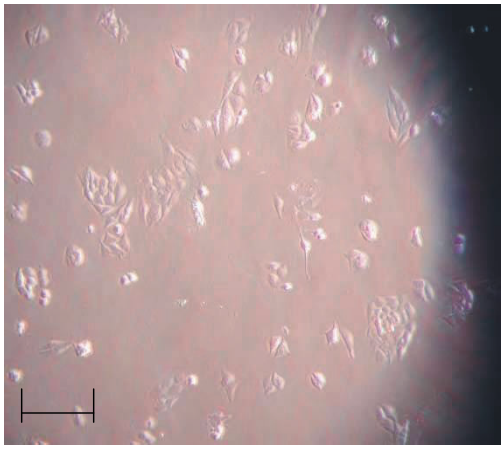


Figure 3.16 The percentage of control cell viability of Undifferentiated Pulpal Fibroblast cell lines when cultured in medium containing substances leached from specimens of the Ormocer Admira (VOCO), the dimethacrylate Filtek™Z250 and the novel RBC namely X-tra fil over immersion/incubation time periods of 1, 2, 3, 7, and 14 days and using a) the Haemocytometer technique and b) the Neutral Red Assay.

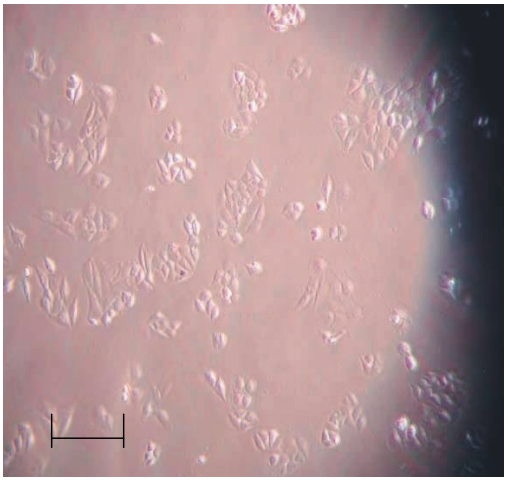
(a) Teflon control – 24hours



(b) Admira –24hours



(c) Filtek™Z250 –24 hours



(d) X-tra fil – 24hours

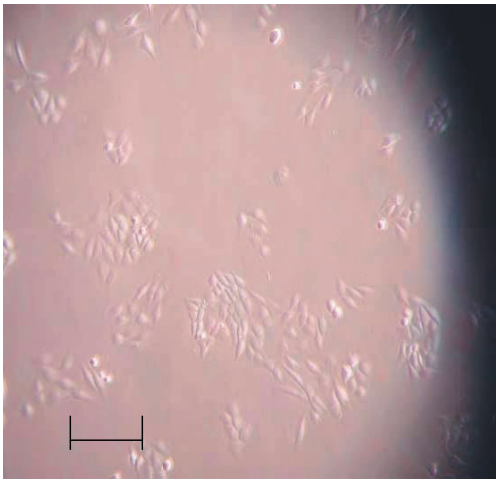
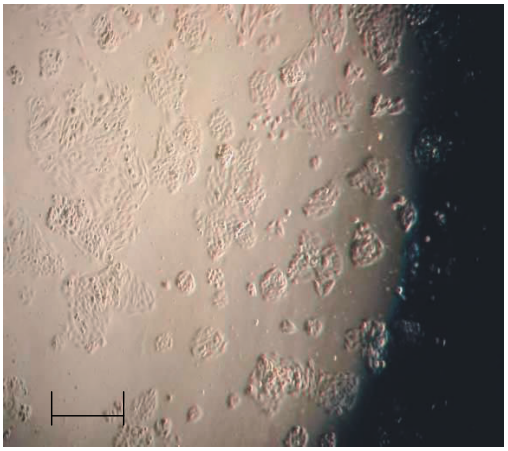
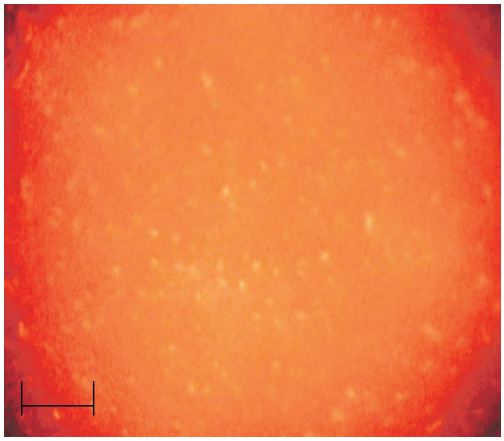


Figure 3.17 Digital images showing the OD21 cell density and morphology following 24hours incubation at 37±1°C in medium containing leached substances from disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek™Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

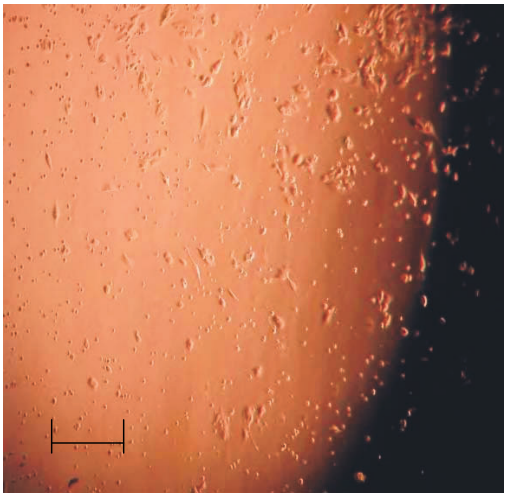
(a) Teflon control – 1week



(b) Admira – 1week



(c) Filtek™Z250 – 1week



(d) X-tra fil – 1week

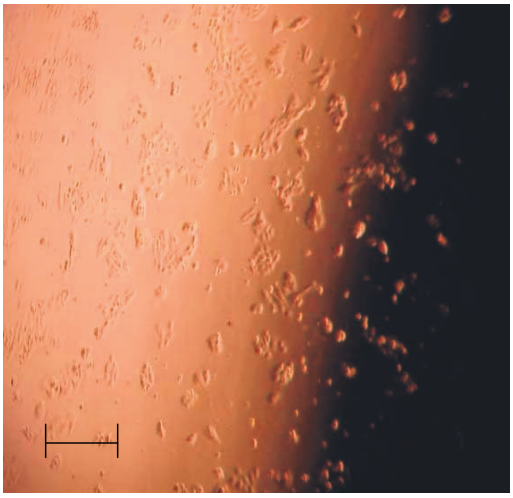


Figure 3.18 Digital images showing the OD21 cell density and morphology following 1week incubation at 37±1°C in medium containing leached substances from disc shaped specimens of (a) Teflon control, (b) the Ormocer Admira, (c) the dimethacrylate RBC Filtek™Z250 and (d) the novel RBC namely X-tra fil. Bar = 100 µm

Overall, the results were not entirely consistent between the cell viability recorded through either haemocytometer cell counting or by the neutral red assay. Whilst trends were identified from inspection of the data, a one-way ANOVA and paired Tukey test analysis revealed that there was no significant difference between the cytotoxic effects of the three composite materials ($P>0.05$). Therefore, it was concluded that X-tra fil appears as biocompatible as Admira and Filtek^{TM}Z250 and does not appear to possess any characteristics that enhance its biological properties.

CHAPTER 4 Discussion

4.1 Light-Curing

The procedure used to manufacture the composite disc specimens was aimed at ensuring consistency between each specimen generated. The polymerisation of the monomers present in RBCs is directly related to the wavelength intensity of the curing unit and the time of exposure. The in the approach applied here there should not be notable differences between each disc specimen generated as the method of production was kept consistent throughout the study (Radzi et al., 2008). The majority of the theories related to spatial prediction assume that the data are generated from a Gaussian random field (Kim & Mallick 2004). The Gaussian distribution is also known as the normal distribution and is a continuous probability distribution that describes data that clusters around the mean. In this case, the curing unit exhibits decreased light intensity towards the tip edge and therefore observes a Gaussian distribution. This is consistent with previous studies which have shown that as the distance from the tip of a light guide increases, the irradiance decreases but the rate of decrease can vary between curing units. Clinically, this difference is important due to the fact that the light guide cannot always be positioned immediately adjacent to the material in question (Vandewalle et al., 2005). Notably therefore when a material is used in clinic the operator's curing technique are a crucial factor in the longevity of the restoration. Notably diminished light output can result in restorations which are incompletely polymerised and as a result can lead to a reduction in their mechanical properties, marginal breakdown, increased wear and even an increase in their water sorption (Martin 1998). With regards to the present study however these factors were not a problem as the specimen

discs were produced outside of the oral cavity and therefore there were no hindrance or restrictions with regards to view and access.

Due to the high operating temperatures of halogen bulbs they tend to exhibit a limited lifespan. This is a significant disadvantage of their use and resultantly a reduction of their curing efficiency occurs after 100 hours of use at which point they undergo degradation in terms of light output (Mavropoulos et al., 2005, Vandewalle et al., 2005). Notably it is the reflector, bulb and filters present in the unit which can all degrade over time and therefore reduce the light's curing effectiveness (Vandewalle et al., 2005). In addition, fluctuations in the line voltage can also contribute to inefficiency of the curing unit (Martin 1998). As well as the normal degradation of light due to usage of the LCU, there are several studies which have demonstrated that many halogen lamps in clinical use do not produce their optimum output due to a lack of maintenance (Mitton & Wilson 2001). In the case of the present study the LCU used was well maintained by wiping clean, although due to time constraints it was not sterilised thoroughly between each specimen disc produced. In future studies however sterilisation should be performed to maintain consistency and eliminate anomalies which might occur due to interactions between the light curing and sterilisation process. Notably the sterilisation of the curing unit between each disc manufacture could potentially pose problem as previous studies have demonstrated that repeated sterilisation can contaminate the light guide and also damage the fibre optic bundle within the unit and hence subsequently reduce the units light output (Martin 1998).

Depth of cure of a resin composite may be affected by both composite-related and light-related factors. The light-related factors identified include irradiance, spectral distribution, exposure

time, post-irradiation time and light dispersion, while the composite related factors include the type of resin used, shade, translucency, temperature of the composite material and thickness of the increment (Vandewalle et al., 2005, Martin 1998). Significantly the presence of a photoinitiator which promotes the polymerisation process, such as Camphorquinone (CQ) can also affect the curing process. CQ is present in the majority of light cured RBCs currently available on the market today and while it is not incorporated into the polymer network of the resin it can leach out of the RBC over time (Volk et al., 2009).

The material components in the present study were kept consistent as the shade, thickness, type of resin and irradiation time (recommended by the manufacturers) in an attempt to minimise discrepancies and facilitate comparison. The specimen discs were also produced in the same environment using the same methodological approach with the amount of material being accurately weighed to produce each disc. Clearly it is important that the synthesis of the discs was standardised as inconsistencies in conversion would affect subsequent the downstream data obtained. Indeed it has been demonstrated in previous studies that the results of material evaluations, including tests for mechanical strength, modulus, hardness and leachable components are associated with polymer conversion with incomplete conversion resulting in poor resistance to wear and poor colour stability (Bala et al., 2005).

4.2 Bi-Axial Flexural Strength Testing

The experimental procedure used to determine the flexural strength in the current investigation needs to be reproducible between different test centres to enable accurate comparisons between materials. As a result, care has to be taken to ensure that the methods planned and carried out are not only an accurate assessment of flexural strength data but also conform to a specification standard recognised world-wide. Although the bi-axial flexure strength method is a laboratory representation of the actual masticatory forces encountered in the oral cavity, measures can be taken to reduce any anomalies and portray an *in-vitro* simulation of the stresses encountered when employed clinically. Previous studies carried out and present in dental literature have shown that the bi-axial flexure strength test has been the most frequently used and is the most reliable method for determining flexure strength (Palin et al., 2003, Higgs et al., 2001). The advantage of this method is that the area of maximum tensile stress is located at the center of the lower face of the plate and as a result this eliminates spurious edge failures commonly associated with three- or four-point flexure strength testing (Higgs et al., 2001). It also offers controllable specimen geometry, simple sample preparation, and the surface to volume ratio is more closely related to that of a posterior filling rather than three- or four-point flexure strength testing. Consequently, the assessment of bi-axial flexure strength has been investigated previously in many dental materials studies and was the basis for the flexural strength testing in the current investigation (Ferracane et al., 1998, Manhart et al., 2000, Fleming et al., 1999).

No significant difference ($P>0.05$) between the mean bi-axial flexure strengths of the Filtek™ Z250, Admira, X-tra fil (t) and X-tra fil (b) investigated in the current study (Table 3.1) were highlighted suggesting that X-tra fil as a novel material is actually very similar in its strength

properties as those materials already available on the market. The materials tested in the current study included a conventional hybrid RBC (Filtek™ Z250) which contained BisGMA, UDMA and Bis-EMA resins which contained minimal amounts of TEGDMA and 61 vol.% zirconia/silica filler. The particle size distribution analysis in Z250 indicate it contains a large number of finer particles when compared with Z100 with the size distribution being from 0.01 μm to 3.5 μm with an average particle size of 0.6 μm. The Ormocer (organically modified ceramic) used here was Admira containing BisGMA, UDMA and TEGDMA with 60.2 vol.% of bariumboroaluminiumsilicate filler (Wolter et al., 1994) with an average particle size of 0.7 μm and micro-fillers from pyrogenic SiO sized at approximately 0.04 μm in diameter. The recently marketed X-tra-fil contained a resin mixture of Bis-GMA, UDMA and TEGDMA with 70.1 vol.% bariumboroaluminiumsilicate filler. With regards to the properties of the different materials, the bi-axial flexure strength results obtained in this study appeared not to be particularly influenced by the filler particle size distribution within the materials. This data is in agreement with that of previous studies which indicated that materials containing different filler substances with different particle sizes exhibited no significant differences in their wear (Nagarajan et al., 2004, Suzuki & Leinfelder 1993). Even though X-tra fil contains approximately 10 vol.% more filler than Filtek™ Z250 and Admira, this property did not appear to have made a difference with regards to its strength as a dental material.

The properties of resin composites depend on several factors, related to the polymer matrix, the filler particles and the coupling between filler and matrix. It is however often difficult on the basis of the published literature to determine what components cause differences in material

properties as these materials differ in several aspects (Asmussen et al., 1998). Notably, the mean bi-axial flexure strength of Filtek™ Z250 has previously been reported (Palin et al., 2005) as 140 (12) MPa which coincided well with the value obtained in the current study (142 (20) MPa). The bi-axial flexure strength and reliability of RBCs have also been reported previously in the literature (Palin et al., 2005) as varying from 115-168 MPa with associated Weibull moduli of 7.0-16.2 depending upon the chemistry of the monomer resins, the extent of polymerisation of the polymer matrix (Ferracane et al., 1998; Peutzfeldt 1997; Johnston et al., 1985), filler particle size and distribution (Keeters et al., 1983; Yearn 1985; Price et al., 2002), the interfacial properties between the filler and resin matrix (Rueggeberg et al., 1994). These results also correspond well with the mechanical properties identified in the current study.

The predicted Weibull modulus of a group of specimens (provided $n \geq 20$) suggests an indication of the flaw distribution in the specimen where a high Weibull modulus can be associated with a narrow distribution of defects and an increased reliability of strength data for a particular material. Weibull analysis applied to bond strength data allows information about bond reliability to be ascertained (Millett et al., 2003). This provides more detailed information required for the prediction of failure stress of brittle materials compared with quoting mean flexure strengths and associated standard deviations alone. Weibull statistics are also essential for predicting the reliability of both bi-axial flexure and three-point flexure strength testing (Palin et al., 2003). It was suggested that the significant increase in Weibull moduli could be a result of the specimen geometry of the bi-axial disc-shaped specimens, which allowed for controlled irradiation of the RBC samples compared with the overlapped curing procedure

associated with the manufacture of rectangular bar specimens for three-point flexure tests (Palin et al., 2003). Previous studies have shown that the reliability of results is determined by whether the confidence intervals for the Weibull modulus overlap such that if an overlap is observed the reliability of the bi-axial flexure strength data recorded is significant (Fleming et al., 2003).

4.3 Water Sorption, Water Solubility and Diffusion Coefficients

The present study showed that the water sorption and water solubility values for Filtek™ Z250 and Admira were significantly increased ($P<0.05$) compared with X-tra fil (t) and X-tra fil (b). The rate of water sorption by dental RBCs has previously been identified as being a diffusion controlled process (Baharav et al., 1988, Cook, 1980, Pilo et al., 1992) and in the current study the plots of M_t/M_e against $t^{1/2}$ remained linear in the initial stages of water sorption (Figures 3.1 and 3.2). The diffusion coefficient of dental RBC materials has also been reported to be dependent upon the chemistry of the monomer resins and the extent of polymerisation of the polymer matrix (Palin et al., 2005; Ferracane, 1994).

Previously, Palin *et al.* (Palin et al., 2005) associated the decreased water sorption and associated lower diffusion coefficient of Filtek™ Z250 compared with Z100 with a decrease in structural heterogeneity of the polymer. The higher molecular weight of Z250 in comparison to Z100 is greater which affects the viscosity of the material measurably with Z100 being 30,000 poise and Z250 being 350,000 poise. The weight of the resin can result in less polymerisation shrinkage, reduced aging and a slightly softer resin matrix (Cunha et al., 2003, Fleming et al., 2005). Palin *et al*. (Palin et al., 2005) also proposed that the ether groups of BisEMA and the urethane groups

of UDMA, predominant in the resins of Filtek™ Z250, can form weaker hydrogen bonds with water molecules than the hydroxyl groups of BisGMA and TEGDMA molecules which are predominant in Z100 and also Admira used in this study (Palin et al., 2005, Pilo et al., 2002, Ferracane, 1994), thereby reducing the hydrophilic nature of the constituent monomer units. Interestingly the diffusion coefficient for the X-tra fil was reduced compared with Filtek™ Z250 despite the presence of TEDGMA, however, the increased filler content would be expected to result in the reduced water sorption and water solubility values determined in the current study. This correlates with the concept behind Ormocer technology and the chemistry of Admira, where there is a considerable widening of the adjustable properties by the incorporation of different fillers. This addition of fillers may bring an improvement of the mechanical and physical properties such as small abrasion rate, low water absorption and low water solubility (Cunha et al., 2003, Ban et al., 1992).

The diffusion coefficient for Filtek™ Z250 identified in the current study was lower than that reported previously by Palin *et al.* (Palin et al., 2005) as the specimen thickness in this study was reduced to conform with the water sorption and solubility testing methodologies stipulated in ISO 4049 for resin-based dental restoratives. The M_e against $t^{1/2}$plot (Figures 3.3 and 3.4) highlighted the fact that Filtek™ Z250 and Admira have larger diffusion coefficients in comparison to X-tra fil which would have been expected as X-tra-fil contains a higher volume% of filler. Interestingly, despite the numerous studies in the dental literature regarding water sorption and water solubility variations in LCU, irradiation times, irradiation protocol and storage condition it is difficult to correlate the data in the current study with the previous

investigations (Sideridou et al., 2004, Sideridou et al., 2003, Kanchanavasita et al., 1997, Kalachandra et al., 1987). The ISO specification standard advocates curing the disc-shaped specimens using an overlapping protocol that results in the 1 mm of material receiving eight times the irradiation that a 2 mm increment would receive when being placed clinically. It is suggested that by employing a larger diameter light curing tip that enables the specimen to be irradiated in one-hit, the values for water sorption and water solubility and the diffusion coefficients obtained in the current study would be expected to be closer to the values when the RBCs are utilised clinically. However, as already explained in Section 4.1, the diameter of the light curing tip is not the only factor that needs to be taken into account, namely the maintenance of the curing units, the life span of halogen bulbs etc.

4.4 Vickers Hardness Testing

Hardness is considered to be related to wear resistance and is the most commonly examined mechanical property indicator for synthetic restorative materials (Loyaga-Rendon et al., 2007). The absorption of water molecules by hydrophilic moieties within an RBC material on exposure to the oral environment may result in plasticization of the resin polymer network (Ferracane et al., 1998) thereby decreasing the mechanical properties of the material. The present investigation combined Vickers hardness testing with short- and medium-term immersion periods for water sorption and solubility testing methodologies similar to those stipulated in ISO 4049 for resin-based dental restoratives (International Standards Organisation, 2000). No significant reduction in the top to bottom hardness of the materials investigated was evident in the current study for

short- and medium-term immersion periods which suggests that any plasticization of the resin polymer network was limited up to the 24 weeks investigated in the current study.

Currently there is little agreement as to a hardness value which reflects an adequate degree of polymerisation for a RBC. A bottom to top hardness value of 80–90% has previously been suggested as an indicator for the minimum depth of cure value which is acceptable (Johnston et al., 1985, Keeters et al., 1983, Yearn, 1995). However, this value has been the subject of considerable controversy amongst dental material scientists and clinicians alike. Indeed previous studies have reported that reducing the depth of cure of RBCs is manifested as a decrease in the surface hardness (Rueggeberg et al., 1994, Baharav et al., 1988, Cook, 1980). For reduced exposure periods the concentration of unexcited CQ molecules decreases in the bottom layers of the RBC specimens following the cessation of light irradiation. With increasing specimen thickness, fewer photons are able to reach the CQ molecules within the resin and as a result fewer molecules are activated and raised to the 'triple' (excited) state (Palin, 2004). Therefore at reduced light exposure periods, the quantity of CQ molecules at the lower surface of the specimen in the triplet state that are able to collide with an amine will be reduced and as a result incapable of producing free radicals to initiate polymerisation. Thus, an increase in light energy density for a constant irradiation time would be expected to result in an increase in the Vickers hardness of both the bottom and top surfaces (Pilo et al., 1992). With regards to the present study you would expect Xtra-fil to exhibit a higher Vickers hardness than Admira and Filtek™ Z250 due to the fact that it is cured for 20 seconds longer (according to the manufacturers recommendations). However, there was no significant difference seen between the materials

suggesting that the novel material is similar in its properties to those already available on the market. Further studies could include the curing of Admira and Filtek™ Z250 for 40 seconds as well as Xtra-fil, and these materials could also all be cured to a depth of 4mm subsequent analysis of their mechanical properties would then be appropriate. Due to time constraints and material availability, this work was not performed in the present study, however it would be interesting to determine the materials mechanical behaviour under these curing conditions.

The reduction of the C=C bond rate represents the degree of conversion of the composite material. This has been shown to maintain a direct relationship with the composite resin microhardness and therefore a hardness test can be used to indirectly evaluate the C=C bond rate (de Araújo et al., 2008). In general, the greater the hardness value is the more extensive the polymerization of the material (DeWald and Ferracane 1987). However, as already stated this may not always be the case. There are several key factors involved in determining the efficiency of cure of the material and these include the monomer structure, amount and type of filler particles, composite shade, light curing time and curing depth. The curing depth in turn is dependant on the intensity of the radiation emitted from the LCU (Cook 1980). Indeed previous studies have determined the transmission coefficient of visible-and ultraviolet-light-activated resin composites and reported that the visible-light-activated resin composites as a group had higher values of cure depth and transmission coefficient than did the ultraviolet light activated resin composites (Tirtha et al., 1982). Also in the same report, it was indicated that darker and more opaque shades of resin composites might be expected to exhibit a lower light transmission coefficient as this value is influenced by the wavelength of light, refractive indices of the resin

and fillers and types and amounts of filler particles (Kawaguchi et al., 1994). In the current study, radiant exposures of 13.4 Jm^{-2} for Filtek™ Z250 and Admira and 26.8 Jm^{-2} for X-tra fil were employed with the results indicating no significant differences between the top to bottom Vickers hardness values. However, the top to bottom Vickers hardness values were material specific for the RBCs investigated. The Vickers hardness results therefore further emphasise that the relationship between the hardness and radiant exposure, namely irradiation and time, is more complex than reported by many researchers.

4.5 Biocompatibility and Cytotoxicity Analysis

In the current study, as well as the mechnical properties of the RBC materials, their cytotoxicity and biocompatibility were also investigated. In modern medicine or dentistry it is important to ensure that every material or substance which is to be placed within the body is biocompatible and exerts minimal cellular cytotoxic effect (Leyhausen et al., 1998). In relation to this, composites have previously been shown to exert significant cytotoxic effects in cell culture systems and this has proven to be caused by residual uncured monomers or oligomers (Ferracane et al., 1990). Several approaches have been used for the determination of this complex biological feature including: *in vivo* systems like usage tests in animals and *in vitro* systems especially utilising cell cultures and microbiological techniques (Leyhausen et al., 1998). Interestingly, several studies have shown that human primary gingival cells are more discriminating in cytotoxic assessment of dental materials than permanent cell lines derived from animals (Holland 1994, Huang et al., 2002). This indicates that cytotoxicity results can vary depending

upon the type of cells used for analysis (Leyhausen et al., 1998). There have been reports that most cytotoxic effects from composites occur during the first 24 hours of testing and correlate with the early leaching of residual monomers (Bouillaguet et al., 2002). However, other studies have reported that resin-based restorative materials may leach sufficient components to cause cytotoxicity as late as 2 weeks after synthesis (Wataha et al., 1999).

Cell culture systems provide convenient, controllable and repeatable means for the initial assessment of the toxicological response to novel biomaterials. Whilst the commonly used cytotoxicity tests for dental materials have cell death as the end point, they however generally do not differentiate between the type of cell death induced (Becher et al., 2005). In the current investigation and in previous studies, *in vitro* cytotoxicity tests have been used to evaluate biological risks of dental resin composites. These reports support the hypothesis that mass release from composites, particularly of lower molecular weight diluents, is responsible for the cytotoxic effects of these materials in vitro (Wataha et al., 2003). When applied to resin composites, some *in vitro* tests have also used an 'aging' of the polymerised specimens in a biological solution such as artificial saliva to help show trends in the biological response and provide additional physiological relevance (Wataha et al., 2003). It has been suggested that identification of leachables as well as further analysis with extracts obtained from longer incubation periods is needed before final conclusions can be drawn about the release of mutagenic components from composites (Eick et al., 2002). However, campherquinone has previously been identified as one of the leachables from resin materials but has been shown to exert a moderate cytotoxicity in comparison to other photoinitiators, although the mechanism of CQ cytotoxicity and its target structures is only partly understood (Volk et al., 2009).

In the current study, the composite materials were placed in direct contact with ATCC Mouse 3T3 fibroblasts and with OD21 pulpal fibroblast cell lines for periods of 1, 2, 3, 7 and 14 days which are the incubation times recommended by Wataha et al. (2003). It has been suggested that rapid release of unbound components of dental composites will proceed as long as there is a concentration gradient and therefore the elution of these molecules is rapid essentially complete within the first 24 hours of culture (Ferracane et al., 1990). However, in the present investigation, this was not entirely supported. Cell counts as determined using the haemocytometer analysis showed that for both 3T3 and OD21 cells the cell their viability decreased over time with the lowest cell density being at the 14 day stage. Fibroblast 3T3 cells, however, displayed a higher cell viability as compared to OD21 cells suggesting that these cell types are more resistant to cytotoxic insults. Overall, this suggests that the hypothesis put forward by Ferracane et al. (1990) is not concurrent with the results of the present study. Notably, the current study utilised animal cells and in recent studies several authors have stated that *in vitro* toxicity tests should be performed with the more appropriate cells i.e. human cells, if these are available (Huang et al., 2002).

The cytotoxicity of RBCs in the present investigation was also studied by culturing 3T3 and OD21 cells in growth medium containing leached substances from each of the RBC materials. Previously it has been stated that incubation of composites in cell culture medium for one to seven days is sufficient to abrogate cytotoxicity (Schedle et al., 1998). The present investigation extended these times periods to 14 days. When cultured with the medium containing leached substances, it was determined that the cell viability of the OD21 cells decreased in relation to the

increase in time that the composite was immersed in the medium. This suggests that the longer the composite was present in the medium, the more toxic substances were released from it. It seems that a wide range of factors may be responsible for the release of unbonded compounds from cured dental composites. An important factor that determines the amount of leachable resin components is the monomer-polymer conversion mechanism (Schweikl et al., 2005). Consequently, curing time, thickness of resin increment and light intensity provided by light units plays a role in the amount of unconverted monomers that may, in turn, remain in the resin composite (de Souza Costa et al., 2003). It has been shown in previous studies that an adhesive resin can induce apoptosis or cell-cycle arrest of cells that are major players in pulp healing and dentin regeneration. However with regards to the development of dental restorative materials it is believed that understanding the mechanisms of cytotoxicity of dental materials is necessary for the selection of a strategy for protection of the dentin-pulp complex that allows for pulp healing and dentin regeneration (Mantellini et al., 2003).

Overall, the results of the current study are not consistent between the cell viability recorded through either cell counting using the haemocytometer or by the neutral red assay, as they do not concur. However, past studies have shown that the haemocytometer analysis is a accurate method for determining cell density and is a method that can be easily reproduced (de Souza Costa et al., 2003, Griggs et al., 2003). Therefore, it can be suggested from the haemocytometer data for both direct contact and media immersion, that the novel RBC X-tra Fil seems to exert less cytotoxic effects on both the 3T3 and the OD21 cells compared with Admira and Filtek^{TM}Z250. This is due to the cell viability of both cell types being greater when in direct contact and in media immersion with X-tra Fil specimens. The composition of RBCs are

continually being improved to help reduce any toxic effects that they may impose, however no dental material meets all the requirements to be considered as an ideal restorative material (de Souza Costa et al., 2003).

The current study utilised methods that have been described in previous studies, and in this case the aging of RBC materials in medium prior to culture seemed to be the more accurate method of determining cytotoxicity. However, a better *ex vivo* model could be utilised in future studies to try and simulate the oral cavity environment more accurately. For example, dentine could have been left covering the cells in culture, therefore buffering any cytotoxic effects and then determining whether the RBCs are still exerting any toxic effect. This system provides a very reproducible, simple technique for the screening of large numbers of compounds. It is less time consuming and relatively inexpensive, especially in terms of animal usage, compared to in vivo tests. It has been suggested that this method, which attempts to mimic cavities in human teeth, may provide a more appropriate test system for comparing the relative toxicities of compounds, especially in view of its reproducibility (Meryon et al., 1983).

The determination of the cell morphology was not particularly clear in the current study, however a more accurate method which can be utilised to observe whether the RBC materials alter the shape of the cells is scanning electron microscopy. In addition to this, a larger sample size should be used to produce a more accurate display of results.

CHAPTER 5 Conclusions

The clinical relevance of this study was to determine the effects of a novel restorative material compared to those materials which are currently available on the market and used in dentistry. The use of resin based materials is dependent on their mechanical (masticatory) stresses and biocompatibility properties.

The current study determined that in comparison to the Ormocer Admira (VOCO, Cuxhaven, Germany) and the dimethacrylate RBC Filtek^{TM}Z250 (St Paul, MN, US), the novel RBC, X-tra Fil (VOCO, Cuxhaven, Germany), exhibited bi-axial flexure strength values similar to Filtek^{TM}Z250 following short-term and medium-term immersion in water. However there was no significant difference detected between the two materials, therefore it cannot be concluded here that the newer material had improved properties over the existing material already available on the market. The hardness testing of top and bottom surfaces of the three different composite materials determined that following immersion in water for short and medium-term periods, the hardness of all three materials varied over time but no significant difference was determined. Therefore, specific characteristics and properties of the novel material cannot be deduced and would need to be further investigated. With regards to curing depth, the X-tra Fil(t) (0-2mm curing depth) displayed better mechanical properties than the X-tra Fil(b) (2-4mm curing depth) however, both displayed much better bi-axial flexure strength, VHN and water sorption and solubility than Admira and Filtek^{TM}Z250. This data suggests that X-tra fil is potentially able to be cured to a depth of 4mm even though the mechanical properties appear to be compromised. However, there was found to be no significant difference between the two different specimens

and in order to better compare the three materials further, the Admira and Filtek^{TM}Z250 specimens would also need to be cured to a depth of 4mm and for the same irradiation time as Xtra-fil (40 seconds). Overall, with regards to the mechanical properties of the materials tested in this study, further investigation would definitely be advised in order to determine the reproducibility of the results presented here. No definite conclusions can be made with regards to the properties of the novel composite Xtra-fil and with regards to this present investigation it can only be presumed that the novel material is similar to those already available and does not demonstrate here any improved mechanical properties.

The current study also investigated the cytotoxic effects of the three composite materials both by direct and indirect contact with two different types of murine fibroblast cell lines ; ATCC Mouse 3T3 Fibroblasts and OD21 undifferentiated pulpal fibroblast cell lines. These biocompatibility tests determined that all three materials exerted a cytotoxic effect on both the cell types. There was no significant difference found between the materials suggesting that the novel material Xtra-fil is just as cytotoxic as Admira and Filtek^{TM}Z250.

Although the present investigation has not shown any significant differences between the mechanical and biocompatible properties of Admira and Filtek^{TM}Z250 in comparison to the novel RBC Xtra-fil, it has laid the foundations for further research.

CHAPTER 6 Recommendations for Further Work

The present study tested samples of the novel RBC X-tra fil cured to a depth of 2mm and 4mm to determine how its mechanical properties and biocompatibility were affected. In order to investigate this further, it is proposed that samples of Filtek^{TM}Z250 and Admira be cured to a depth of 2mm and 4mm and then these materials can be compared further.

Fourier transform infrared spectroscopy (FTIR) offers a direct approach to evaluating the depth of cure for light-activated resins. It is proposed that in order to determine the degree of conversion (DC) of each of the composite materials i.e. the percentage of carbon double bonds converted to single bonds during the polymerization reaction, specimen samples be evaluated using FTIR.

Furthermore, for quantitative evaluation of the relationship between translucency and depth of cure for each of the composite materials, comparison of the transmission coefficient and the cure depth would be a useful method.

Article written by Mumtaz Awan

The potential of a resin-composite to be cured to a 4 mm depth
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Dental Materials

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