EFFECT OF PALM OIL BASED MICROCRYSTALLINE CELLULOSE ON PROPERTIES OF ACRYLIC DENTURE BASE RESIN

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ABSTRACT

The objective of this study was to assess whether the reinforcing of acrylic denture base resin poly methyl methacrylate (PMMA) with oil palm based microcrystalline cellulose (MCC) has an impact on the flexural properties in addition to studying the biocompatibility, chemical and microstructure of the resultant composite (PMMA-MCC). For the purpose of flexural strength and flexural modulus evaluation, three MCC reinforced PMMA groups were fabricated and compared with the conventional and commercially available PMMA. The three test groups were represented by addition of MCC by 2% ($_+$ equal amount of MMA liquid increased) only, 2% ($_+$ equal amount of PMMA powder reduced) and 5% ($_+$ equal amount of PMMA powder reduced)respectively. Twenty specimens (65 mm x 10 mm x3 mm) for each group were prepared according to the manufacturer instructions. In addition to the establishment of cytotoxicity, fourier transform infra-red and scanning electron microscopy tests were also done. One-way analysis of variance (ANOVA) was used to determine statistical differences among the experimental groups. The three test groups with MCC addition demonstrated statistically significant differences in flexural strength and flexural modulus values compared to the conventional resin ($P \leq 0.05$). The highest mean flexural strength was corresponding to the PMMA reinforced with 5% MCC followed by 2% MCC when compared to the conventional PMMA. The improvement in the flexural quality of a denture base acrylic material utilizing MCC supports the potential application of natural fibres in MCC form in the future fabrication of denture base resin. MCC of oil palm empty fruit bunch represents a viable alternative to the current commercially available synthetic reinforced acrylic denture base resin.

Abstrak

Objektif kajian ini adalah untuk menilai sama ada mengukuhkan daripada akrilik resin asas gigi palsu Poly metil metakrilat (PMMA) dengan microcrystalline berasaskan kelapa sawit selulosa (MCC) mempunyai kesan ke atas sifat-sifat lenturan di samping belajar daripada biocompatibility, kimia dan mikrostruktur komposit paduan (PMMA-MCC). Bagi maksud lenturan kekuatan dan lenturan modulus penilaian tiga MCC bertetulang kumpulan PMMA telah direka-reka dan dibandingkan dengan PMMA konvensional dan boleh didapati secara komersial. 3 kumpulan ujian diwakili penambahan MCC sebanyak 2% (jumlah yang sama cecair MMA ditambah), 2% (jumlah yang sama serbuk PMMA dikurangkan) dan 5% (jumlah yang sama serbuk PMMA dikurangkan) masing-masing. Selain penubuhan ujian MTT, FTIR dan SEM. Ketiga-tiga kumpulan ujian dengan MCC samping menunjukkan kekuatan lenturan yang lebih baik statistik yang signifikan dan modulus lenturan berbanding resin konvensional. Kekuatan lenturan tertinggi min yang sepadan dengan PMMA bertetulang dengan 5% MCC diikuti oleh 2% MCC berbanding PMMA konvensional. Peningkatan keterangan dalam kualiti lenturan bahan akrilik asas gigi palsu menggunakan MCC menyokong permohonan potensi gentian asli dalam fabrikasi masa depan resin asas gigi palsu. MCC kelapa sawit tandan mewakili alternatif yang berdaya maju untuk masa ini boleh didapati secara komersial sintetik diperkukuhkan resin akrilik gigi palsu asas.

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LIST OF SYMBOLS AND ABBREVIATIONS

	:	
ANOVA	:	Analysis of variance
FFB	:	Fresh Fruit bunches
FTIR	:	Fourier transform infra-red
GPa	:	Giga pascal
IR	:	Infra-red resonance
MCC	:	Microcrystalline cellulose
MMA	:	Methyl methacrylate
MPa	:	Mega pascal
MPOB,	:	Malaysian palm oil Board
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N	:	Newton is The International System Of Units (SI) derived unit of Force.
NHOFs	:	Normal human fibroblasts cells
nm	:	One nanometre is equal to 10^{-9} metre
OPEFB	:	Oil palm empty fruit bunch fibres
PMMA	:	Poly(methyl)) methacrylate
p-value	:	Statistical significance (generally interpreted as “$p \leq 0.05$”
SD	:	Standard deviation
SEM	:	Scanning electron microscopy
	:	
	:	
	:	

CHAPTER 1: INTRODUCTION

The demands for the discovery and improvement of man-made materials involving the treatment of the human body have attracted many to the field of biomaterials (Leist *et al.*, 2012). Several challenges in biomaterial science need to be addressed to produce/improve newer effective materials. In both situations, the aim is to make the application or utilization of these materials hazard-free, stable and comfortable.

Advancements in the area of dental biomaterial science are driving forward the growth of the dental material industry. Consequently many traditional popular materials used in restorative treatment and oral rehabilitation have been improved or replaced with biomaterials that are more appropriate and of better quality.

In dentistry, tooth loss occurs frequently due to causes such as dental caries, periodontal disease and trauma affecting the patients' appearance, speech, food intake habits and social life. Due to these circumstances, the demand grows for an ideal substituting material to restore patients' functional and aesthetic loss. In 1933, the synthetic polymer of methyl methacrylate known as poly methyl methacrylate (PMMA) was introduced to the market (Cheng *et al.*, 2010). Poly methyl methacrylate has an amazing degree of compatibility with human tissue, and therefore considered one of the most important materials to be used in plastic surgery, orthopaedic surgery and dentistry.

In dental practice, PMMA plays a major role in the replacement and rehabilitation of teeth and it has been the principal denture base material for approximately eight decades (Frazer *et al.*, 2005). The main appeal for the use of PMMA as a denture base material is its good aesthetic properties, reasonable cost effectiveness as well as its ease of handling, accuracy and stability in the oral cavity. Despite its long history of clinical success, PMMA has had a number of problems that need to be resolved such as poor flexural properties and low tensile and bending strengths (Jagger *et al.*, 1999), making it highly prone to fracture, which is considered as one of the most habitual clinical problems in the field of prosthodontics (Takamiya *et al.*, 2012). A study by Johnston (Johnston *et al.*, 1981) showed that there have been many studies on the strength problems of PMMA and they are summarized in the following two approaches: the first approach is to increase the strength of denture base polymer through chemical modifications by adding a cross-linking agent of a multi-functional monomer, such as polyethylene glycol di-methacrylate or other chemical changes to enhance the existing material in order to ensure life stability (Yu *et al.*, 2014). The second approach is to devise a reinforcement of denture base polymer with fibres or rods such as metal wires or metal nets. Many methods of improving its strength and toughness have been investigated. Nevertheless, most of these methods have not been utilized either due to their high cost, the need for specialized processing equipment or increased laboratory time due to more complicated procedures. It is stated that 68% of acrylic resin dentures break within a few years after fabrication (Kim *et al.*, 2004).

In addition to mechanical strength assessment, an in vitro cytotoxicity assays are a potentially useful tool in the study of toxic compounds of complex mixtures. Cytotoxicity assays using cells in tissue culture may provide an answer to the problem of assessing the biological activity of complex mixtures (Žegura *et al.*, 2009). Also, detailed examination of materials in the aspects of their microstructure and chemical composition is vital to any investigation relating to its processing properties and behaviour. Characterization includes all information relating to topographical features, morphology, habit and distribution, identification of differences based on chemistry, crystal structure, physical properties, and subsurface features (Gusev *et al.*, 2004).

Scanning electron microscopy (SEM) and fourier transmitter infrared (FTIR) are useful methods to investigate materials for microstructure and chemical composition, respectively.

1.1 The hypothesis of the research

Microcrystalline cellulose (MCC) of oil palm empty fruit bunch (OPEFB) is a material used to improve the mechanical properties of some polymers. In this study, through the addition of MCC to conventional PMMA, an improvement in flexural and modulus strengths is expected to be achieved.

1.2 The aim of the research

The overall aim of the research is to investigate and assess the influence of MCC when added to PMMA denture base in promoting flexural strength and resistance to fracture. In addition, attention will be focused towards the cytotoxicity of the denture base acrylic resin reinforced with various concentrations of MCC.

1.3 Objectives of the research

An in vitro study was conducted:

1. to determine the effects of MCC addition on the mechanical properties of PMMA through flexural strength and flexural modulus before and after thermocycling;

2. to determine the cytotoxicity of processed acrylic denture base resins reinforced with oil palm based MCC; and

3. to compare SEM microphotograph and chemical composition of processed conventional and high impact PMMA to PMMA with oil palm based MCC.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Numerous research has been conducted to investigate and improve the mechanical properties of PMMA as a denture base material. In spite of the fact that the literature covers a wide assortment of such speculations, this review focuses on six important subjects which arise most frequently throughout the literature. These subjects include traditional and basic ways of acrylic denture base reinforcement, different materials and methods that previously had been studied before on reinforcement and their influences on the mechanical properties of PMMA, fibres as resources for reinforcing, natural fibres as essential assets in green composite and denture base augmentation, the increasing importance of new and more efficient means of PMMA fortification and the essentiality of biodegradability and biocompatibility in these means. However, the literature presents these means in different backgrounds through which major and related work to our study is highlighted in this chapter. Initially, the PMMA material as one of the principal materials in this study will be discussed from the chemical, physical and applications perspectives.

2.2 Ideal properties of acrylic denture base material

The ideal denture base material must have some properties such as biocompatibility, good bond strength with denture teeth, good esthetics and ease of repair. The denture base should be tough enough to resist fracture during mastication and sudden impact forces (Swaney *et al.*, 1953). It is reported that the ideal denture base material should also possess a desired balance of stiffness and toughness. In addition it should be capable of matching the appearance of the natural oral soft tissues (Doğan *et al.*, 1995).

A polymer which is used to construct a denture base should have a value of glass transition temperature (Tg) which is high enough to prevent softening and distortion during usage. The material should, ideally, have a low value of specific gravity in order that dentures should be as ‘light’ as possible. This reduces the gravitational displacing forces which may act on an upper denture. (McCabe *et al.,* 1980).

2.3 Poly methyl methacrylate resin

2.3.1 Chemical and physical properties

PMMA is an organic biological material; this material is also referred to as acrylic or acrylic glass. Chemically, it is known as synthetic polymer methacrylate, with the chemical formula ($C_5H_8O_2$) Figure A-1.

From the physical view, the material is a flawless, colourless polymer; it is accessible in both pellet and sheet structure (Banks-Sills *et al.*, 2016). It has a wide range of reasonable properties such as having low moisture level and water absorbing capacity, due to which products made from this material have good dimensional stability as well as good optical properties as it transmits more light (up to 93% of visible light) than glass.

2.3.2 Applications of PMMA

Different sorts of acrylics are utilized in a wide range of applications including in vehicles, electrical engineering, construction, optics, medicine and dentistry (Balen *et al.*, 2016). PMMA is a critical polymer in light of its applications in numerous fields. Therefore, it is possible to hypothesize that the significance of PMMA can be represented by the various studies conducted to obtain more data and information.

2.3.2.1 Applications of PMMA in medicine

PMMA presents a virtuous degree of compatibility with human tissue. As a result, it is used in cosmetic surgery, for instance in the fabrication and replacement of intraocular and contact lenses (Song *et al.*, 2013). In orthopaedic surgery (Jaeblon, 2010), PMMA has been used as bone cement, vertebrae fixer in cases of osteoporosis, filler for bone defects and screw fixation in the bone (Frazer *et al.*, 2005).

2.3.2.2 Applications of PMMA in dentistry

In dentistry, PMMA is used in the making of artificial teeth, impression trays and provisional crowns. In addition, it is one of the customarily used materials for replacement in dental practice as a base for dentures. It has been argued for approximately seven decades that PMMA is the material of choice for the fabrication of removable partial and complete dentures (Cheng *et al.*, 2010). The popularity of the acrylic denture base material is attributable to its satisfying an aesthetic need, cost effectiveness, ease and uncomplicatedness of handling, as well as accuracy and stability in the oral cavity (Cucuruz *et al.*, 2016).

2.3.3 Limitations of PMMA in dentistry

Despite its extended clinical success as a denture base resin, non-modified PMMA has a number of limitations. It acts in a weak mode when loaded, especially under repeated and impact forces. Besides that, the material is also more liable to scratching.

Much of the existing literature on PMMA as a denture base material pays particular attention to its undesired characters, namely brittleness and ease of fracture. As a result, many studies have been conducted to overcome these drawbacks as well as to enhance and improve the lifespan and proficiency qualities of the material.

2.4 Acrylic denture base failure (fracture)

Denture base fracture (Figure A-1) is a common problem faced by patients who are partial or complete denture wearers; literature has shown that 68% of acrylic resin dentures break within a few years after fabrication (Mowade *et al.*, 2012). Denture failure is a time consuming and costly problem, as well as troublesome for patients as functional and aesthetics matters are affected; in some cases, fractured denture portions can carry a significant health threat if inhaled (Hashmi *et al.*, 2004).

The mechanical properties of PMMA vary as the temperature changes. This material tends to creep. It is not suitable for operation under multiple dynamic load. The fracture of brittle materials, such as PMMA, occurs through the propagation of pre-existing cracks under tensile stress. These cracks may be produced by voids incorporation during processing of the material, polishing or may appear due to microstructural imperfections. The characteristically low fracture toughness of PMMA is one of the major shortcomings of the PMMA based denture base resins. These properties affect the clinical performance of PMMA denture bases (Landel *et al.*, 1993).

Several factors are related to the failure of dentures, including properties of PMMA material, technical shortages that may occur during denture processing and stress. These variables can be associated with the following circumstances: porosity, residual monomer, presence of cracks, or poor adaptation of the denture to the residual ridge.

Dentures exposed to various sorts of loads and exertions inside the mouth (i.e. repeated masticatory force) or outside the mouth by sudden, high-effect powers (i.e. denture fall) will result in the phenomenon of denture weakness. Subsequently, cracks in the denture base can occur. It has been shown that midline cracks are a typical issue in maxillary complete dentures (Ray *et al.*, 2014).

Previous studies expressed that such cracks brought about by flexural exhaustion phenomena and stress application enable the commencement and transmission of cracks, thereby influencing the rate of failure (Sahin *et al.*, 2013).

Collectively, reported data in the area of denture base failure mostly support the assumption that fatigue failure and impact forces are considered as the most frequently stated primary mechanical problems affecting PMMA usage and success in dental practice as a denture base material (Ladha *et al.*, 2011). In addition, during processing or handling, other variables assist denture fracture (Akil *et al.*, 2011). These variables can be associated with the following circumstances: porosity, residual monomer, presence of cracks, or poor adaptation of the denture to the residual ridge (Faot *et al.*, 2006).

2.5 Advancements to improve PMMA performance

Over the recent years, a few endeavours have been carried out to overcome the issue of shortcoming and fragility as the drawbacks of mechanical properties affecting the utilization of PMMA as a denture base material. On the other hand, available knowledge in polymer reinforcement and progress in material science have assisted in the invention and creation of new methods or procedures that have resulted in the improvement of PMMA (Kim *et al.*, 2004). The previous and ongoing investigations and studies on the enhancement of PMMA behaviour towards flexural and sudden impact fall under three headings: the search for or development of an alternative material, chemical modification, and insertion of materials like fibres, metal nets and ultra-high modulus polyethylene. Trials on the reinforcement of the acrylic denture base can be outlined in two categories: chemical alteration and addition of fillers or fibres to the heat-polymerized conventional acrylic resin.

2.5.1 Alternative materials for PMMA

One of the proposed solutions to avoid unwanted mechanical behaviours of the acrylic is to substitute it with another polymer in the manufacturing of denture bases. Some well-known examples for the substitute are polystyrene, polyvinyl acrylic and polyamides (i.e. nylon) (Machado *et al.*, 2007). Nevertheless, they have not been indicated to deliver dentures of more noteworthy precision or with better execution. Meanwhile, studies on the strengthening of the existing material continue to be conducted.

2.5.2 Chemical modification to PMMA

To date, a number of studies have evaluated the impact of chemical modifications on the performance and mechanical characteristics of PMMA. Different theories exist in the literature regarding the chemical modifications of PMMA such as addition of a copolymer or a cross-linking agent of a poly-functional monomer, for example polyethylene glycol di-methacrylate (Abuzar *et al.*, 2010). Another method is addition of rubber to the acrylic denture base, the aim of which is to produce a resin that absorbs greater amounts of energy at a higher strain rate before fracture than the standard resins. The problem is that the impact strength is often improved at the expense of the Young's modulus producing a denture base with increased impact strength but is too flexible. Addition of elastic to PMMA as a strategy for fortification is, to date, the best and generally acknowledged as a different option to the ordinary PMMA denture base material. In any case, the high cost - often up to 20 times that of traditional resin- limits its routine utilization. Nevertheless, it has multiple restrictions, particularly diminished firmness, increased creep and water sorption, which therefore may help to ease microbial plaque (Jagger *et al.*, 1999).

2.5.3 Metal wires and frameworks

This approach suggests devising dentures fabricated from PMMA with metal wires or nets. However, approaches of this particular kind come with various well-known limitations. A serious drawback of this approach is stress concentrations around embedded materials. In addition, the net effect of embedded metals result in the weakness of the polymer; this is often due to poor adhesion between the resin matrix and the metal inserted. Another limitation is that metal-reinforced dentures may be unaesthetic (Balch *et al.*, 2013).

Metal bases and frameworks have been consolidated into denture bases to enhance crack resistance. This method certified the support of the prosthesis. Nevertheless, a degree of troubles arise in the placement or alteration of the metal nets and frameworks position during processing.

2.5.4 Fillers as PMMA reinforcer

Recently, composites (polymer + filler) have replaced materials/minerals in many applications and systems. The main reason behind this is its favourable characters and performance that polymers can offer over routine materials, such as simplicity of preparing, efficiency and cost-effectiveness. Generally, a polymer composite is an engineered material made up of more than one component. These components have distinct and different properties. In a polymer composite, one of the components is called the matrix, while the other is the filler material. Fillers are artificial or inorganic materials utilized to enhance the desired properties of the polymers (Zhang *et al.*, 2003).

Many researchers argued that the mechanical properties of modified polymer are affected by fillers flexural strength and flexural modulus, impact strength and surface micro-hardness. In reinforced composites, adequate bonding of filler particles to the polymer matrix is needed to transfer stresses from the weak matrix to more durable fillers. Earlier examples of fillers studied to reinforce PMMA include glass flakes and hydroxyapatite whiskers.

2.5.4.1 Glass flakes

The addition of glass flakes (Figure A-1) to the heat-polymerized PMMA material results in the improvement of some mechanical properties of the PMMA denture base and a study reported that the addition of glass flakes essentially enhanced fracture toughness and hardness of the material (Franklin *et al.*, 2005).

2.5.4.2 Hydroxyapatite whiskers

Hydroxyapatite (HAP, Ca10 $(PO_4)_6$ $(OH)_2$), is a natural organic material that has been broadly used as a substitute material for damaged teeth or bone over the past three decades. Its compatibility with the surrounding tissues has been experimentally proven. Furthermore, it is an important bioeramic with tremendous potential for biomedical applications. PMMA-HAP filler has been tested with acrylic resin and shown enhance the mechanical properties of the acrylic denture base (Pan *et al.*, 2013).

2.5.5 Nanocomposite polymers

The word Nano originated from the Greek word *νανος*, meaning “dwarf”. Nanocomposite refers to a composite polymer in which one of its components has one or more measurements below one hundred nanometre (nm). One nanometre is equal to 10^{-9} M (Alemdar *et al.*, 2008), or structures having nano-scale separations between the different parts that make up the nanocomposite. The strong combination of a matrix and nano-dimensional phase(s) results in contrasting material properties due to dissimilarities in structure and chemistry (Anttila *et al.*, 2008). Although the term nanocomposite is fairly new in the field of material science and innovation, the nanocomposites have really been utilized for a considerable length of time. It is made up of some natural materials and polymers such as proteins, lipids, and starch, nature. The structure of shells, wood and bones are good examples of nanocomposites that can be found in nature (Azwa *et al.*, 2013).

The mechanical properties of the nanocomposite are different from the polymer and nano filler materials from which they are composed In mechanical terms, nanocomposites vary from routine composite materials because of the extraordinarily high surface-to-volume proportion of the fortifying stage and/or its particularly high aspect proportion. The strengthening material can be comprised of particles, sheets or filaments (Cho *et al.*, 2006).

The space of the interface between the matrix and the reinforcing phase(s) customarily and fundamentally influences the support and fortification of the polymer. Addition of nano-particulates to a polymer matrix can upgrade its performance. The increase of these nanoparticles in the polymer framework at low amounts, approximately 0.2 weight %, prompts critical changes in the compressive and flexural mechanical properties of the resulted polymeric nanocomposites (Balen *et al.*, 2016). A series of polymeric nanocomposites are utilized for biomedical applications, such as tissue engineering. In addition, a range of natural and synthetic polymers are used to design polymeric nanocomposites for biomedical applications; they include including starch, cellulose and collagen (Fratzl *et al.*, 2004).

So far, nanoparticles have been used to examine three factors which are potentially important characters for a denture base material, namely weakness resistance, flexural quality and strength. Recently, two studies of PMMA-nanoparticles reinforcement - zirconium oxide and carbon nanotubes, in particular - have been conducted.

2.5.5.1 Zirconium oxide (ZrO_2) nanotube-PMMA

ZrO_2 nanotube has significant properties, including phenomenal durability and mechanical quality, scratch resistance, rigidity to physical erosion and biocompatibility. It has been employed in clinical materials, particularly for denture and artificial bone generation and repair. In a recent study (Yu *et al.*, 2014) used ZrO_2 nanotubes and nanoparticles to augment the PMMA denture base and reported an increased flexural performance of the PMMA.

2.5.5.2 Carbon nanotubes

Carbon nanotubes (Figure A-1) are used to strengthen the acrylic denture. The study has concluded that even a small amount of nanomaterial (0.5% weight) could result in an important and significant improvement in flexural strength and resilience. The augmented PMMA resin performed better than the control group during the static flexural test. The addition of more than 2% weight of multi-walled carbon nanotubes has no favourable effect on the mechanical properties of the PMMA because of the inadequate dispersion of multi-walled carbon nanotubes in the matrix of the polymer (Yu *et al.*, 2014).

2.5.6 Fibre reinforced composites (FRC)

Fibres are a type of filler that may be utilized to alter the undesired properties of polymers and increase their productivity and quality in many applications (González *et al.*, 2007). Fibres are applied to enhance or adjust certain thermo-mechanical properties of the polymers for particular applications or to diminish the expenses of their manufacturing and production (Daud *et al.*, 2011).

As previously mentioned, the two most important perceivable properties for materials used in significant and vital constructing applications are modulus of elastic and impact strength. Basically, the systems for most of these applications are hugely dependent on material properties in order to give the desired stiffness. However, some limitations may be present and need to be improved, such as flexural properties, shrinkage due to polymerization and impact strength. Fibres are used in polymers to overcome unwanted characteristics (Ku *et al.*, 2011) as well as to enhance the strength, chemical resistances, and electrical or thermal conductivity properties (Maruo *et al.*, 2015). However, there are some known disadvantages of FRC; they are easily affected by humidity and temperature in an adverse manner, and in transverse direction these composites behave inelastically.

In fibre-reinforced composites, the properties of both polymer and fibre are crucial for the improvement of the mechanical properties of the resulting material. Rigidity is more affected by characteristics of the matrix, while elasticity is affected by those of the fibre. A strong interface between components of the composite, concentration, orientation and impregnation of fibres is a significant factor that affects flexibility (Bahramian *et al.*, 2015).

In clinical applications, FRC plays a great role in the medical field and prosthetic dentistry as well as in reconstructive medical treatment. In dentistry, FRCs are utilized in prosthodontics, restorative dentistry (Al Twal *et al.*, 2012), periodontology, orthodontics and in repairing prosthetic devices. Moreover, there are also cranial inserts made of glass FRCs.

Fibre-fortified composites are a recently known collection of dental materials characterized by the incorporation of fibres as filler. The most commonly utilized applications of FRCs are as a part of removable dentures, fixed partial dentures, periodontal supports, root canal posts and orthodontic devices(Butterworth *et al.*, 2003).

The most significant findings that have emerged from the literature review regarding fibre addition to polymers can be concluded as follows: fibre addition separates the consistent matrix of acrylic resin because of the deprived interface between them, which subsequently influences the mechanical properties as mentioned earlier. To overcome this problem and to maintain the goals of fibre addition to polymer, several studies support the concept of fibre surface treatment for the purpose of enhancing contact quality between matrix and fibre. It has been argued that two sorts of fibre are used to reinforce polymers: natural fibre and man-made (synthetic) fibre.

2.5.6.1 Synthetic fibre

Synthetic fibres account for 50% of the used fibres in industry (Hearle, 2001). They are made from synthetized or small polymers. The raw materials of these polymers which compose this type of fibre are petroleum-based chemicals or petrochemicals. The processing of that raw material includes polymerization into a long, linear chemical that bonds two adjacent carbon atoms. Examples of some synthetic fibres that have been used to strengthen PMMA include carbon (Kumar et al., 2002), aramid (John *et al.*, 2001) and glass (Stipho, 1998) fibres. The last has been used since 1960s as a polymer reinforcer (Chen et al., 2004).The compounds used to create these fibres come from petroleum-based chemicals or petrochemicals. The compounds are polymerized into a long, linear chemical chain that bonds two adjacent carbon atoms. Different chemical compounds are used to produce different types of fibres. Synthetic fibres have many disadvantages; they are non-biodegradable, potentially flammable, can be damaged easily by heat to the extent of melting and are liable to electrostatic charge. In the case of usage of artificial fibres in clothes, they are not skin-friendly and thus can cause discomfort after long-wearing or might cause an allergy reaction for some people. In this section, four well-known types of synthetic fibres have been studied in fortifying the PMMA denture base resin are described nylon, aramid, carbon and glass fibres.

(a) ***Glass fibre***

Studies that emphasize the significance of glass fibre (Figure A-1) in dental materials fortification are abundant and utilization of the fibre is advocated in the view of its great cosmetic–aesthetic properties as well as its capacity to adhere accurately to the resin when silane coupling agent(s) is used; this is apparently inconceivable in the case of polyethylene (Vallittu, 2012).

It has been expressed that glass fibre is a known material in fortifying PMMA. It has the potential to impressively improve the fatigue, impact and transverse qualities and modulus of flexibility of multiphase dental polymers (Vojdani *et al.*, 2006). Glass fibre reinforcement for acrylic denture base was thoroughly studied. In dentistry, S-glass (high strength glass) fibre, E-glass fibre and R-glass fibre are used for commercially available FRCs. However, glass filaments created particularly for the purpose of dental application through dental producers are extremely expensive for more extensive clinical uses especially in nations with lower living and health standards (Goguta *et al.*, 2006).

(b) ***Carbon fibre***

Carbon fibre (Figure A-1) has been examined as an acrylic denture base reinforcement agent; the bulk of carbon fibre is made by heating poly acryl nitrate in air at 200–250 °C and then in an inert atmosphere at 1200 °C. This process removes hydrogen, nitrogen and oxygen, leaving a chain of carbon atoms and thus forming carbon fibre (Demirci *et al.*, 2008).

(c) ***Nylon fibre***

Nylon fibres (Figure A-1) are polyamide fibres and are based primarily on aliphatic chains. The chief advantage of nylon lies in its resistance to shock and repeated stressing. However, water absorption affects the mechanical properties of nylon. In the study, nylon-reinforced specimens bases had a higher fracture resistance than the control PMMA specimens (Bledzki *et al.*, 2010).

(d) ***Aramid fibre***

Aramid (A-1) is an ordinary term for entirely aromatic fibres. These fibres are impervious to chemicals, thermally steady and they also have a high mechanical reliability, liquefying point and glass transitional temperature. In addition, they have creased structure (atoms are radially orchestrated as sheets) that makes aramid weak as far as flexural, pressure and scraped area conduct are concerned. As demonstrated in the comparative study by (John *et al.*, 2001), aramid fibre-strengthened samples showed lower flexural quality than those strengthened with glass.

Studies on the science of materials with the purpose of examining and investigating alternative strengthening agents from renewable assets are in progress and are being conducted by specialists over the world (Bras *et al.*, 2010).

Over the last few years, a number of researchers have been involved in investigating the performance of natural fibres as load bearing constituents (reinforcements) in composite materials. The environmental advantages of such materials are clear and obvious, along with the fact that they originate from renewable resources. In addition, there are other significant benefits such as ecological stability and the fact that they do not have a negative impact on health. In brief, natural fibres have attracted the attention of scientists because of their promising advantages in comparison to synthetic ones (Butterworth *et al.*, 2003).

2.5.6.2 Natural fibre

Over the past two decades, natural fibres have received considerable attention as reinforcement for polymeric materials (Brahmakumar et al., 2005). Natural fibres such as coir, sisal, jute, flax, pineapple, etc. are extracted from low-cost raw materials and renewable sources (Raquez et al., 2010), and they have many advantages such as their quality as being non-abrasive, having good thermal stability and thermal insulation, biodegradable and environmentally safe (George et al., 2001). Chemically, they are composed of micro-fibrils embedded in an amorphous matrix of lignin and hemicellulose. These fibres consist of several fibrils that run along the entire length of the fibre. The hydrogen bonds and other linkages provide the necessary strength and stiffness to the fibres.

Natural fibre originates from diverse origins: plants, animals or minerals. Proteins are the basic material that animal fibres are comprised of, while plant fibres are made out of cellulose (Engelbrecht, 2010). Almost all research revolves around the so-called cellulosic fibres. The utilization of such materials in composites has expanded because of their relative efficiency, capacity to reuse and because they contend well as far as quality per weight of material (Faot *et al.*, 2006). Natural fibres have been widely utilized as basic filler material in auto parts. Sisal, jute, coir and oil palm fibres are some common examples of cellulosic natural fibres that have been used in the fortification of thermoset and thermoplastic composites (Ray *et al.*, 2014). Within the scope of characteristically natural fibres, oil palm empty fruit bunch (OPEFB) fibres are promising biomaterials due to its biocompatibility (Rosa *et al.*, 2012).

(a) ***Composition of natural fibres***

Natural fibres mainly consist of cellulose fibrils embedded in lignin matrix. The cellulose fibrils are aligned along the length of the fibre, which condense and provide maximum tensile and flexural strengths, in addition to rigidity (Chuayjuljit *et al.*, 2010). The reinforcing efficiency of natural fibre is related to the nature of cellulose and its crystallinity. Moreover, the hydrogen bonds and other linkages deliver the necessary strength and stiffness to the fibre (Cucuruz *et al.*, 2016).

It can be concluded that natural cellulosic fibre consists of micro fibrils in an amorphous matrix of lignin and hemicellulose. Generally, the fibre contains approximately 60–80% cellulose, 5–20% lignin and up to 20% moisture. Research has been carried out all over the world on the use of natural fibre as a reinforcing material for the preparation of various types of composites that can be used in different fields (Darbar *et al.*, 1994).

Natural fibres from cellulosic origin have received much attention because of their lightweight, nonabrasive, combustible, nontoxic, low-cost and biodegradable properties (Cho *et al.*, 2006). Coir, alfa and hemp are examples of natural fibres that have been examined as suggested substitutes for the manufacture of reinforcing materials (carbon dioxide or even glass). Its cellulosic composition when mixed with the polymer surface zone has a tendency to be linked. However,, this can also prompt de-bonding and void expansion. This situation could be overcome by subjecting the material to some compound substance or even genuine surface region changes to improve the relationship with the polymer reinforced network (Cheng *et al.*, 2010). A few investigations have focused on altering or minimizing the hydrophilic part of the fibre in order for it to be compatible with the hydrophobic character of polymers.

Nonetheless, other shortages still exist. These include the absence of good interfacial grip with the matrix, low liquefying point and poor resistance towards dampness, which make the utilization of natural fibre-fortified composites less appealing. As a result, pre-treatment of the natural fibre in industries has become a significant process that allows the physical, chemical and structural characteristics of the surface of natural fibre to be improved. The artificial alteration of the fibre surface stops the dampness retention process and expands the surface roughness. Among the different pre-treatment techniques, the best methods for man-made surface modifications of natural fibre are graft copolymerization and plasma treatment (Banks-Sills *et al.*, 2016). Generally, construction cement is a brittle material that has lower strain and tensile competences. Natural fibres have been used to strengthen cementation materials and are assumed to be effective in the production of lightweight materials. In addition, they are also expected to result in better process ability, higher strength and reduction of final material brittleness (Bentur et al., 2006).

2.5.7 Bio-composites

Bio-composites can be categorised as materials made by combining natural fibre (filler) and petroleum-inferred non-biodegradable or biodegradable polymer. They have been used since the earlier times to reinforce blocks and buildings. Bio-composites obtained from natural fibre and plastic (bio-plastic) are likely to be more eco-compliant, and such composites are termed ‘green composites’. In the present day, they are used where high static component quality and toughness are essential properties especially in association with weight. Valid examples include dental and medical constructive devices, which are ordinarily subjected to an immense number of loading cycles by masticatory activity or the weight of the body in the midst of physical movement (Deraman, 1994).

Cellulosic bio-composites are fundamentally attractive to scientists because of their high modulus and low specific weight. As a result, higher quality and solidness as well as more secure processing conditions and properties were found when compared and contrasted with glass fortified composites (Faruk *et al.*, 2012). The fact that it does not damage or cause abrasiveness on the processing equipment is yet another of its advantage which also translates to cost reduction. Other interesting benefits include their positive natural effects, biocompatibility, high electrical and thermal resistance, as well as the ability to reduce dermal and respiratory irritation.

2.5.7.1 Denture base PMMA-Sisal bio-composite

In 2008, a study on the development of the mechanical properties of denture base (PMMA) material by utilizing plant fibre sisal filaments (Figure A-1) as a reinforcement was conducted (Kondo *et al.*, 2008). The reinforced composites illustrated a slight development in flexural modulus at an incorporation of 2.5 weight % of sisal fibres and afterwards, a constant deterioration up to 10 weight % had occurred. However, flexural strength clearly diminished and changed relative to fibre content. The study concluded and proposed that further studies ought to consider fibre ratio proportion and surface treatment of added fibres for further improvement of the acrylic material.

2.5.7.2 Denture base PMMA-Ramie bio-composite

Ramie (Figure A-1), cellulosic short vegetable fibre, has been used in the textile industry since a long time ago. Recently, it has been studied to reinforce PMMA, the denture base material, and improve its strength. Different fibre lengths (1.5 -3.0 mm) and volumes % were examined. Mechanical dispersion was used to ensure even and good distribution of fibre in the dough of PMMA during processing.

During the handing out stage of PMMA denture base, the dough is highly-viscous. As a result, it is difficult for the dough to enter into and fill the areas between fibres. Consequently, introduction of voids and porosity in composites and reduction of its strength in most cases may result. Furthermore, the prepared mixture of fibres and resin may shift apart under pressure; due to even higher viscosity, and this also cause inhomogeneous dispersion of fibres in the matrix. The author recommended finding of a suitable surface modification for ramie fibre, so, increase the efficiency of stress transfer in the composites. According to the study, short ramie fibre allowed good dispersion and increased the flexural modulus of the composites. Although adding long fibres increased the flexural modulus, the modulus was reduced at higher contents as a result of apparent accumulation. The flexural strength of short ramie fibre-reinforced denture base PMMA was decreased due to the weak interfacial adhesion between the fibre and the matrix (Xu *et al.*, 2013).

The author proposed a suitable surface change or modification of ramie fibre to decrease the output of stress that causes their movement in the composites.

2.5.7.3 Denture base PMMA-OPEFB (fibres) bio-composite

Recently, the effect of adding 0.5 mm- and 2.0 mm thick of (OPEFB) (Figure G-1) and its cellulose on the flexural strength of PMMA was tested. The mean flexural strength for all experimental groups showed a statistically significant improvement compared to the conventional group. The reason for improvement can be attributed to the modulus of elasticity of the OPEFB fibre which is very high, thus enabling it to absorb most stresses without deformation. There are certain significant inherent properties of OPEFB fibre that make it suitable for composite application. The transverse section of OPEFB shows a lacuna-like portion in the middle surrounded by porous tubular structures. This porous surface facilitates better mechanical interlocking with polymer matrix in composite. This advantage makes OPEFB fibre superior to ramie as the latter needs chemical surface treatment to get a good interface relation with PMMA polymer.

2.5.8 Oil palm empty fruit bunch (OPEFB)

Oil palm (*Elaeis guaineensis*) is the most noteworthy eatable oil crop which is vital in the food industry and for day-to-day use (Sahin *et al.*, 2013). Malaysia is one of the top palm oil producers in the world. Other countries in which oil palm trees can be found include countries in South East Asia, Africa, Latin America and India (Mekhilef *et al.*, 2011).

Fresh fruit bunches (FFB), as shown in Figure G-1, are reaped and prepared in the palm oil factories, bringing about the creation of palm oil. The vacant product bundles or empty fruit bunch (EFB) remains of the fruits left after the extraction of palm oil . The process of oil squeezing results in a lot of waste, known as biomass. Biomass is a fundamental asset which is renewable and manageable. It is also among resources which are available in the production of ethanol and other chemicals. It contributes approximately 14% of the total global final energy demand. Common uses include cooking, heating and electricity. On the other hand, bioethanol is a new industry which is an alternative source of fuel (Sakthivel *et al.*, 2013).

2.5.9 Composition of oil palm empty fruit bunch

OPEFB is a lignocellulosic waste isolated after the fruits are stripped from the oil palm fruit bunch (Figure G-1) to be utilized as a part of oil extraction; it has high fibrous content, amounting to as high as 73% fibre. The EFB is composed of fairly high cellulose content (45-50%) and approximately equal amounts (25-35%) of hemicellulose and lignin (Deraman, 1994).

2.5.10 Oil palm fibre

Oil palm fibre (Figure G-1-3) is taken out from the empty fruit bunch by a process known as retting. It is harsh and firm, containing silica and starch. As mentioned earlier, it has a special surface structure that assists in their interfacial relation with polymers when used to strengthen them (Abdul Khalil *et al.*, 2008).

2.5.11 Cellulose

Cellulose is the world's most commonly existing biopolymer which is organised and produced by plants, algae as well as some microorganisms (Fernandes *et al.*, 2011). Cellulose serves as the predominant fortifying substance in plant structures. Regardless of its relative compound simplicity, the physical and morphological structures of cellulose in higher plants are complicated and heterogeneous. Cellulose particles are connected with different polysaccharides and lignin in plant cell dividers (Engelbrecht, 2010).

The regular polymer cellulose is a direct homo polymer of glucose $(C_6H_{10}O_5)_{n,}$ as shown in Figure H-1, with rehashing units comprising of d-glucose in a $^4C1$ compliance which is insoluble in water. The repeating unit contains three hydroxyl groups. These hydroxyl groups and their ability to hydrogen bond play a major role in directing the crystalline packing and also govern the physical properties of cellulose (Foulk *et al.*, 2011).

Cellulose has several uses, including as an anti-caking agent, stabilizer, preservative, dispersing agent, thickener and gelling agent. Despite that, these uses are generally secondary compared to its most important use of holding on to water.

In nature, the cellulose atomic chains are biosynthesized and self-amassed into micro fibrils, which are made from crystalline and amorphous areas (Fernandes *et al.*, 2011). These aggregated cellulose molecules are stabilized laterally by hydrogen bonds between the hydroxyl groups and oxygen of adjacent molecules (Franklin *et al.*, 2005). The amorphous areas of natural cellulose can be quickly hydrolysed when subjected to strong acid hydrolysis; typically, when wood sources are utilized, the resulting particles of hydrolysed cellulose are ∼100–300 nm long and ∼3–10 nm wide. These nanoparticles are alluded to as "Nano crystalline cellulose" or "microcrystalline cellulose" in spite of their nano scale measure (Bras *et al.*, 2010).

2.5.12 Microcrystalline cellulose

Microcrystalline cellulose is pure, partially depolymerized cellulose. It is synthetized from α-cellulose through the hydrolysis process. Its exceptional fortifying ability, high mechanical properties, low thickness and environmental advantages have drawn the consideration of researchers to use microcrystalline cellulose for the purpose of growing ecologically-friendly polymer composites or green composites. The creation of nano-scale cellulose filaments and their application in composite materials have increasingly gained significant consideration due to their high quality and firmness, along with their low weight, biodegradability and renewability (Frazer *et al.*, 2005).

MCC is a fine, white, unscented, crystalline powder and a biodegradable material which can be utilized as a suspension stabilizer and water retainer in beauty care products, nourishment, and pharmaceuticals commercial ventures (Chuayjuljit *et al.*, 2010). Lately, there has been great enthusiasm in delivering nano-composite materials. This considerable interest is because of the potential properties supported by the nano-scale of the MCC which offers significant characters such as the high surface range for interfacing with matrices of polymers, encouraging optical effects and extra/multifunctional properties, e.g. electrical conduction (Fratzl *et al.*, 2004).

With a specific end goal to create completely renewable and biodegradable nano-composites, both the polymer framework and the nano support must be obtained from renewable assets. MCC has captured the interest of scientists to utilize it as a potential beginning material for cellulose fortified nanocomposites(John *et al.*, 2001). Additionally, a highly mechanical characteristic of the MCC is reported as it can be utilized as an all-inclusive filler. Cellulose nano-fibres show outstanding mechanical properties and this can be attributed to its high modulus property (Šturcová *et al.*, 2005).

They are likewise perfect materials to use as strengtheners in an opaque polymeric framework, provided that they are free from light diffusion. This is because of their sidelong measurements being smaller than the wavelength of obvious light, e.g. bacterial cellulose fibrils (Yano *et al.*, 2005).

Haafiz and colleagues in their study concluded that the prepared oil palm based MCC has the potential to be a good reinforcing material for polymeric materials, where large surface area and good thermal properties are required (Haafiz *et al.*, 2013).

2.5.13 MCC Vs. fibre

High fibre fineness should result in better embedment during compression and moulding, and therefore higher mechanical composite properties. Furthermore, higher fineness should lead to an improvement in the ratio between surface and volume, which also means an increased contact surface between fibre and polymeric matrix. The utilization of fibre with fewer diameters also increases the number of actually embedded fibre, which in turn reduces the concentration of peak stresses at the end of the fibre. Fibre with fewer diameters offers the advantage of increased absorption for impact energy.

The incompatibility problem due to the polar and hydrophilic nature of cellulosic fibre and the non-polar features of most thermoplastics prompts a non-uniform scattering of fibre inside the matrix of polymers. Subsequently, this debilitates the productivity and quality of the composites (Frazer *et al.*, 2005). This is a remarkable problem regarding natural fibre-strengthened composites. The fact that the handling temperature of composites is confined to 200° C - as vegetable fibres experience debasement at higher temperatures - is also another issue; this limits the firmness of matrix material (Frone *et al.*, 2011). There is also the problem of high humidity absorption of natural fibres which contributes to swelling and the vicinity of void formation at the interface between the polymer and filler. This leads to poor mechanical properties and lessens the dimensional steadiness of composites. Another limitation to the effective usage of cellulosic bio-fibre for tough composite application is the low microbial resistance and defencelessness to spoiling. These properties constitute the major issues in the processing, storing and handling of bio composites.

The variability of mechanical properties between natural fibres and polymers presents another key issue of diversity that may affect the mechanical properties of the resulting composites positively. Chemical treatment for natural fibres is a helpful measure to resolve the problems that may arise due to the differences between the matrix and fibre. However, it is clear that the advantages exceed the drawbacks.

Native cellulose is one of the most grounded and stiffest regular strands available; the theoretical modulus is assessed at 167.5 GPa (Hamad, 2013) and it has a high potential to act as a strengthening agent in biopolymers. MCC has the benefit of having high particular surface area as opposed to other traditional cellulose fibres. Wilkinson reported that the best known renewable assets equipped for making biodegradable plastics are starch and cellulose (Wilkinson, 2001). As of now, typical fibre-fortified polymer composite innovations revolve around minimizing the cost, enhancing the performance and exploring lightweight materials to replace pure polymer or glass fibre composites.

There have been concentrated examinations and improvements of composite materials from petroleum-based polymers, such as polypropylene and polyethylene strengthened with regular fibre. These composite materials are utilized widely in automotive, construction and household products. The benefits of utilizing lignocellulosic filaments as fortification and as a part of distinctive polymers are their low weight, great solidity, high quality as well as minimal effort and simplicity of transfer. The potential change in the properties of any composite material depends on the level of scattering and the level of cooperation/grip between the network and strengthening phase (John *et al.*, 2001).

It can be concluded that the fortifying limit of the fibre depends on the type of fibre, attachment to the matrix and impregnation in the resin. Moreover, green polymers get their quality from the characteristics of predominant mechanical properties.

Recently cellulose reinforced composites have been used in car business, particularly for inside applications, development hardware, beauty care products, buildings and furthermore in biomedicine purposes (Khalil *et al.*, 2012).

The utilization of MCC in polymer fortification is still new and thus, further research in this area is needed. Some examples of its application include the consolidation of MCC with polyvinyl liquor (PVA), poly lactic corrosive (PLA), starch and polyethylene (Frone *et al.*, 2011). The PVA bio-composite film that is produced is water soluble, biodegradable, and can be used in the manufacturing of medicine cachets, for surgical purposes, and in controlled drug delivery systems since it has no toxic effect on the human body (Fuqua *et al.*, 2012).

Based on the discussion earlier in this chapter, we can conclude that PMMA is the material of choice in the fabrication of the bases of complete and partial dentures, although its low mechanical properties need to be overcome. Despite there being studies that have investigated strengthening PMMA, most of them illustrate shortages. Biocompatible, biodegradable and suitable fillers may be obtained from natural resources. The favourable mechanical properties of the filler such as the size of its particles and good spread in the matrix can produce significant results in improving the properties of the polymer.

Oil palm empty fruit bunch (OPEFB) is a by-product left in the palm oil mills. The biomass from oil palm consists of over 14 million tonnes of OPEFB fibres (Rupani *et al.*, 2010). Generally, construction cement is a brittle material that has lower strain and tensile competences. Natural fibres have been used to strengthen cementation materials and are assumed to be effective in the production of lightweight materials. In addition, they are also expected to result in better process ability, higher strength and reduction of final material brittleness (Bentur *et al.*, 2006).

Dentures in the oral cavity are subjected to various loads, forces, contact to water, saliva and other fluids; all these are factors related to their success, durability and dimensional stability. PMMA denture base has some poor mechanical properties that affect the denture quality, and reinforcement of the denture resin has emerged as a powerful platform to overcome its undesirable properties. Natural fibres as strengtheners have in the recent past attracted researchers' attention (Sung *et al.*, 2013) due to their superior properties, such as being free of bio-hazards, more cost-effective in comparison to synthetic fibres, biodegradable, bio-compatible and are renewable resources. However, there are some difficulties during manipulation and clinical problems such as mucosal irritation might occur from direct fibre usage as a mechanical reinforcement in the denture base (Bayraktar *et al.*, 2004). John in his study concluded that OPEFB in its cellulose form can be considered as a viable alternative to the existing commercially available synthetic fibre-reinforced denture base resins (John *et al.*, 2015).

Cellulose is the world's most abundant naturally occurring polymer, which is produced by plants as well as microorganisms (Khalil *et al.*, 2012). It is a classic example of a renewable and biodegradable structural plant polymer which can be processed into whisker-like micro-fibrils. Its reinforcing capability, excellent mechanical properties, low density and environmental benefits have drawn the attention of scientists to utilize cellulose to develop environmentally friendly polymer composites, also known as green composites (Eichhorn *et al.*, 2010). MCC is a bio-degradable, purified white, odourless, fine non-fibrous and partially depolymerized crystalline-powder. It is usually isolated from cellulose through the hydrolysis process and usage of mineral acid.

2.6 Testing methods

The study of microstructure, chemical composition and cytotoxicity can be achieved by performing some tests.

2.6.1 Scanning Electron Microscopy (SEM)

SEM is used very effectively in microanalysis and failure analysis of solid inorganic materials. SEM is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects (Goldstein *et al.*, 2012). The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample, including the external morphology (texture) and orientation of materials that make up the sample. SEM uses electrons for imaging, much as a light microscope uses visible light. The advantages of SEM over light microscopy include much higher magnification (>100,000X) and greater depth of field up to 100 times that of light microscopy. Qualitative and quantitative chemical analysis information is also obtained using an energy dispersive x-ray spectrometer (EDS) with the SEM (Brundle *et al.*, 1992).

2.6.2 Fourier transform infra-red (FTIR) analysis

Infrared spectroscopy method has been used for more than seventy years to understand the substance structure and bonds in a material(Reis *et al.*, 2006). . It can determine the quality or consistency of a particular specimen. An infrared range represents a unique profile of a material, showing its molecular and chemical structure. The range consists of an absorption spectrum which is shown in peaks; it relates to the frequency of vibrations between the particles making up the material and indicates functional groups (Papageorgiou *et al.*, 2010). Furthermore, the span of the peaks in the spectra is an immediate indication of the measure of material present. Since each distinctive material is a unique blend of particles, no two mixes produce the same exact range. In this manner, infrared spectroscopy can bring about a positive recognizable proof (subjective investigation) of each diverse sort of material. Considering the current programming calculations, infrared is a remarkable instrument for quantitative investigation.

2.6.3 Cytotoxicity test

In vitro cytotoxicity tests are required for the screening of new materials for and before their usage in vivo, using a cell culture or constituents. Cytotoxicity tests of new or modified materials permit the usage of certain techniques to screen and increase the understanding of their use in clinical application. In the science of biomaterials, a non-toxic effect on cells is one of the indications for material biocompatibility (O'Brien, 1997). Cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. Nowadays, various reagents are used for cell viability detection. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production and nucleotide uptake activity. Many have established methods such as colony formation method, crystal violet method, tritium-labelled thymidine uptake method, MTT and WST methods, which are used for counting the number of live cells. Moreover, trypan blue is a widely used assay for staining dead cells. In this method, cell viability must be determined by counting the unstained cells with a microscope or other instruments (Sumantran, 2011).

The dominant part of denture bases is produced using heat cure acrylic resin which is known to bring about the onset of certain dangerous chemicals that may create genuine responses in the encompassing tissues (Jorge *et al.*, 2003). As reported and discussed in the literature, the monomer-to -polymer ratio is one of the factors that influences the cytotoxicity of denture made of PMMA denture base materials. As stated by Kedjarune *et al* (1999), if extra monomer is added to the mixture, the more the quantity of leftover monomer will be, and this in turn increases the likelihood for cytotoxicity. In this study, all test groups that contained oil palm based MCC had a change in powder: liquid ratio.

Therefore, the screening of the incorporation of oil palm based MCC in the acrylic denture base material for the purpose of strengthening in this study should be examined and evaluated for toxic –free and biocompatibility aspects. In addition to that, MCC extracted from the OPEFB residue was subjected to harsh and long chemical alterations to reach the final form (Yahya *et al.*, 2015) that was used as a strengthener in this study.

2.6.4 Thermocycling.

Thermocycling is one of the broadly conducted methods to mimic the physiological maturing experienced by biomaterials in clinical practice. Hence, it is routinely utilized in *in-vitro* studies to assess the effect of aging and thermal changes on biomaterials. In thermocycling, consecutive cycles of heating and cooling occur, and each heating-cooling arrangement presents mechanical stresses as materials stretch upon warming and contract upon cooling (Kulak-Ozkan *et al.*, 2003). During the presence of rehabilitating devices in the oral cavity, they are subjected to thermal changes and expansion. Therefore, thermal variations can be considered a factor that may affect the strength and behaviour of oral prosthetics. Flexural quality of the specimens were assessed following thermocycling, which is one of the more practical approaches to imitate the aging procedure occurring on the denture base inside the mouth (Machado *et al.*, 2012).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials used in preparation of the study sample

3.1.1 Poly methyl methacrylate (PMMA)

The product name, code, batch number, composition, manufacturer details, proportions and polymerization conditions of the conventional and high impact acrylic denture base materials used in this study are listed in Table 3-1.

Table 3-1: Materials, products information and manufacturers

Heat cure acrylic denture base, Acron Duo	
Powder Pink A28/1. Associate Dental Products Ltd Kemdent Works, Purton ,Swindon, Wiltshire Lot: 102075 SN5 4HT, UK	*Liquid* Acron universal (Heat Cure) Liquid Lot: 913300 Associate Dental Products Ltd
High impact strength heat cure acrylic denture base, Acron HI	
Powder Shade 263 Associate Dental Products Ltd	*Liquid* Hi-Impact Liquid Lot: 902322 Associate Dental Products Ltd

Generally, the ratio between monomer and polymer in the PMMA dough preparation is 1:3 (Chu *et al.*, 2004) :(Silikas *et al.*, 2005).

3.1.2 Palm oil based microcrystalline cellulose

The powder of MCC used in this study, has been extracted from the fibres of empty fruit bunch of oil palm, obtained from the Malaysian Palm Oil Board (MPOB), Kajang, Malaysia.

3.2 Preparation of test specimens

For the purpose of testing mechanical properties, five groups of test specimens were fabricated into rectangular shapes. Specimens were prepared according to ISO 20795-1:2013 (Zuo *et al.*, 2016) in the dimension of 65mm x 10mm x 3mm. The powder-liquid ratio used in this study for each group of specimens is illustrated in Table 3-2

Table 3-2: Composition of all test group specimens

		Powder (g)	Liquid (ml)	Cellulose (g)
A	Conventional heat cure PMMA	12	4	
B	High-impact PMMA resin	12	4	
C	Conventional PMMA (A) reinforced with 2 % cellulose; same percentage of liquid increased	12	4.25	0.24
D	Conventional PMMA (A) reinforced with 2 % cellulose; same percentage of powder decreased	11.76	4	0.24
E	Conventional PMMA (A) reinforced with 5 % cellulose; same percentage of powder decreased	11.4	4	0.60

3.2.1 Preparation of control samples (without MCC)

Nine stainless steel moulds were designed and fabricated in the dimension of 10 mm wide, 65 mm long and 3 mm thick (Mundra *et al.*, 2016), as illustrated in Figure 3-1. In the usual dental flask, every three moulds were invested in dental stone after they had been coated with a thin layer of petroleum jelly (Figure 3-2). The dental flask was pressed to allow the flow of excess material. After the stone set, the moulds were removed carefully, gently and washed under running hot water to make them clean and ready for usage again. The same procedure was followed to clean the cast from any unwanted traces and to facilitate easy application of the separating medium (Sodagar *et al.*, 2013).

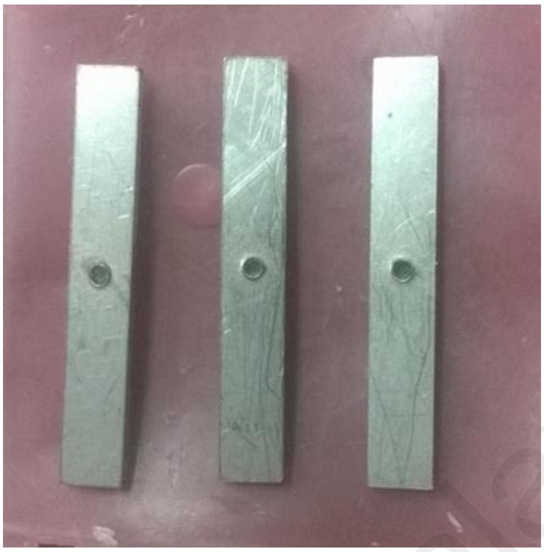

Figure 3-1: The stainless steel moulds

Figure 3-2: Each three moulds placed in one flask

The obtained compartments were used for the preparation of all test specimens. Specimens in group A and B were prepared according to manufacturer’s instructions. The right amounts of powder and liquid were mixed, and the powder was allowed to be wetted by liquid until reaching the dough stage. The dough was then divided into three equal portions to fill the prepared cavities in the set dental stone. Closure at 40.000 N was performed with a hydraulic press (MESTRA, MOD 030350, Sondika Bilbao, Spain) as shown in Figure 3-3 (Hamouda *et al.*, 2014). The flask was clamped, and low pressure was maintained for 30 minutes to allow proper penetration of monomer into the polymer, even flow of the material, and outward flow of excess material. It was then placed in an acrylizer (MESTRA [Paco bath]/LP-E2 EVOLUTION, Sondika Bilbao Spain) at 71°C and left overnight (Kohli *et al.*, 2013). After the completion of polymerization cycle, the flasks were allowed to bench cool at room temperature for 30 minutes before the rectangular bars were expelled and cleaned of stone particles. The edges of the rectangular bars were smoothened with polishing paper to remove abnormalities (Abuzar *et al.*, 2010).

Figure 3-3: Hydraulic press

3.2.2 Preparation of samples containing MCC

The MCC quantity was calculated from the initial weight of the recommended powder weight to an accuracy of 0.01 mg using a digital weighing device (Sartorius-AX224, Göttingen, Germany). The weight of MCC was determined at a ratio of 2% of the liquid weight and 2% and 5% of the powder weights respectively. The PMMA powder and MCC were mixed thoroughly to disperse the MCC powder crystals. Upon reaching dough stage, the mixture was kneaded and packed into the prepared mould. The specimens were polymerized and recovered in the same manner as the control group. After de-flasking, the specimens were polished and finished in the same way as the conventional and high impact PMMA. The test specimens were polished with wet and dry polishing paper until they reached 3 mm in thickness and 10 mm in width. All specimens were visually examined to ensure they were void-free and measured with a digital Vernier calliper at three locations; they are required to maintain a ±0.03 mm tolerance for the thickness and width. All specimens were stored for 48 hours in water at room temperature prior to testing (Meng *et al.*, 2005). Specimens were labelled on each end before testing so that fractured pieces could be reattached and examined subsequent to testing.

3.3 Three-point bending test

Flexural strength was determined using a three-point bending test with a universal testing machine (AG-X Series SHIMADZU, Kyoto, Japan) as displayed in Figure 3-4 at a crosshead speed of 5 mm/min with a span of 50 mm (Meng *et al.*, 2005).

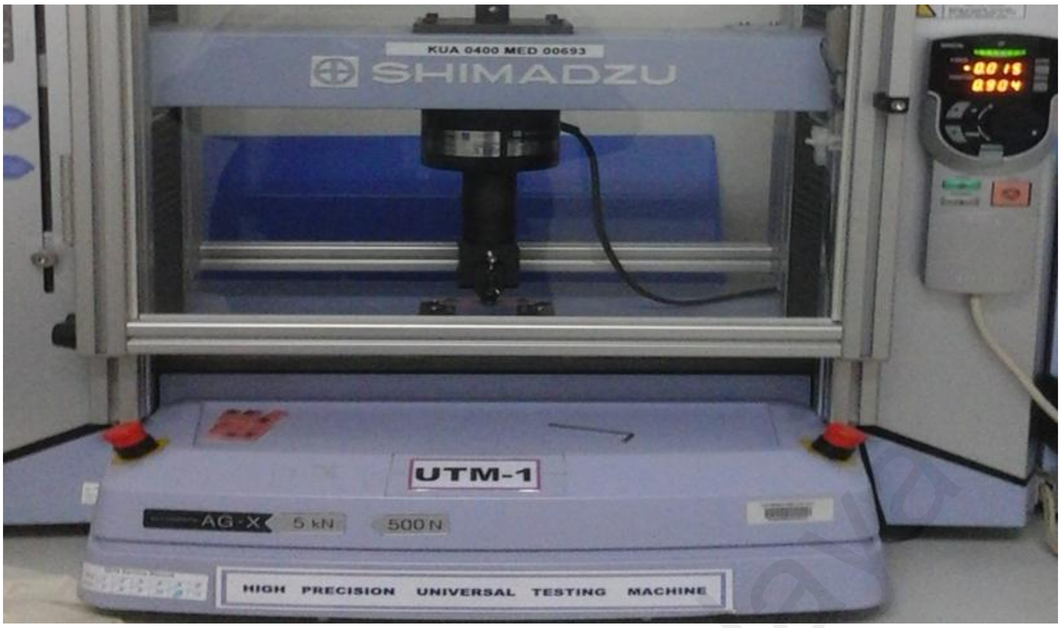

Figure 3-4: UTM (universal testing machine)

The specimens were deflected until a rupture occurred. The flexural strength (F_S) and the flexural modulus (F_E) were calculated using the following formula:

$$F_S = \frac{3P_m l}{2bh^2}$$

$$F_E = \frac{l^3 P_x}{4bh^3 d}$$

Where: P_m is the maximum load, l the span length, b the width of the test specimen, h the thickness of the test specimen, and d the deflection corresponding to load $P_x = 9.8$ N.

Ten specimens from each group were used for the testing. The data from the evaluation of mechanical properties which were obtained from the 3-point bending test were analysed. Statistical analysis of the results was achieved using the Statistical Package for Social Sciences (SPSS) version 20.0 for Windows, by means of descriptive statistical analysis. The one-way ANOVA (analysis of variance analysis) test was run to compare the means of all independent variables in the test groups.

3.4 Measurement of the flexural properties after thermocycling.

Specimens from all five groups were submitted to turns/periods in a water bath (ATDM-T6-PD, AMMP Centre, Kuala Lumpur, Malaysia) which was thermostatically kept at 37° C. Specimens were submitted to 5,000 cycles at 5°C and 55°C with a 30-second dwell time ,. The temperature was kept at 55°C in the hot path and 5°C in the cold cycle; this frequency was used to keep the testing methodology (Ayaz *et al.*, 2015) within a reasonable time limit. For every material, the cyclic loading was directed at utilizing the load as far as possible. After cyclic loading, the samples were submitted to the flexural quality test as described previously (Reis *et al.*, 2006).

3.5 SEM examination

In this study Scanning Electron Microscopy (SEM) as shown in Figure 3-5, was employed to characterize the surface morphology of the samples, the dispersion and compatibility of cellulose in the matrix via the surface and fracture section of the specimens. The side surfaces of each control and fortified test sample were polished with polishing paper. SEM (FEI Quanta 250 FEG SEM, Oregon, USA) photomicrographs were then brought with a speeding up potential of 15 kV at ×400 magnification. A dry and polished prepared sample from each test and control groups was placed in sample mount in sample chamber and the wide top surface was examined and images of the sample surface was obtained and saved. Same process was repeated on fracture surface to study the MCC and matrix at failure side,

Figure 3-5: SEM machine

3.6 Fourier transform infra-red (FTIR) analysis

FTIR (Figure 3-6) measures direct transmittance by using an infrared spectrometer with a resolution of 4 cm^{-1} and within the wave number range of 370–4000 cm^{-1}. The position of significant transmittance peaks was determined using the provided software, SPECTRUM ^{TM} 10 (designed for PerkinElmer FT-IR spectrometers, Beaconsfield, UK).

Figure 3-6: FTIR spectrometer

Comparison of the chemical structure between groups before and after cellulose addition was carried out using the FTIR (Spectrum 400 FT-IR/FT-FIR, Beaconsfield, UK). The spectra were recorded in transmittance (Rozman *et al.*, 2003). The infra-red spectra determining the chemical structure of investigated materials were recorded and scans were collected for each measurement over the spectral range of 4000 – 400 cm^{-1} with a resolution of 4 cm^{-1}. Polymers, MCC, and matrix of all test samples were analysed and compared.

3.6.1 FTIR sample preparation

All samples tested for determining the micro-structure of Group A, B, C, D and E were prepared in a grinded form. After the mechanical testing, one half of the fractured samples from each group were ground using a diamond bur. Each sample was then carefully kept in a dry, clean and tightly closed container before FTIR testing.

3.6.2 Testing of MMA (methyl methacrylate) polymer, monomer and MCC

The window in the FTIR machine was wiped with a clean and dry tissue paper, then washed several times with ethanol. The cleaned surface was clear and a small drop of methyl methacrylate monomer was placed on the plate. Then, spectrum was run, recorded and smoothed using the software. After thorough cleaning from the previous sample, a small amount of polymer powder was taken with a spatula, placed in the small window and spectrum was run.

A small amount of powder was used to define the spectrum of MCC using the same procedure that was used to analyse the poly methyl methacrylate powder(Li *et al.*, 2008).

3.7 Cytotoxicity test

In this study, all test groups that contained oil palm based MCC had a change in powder: liquid ratio. Therefore, the screening of the incorporation of oil palm based MCC in the acrylic denture base material for the purpose of strengthening in this study should be examined and evaluated for toxic –free and biocompatibility aspects. In addition to that, MCC extracted from the OPEFB residue was subjected to harsh and long chemical alterations (Haafiz *et al.*, 2013)to reach the final form that was used as a strengthener in this study.

Hence, the aim of cytotoxicity test was to study if oil palm-based MCC that had been used as reinforcement in the heat cure conventional acrylic denture base would exert toxic effects onconventional PMMA.

3.7.1 Sample preparation

Cylindrical samples measuring 5.0±0.2 mm in diameter and 3.3±0.2 mm in height Figure 3-7 were prepared. Samples were prepared by cutting segments from the test specimens with a diamond cutting disk. Afterwards, the corners were ground with grinding papers to form the cylindrical specimens. Three samples (n=3) were prepared for each group. Prior to testing, the samples were stored dry. Two types of samples before and after thermocycling were used.

Figure 3-7: Cylindrical samples prepared for cytotoxicity test

3.7.2 Cell culture

3.7.2.1 Thawing of cells

A vial containing frozen normal human oral fibroblast (NHOF) cells was transferred from liquid nitrogen to a room-temperature water bath. The vial was held on the surface of the water bath with an occasional gentle push during thawing, which only takes a few seconds. It is important for cell viability that the cells are thawed and processed quickly. The vial was dried off from the outside and wiped with a 70% ethanol solution before opening to prevent contamination. The cell vial contents were transferred to a T-75 culture flask (Sigma-Aldrich, Corning® cell culture treated flasks, New York, USA) containing 30 ml of growth medium. Afterwards, the NHOF cells were incubated at 37°C/5% CO_2 overnight, keeping the flasks in a horizontal position. The next day, the medium was removed and replaced with 15 ml of fresh growth medium. The medium was changed after 2-3 days until the cell monolayers were confluent.

3.7.3 MTT test

Three samples from each specimen group, both before and after thermal cycling, were placed into the test tubes containing Dulbecco's modified Eagle's medium (DMEM). One test tube was filled with cells and normal medium only as control. A total of 15 test tubes were used. The volume of medium used was calculated from the surface area of the sample: 1.25 cm^2/ml. All test tubes were placed into a water bath at 37° C for 24 hours. The specimens were removed and the extracts were filtered using 0.22um syringe sterile filters (Sartorius-Ministar® syringe filter units, Göttingen, Germany).

(A) T-75 culture flask

(B) Minisart® high flow Syringe Filters16541----------K

Figure 3-8: Culture flask and syringe filter

NHOFs were maintained in the DMEM supplemented with 10% foetal bovine serum (FBS) and antibiotics 1% penicillin streptomycin. Cultures were maintained at 37° C in a humidified atmosphere of 5% CO_2 and 95% air. NHOFs were cultivated with 5% CO^2 at 37° C in DMEM and 10% FBS containing penicillin/streptomycin and amphotericin-B. The cells were plated in 24-well tissue culture trays (10^4 cells /cm). The test specimens were placed in the centre of 24-well tissue culture trays. After a 24-hour incubation period, the test specimens were removed from the culture wells and the cytotoxicity of the materials was assessed using the MTT.

3.7.4 MTT assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. MTT (5 mg/mL in Hanks balanced salt solution) was added to each well, and the microplates were further incubated at 37°C for 4 hours (Sipahi *et al.*, 2006). After the incubation period, 10 µL of acidified isopropanol (0.04 M HCI in isopropanol) was added to the cultures and mixed thoroughly to dissolve the dark blue formazan crystals. The solubilized reaction products were transferred to a 96-well plate, and the absorbance value of each well was determined using a microplate enzyme-linked immuno-assay (ELISA) reader (Tecan. Infinite M200 PRO, Männedor, Switzerland) Figure 3.6 equipped with a 570-nm filter.

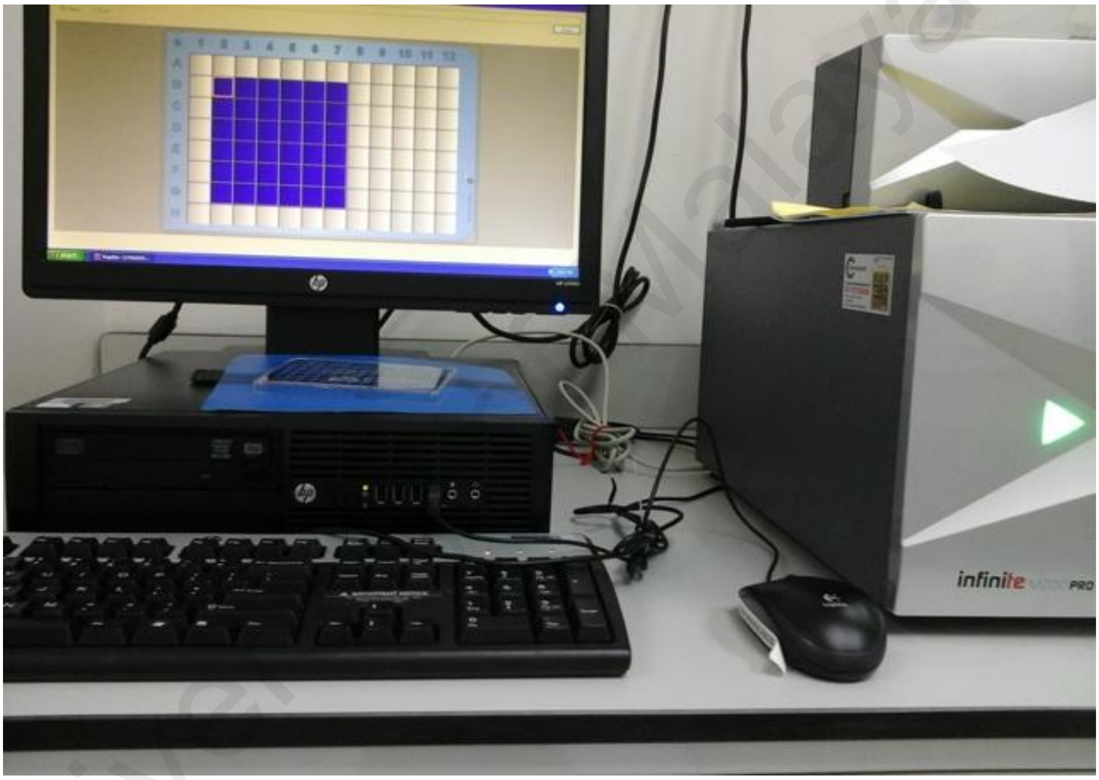

Figure 3-9: Microplate reader

Survival rates of the controls were set to represent 100% viability. Each specimen was tested three times and untreated cell cultures were used as controls. Results were expressed as “percentage of cell viability” determined as: Cell viability % = 100 - [Absorbance at experimental well – absorbance at positive control well] x 100 cytotoxicity was rated based on cell viability relative to controls as reported by (Hubbe *et al.*, 2008); values demonstrated in table 3.3.

Table 3-3: Cell viability rates

Cell viability	Rate
> 90 % cell viability	**non-cytotoxic**
60 – 90 % cell viability	**slightly cytotoxic**
30 – 59 % cell viability	**moderately cytotoxic**
≤ 30 % cell viability.	**severely cytotoxic**

CHAPTER 4: RESULTS

4.1 Three-point bending test before thermocycling

The first question in this study aimed to investigate the effect of addition of MCC as a reinforce material on the flexural strength and modulus of the heat cured PMMA and to compare that with the commonly used high impact acrylic denture base. This test is adopted from the widely available prior studies (Jain *et al.*, 2013) about mechanical properties of denture base materials that has been improved with different types of reinforces.

4.1.1 Flexural strength

Table 4.1 shows the results of the one-way ANOVA test (p<.0001). The mean values of flexural strengths of all test groups with and without reinforcement, shows significantly different flexural strength among the groups contain MCC than the controls (group A and B).

Table 4-1: Flexural strength of the specimens before thermocycling

Groups	N	Mean(MPa)	(SD)	F statistics (df)	p-value
A	**10**	**94.90**	**(20.48)**		
B	**10**	**102.66**	**(30.32)**		
C	**10**	**123.93**	**(11.30)**	**4 (8.60)**	**0.000**
D	**10**	**117.54**	**(23.91)**		
E	**10**	**156.44**	**(35.61)**		

The addition of oil palm based MCC has significantly increased the flexural strength of plain acrylic denture base resin; this was supported by one-way ANOVA which had revealed significant difference in flexural strength proportionate to the amount of the added cellulose. Interestingly for those groups with oil palm based MCC incorporation in different concentrations; the flexural strength is noticeably increased as compared to commercially available high impact acrylic denture base resin. The Scheffe's posthoc procedure, to point out where any distinctions lay between the groups is presented in Table 4.2. This uncovered that, for every cellulose reinforced group, the flexural strength was fundamentally higher than the control groups A and B. Moreover the 5% cellulose in group E had an essentially higher flexural strength than even group C and D. Flexural strength increased with the increasing of cellulose amount. Taken together, these results suggest that there is an association between cellulose incorporation in the conventional PMMA denture base resin and improvement in flexural strength.

Table 4-2: Multiple comparisons* of the mean flexural strength of the specimens before thermocycling

Group	A	B	C	D
B	**.977**			
C	**.193**	**.498**		
D	**.434**	**.794**	**.989**	
E	****.000**	****.001**	**.111**	****.034**

**by post hoc test (Scheffe's procedure) ** Statistically significant (p<.0001).*

The vertical bar graph in Figure 4.1 indicates that the flexural strength increased when more cellulose was added. The best flexural strength was reached at 156.44 MPa, which is more than 60 MPa higher than the plain (conventional) samples (group A), with the optimal content of 5% wt cellulose (group E).

Figure 4-1: Flexural strength of the specimens before thermocycling

4.1.2 Flexural modulus

A definite variation was seen in the flexural modulus values of conventional heat cure and high impact PMMA when compared to reinforced specimens. The flexural modulus of all MCC reinforced groups was significantly greater than those of group A and B as shown in Table 4.3, and the flexural modulus of the reinforced PMMA specimens in group E was significantly greater than those of group C and D. The flexural modulus of the reinforced specimens was higher than those of the standard commercially available specimens as shown in Figure 4,2.

Table 4-3: Flexural modulus of the specimens before thermocycling

Groups	N	Mean (GPa)	(SD)	F statistics (df)	p-value
A	**10**	**5,64**	**(0.26)**		
B	**10**	**7.18**	**(0.16)**		
C	**10**	**8.22**	**(0.10)**	**4 (4.68)**	**0.003**
D	**10**	**8.33**	**(0.18)**		
E	**10**	**9.20**	**(0.24)**		

In the multiple comparison by post hoc test (Scheffe’s procedure) as shown in Table 4.4, there was no statistically significant difference between groups except group E, a significant difference was found between the conventional and 5% MCC acrylic denture bases.

Table 4-4: Multiple comparisons* of means of flexural modulus of the specimens before thermocycling

Group	A	B	C	D
B	**.563**			
C	**.096**	**.848**		
D	**.075**	**.795**	**1.000**	
E	****.007**	**.291**	**.877**	**.916**

**by post hoc test (Scheffe’s procedure) ** statistically significant (p<.0001).*

Figure 4-2: Flexural modulus of the specimens before thermocycling

4.2 Three-point bending test after thermocycling

Ten specimens from each group were subjected to thermocycling to test the effects of loading and stresses over time on their flexural and modulus strengths behaviour..

4.2.1 Flexural strength

The ANOVA test revealed a significant difference in flexural strength between MCC added groups and the control groups ($p<0.01$) as can be seen in Table 4.5. Moreover the bottom half of the table showed higher values of the means of flexural strength (group C, D and E). The most striking observation that emerges from the data comparison between tables 4.1 and 4.3, group C (2% MCC) has the same mean value of flexural strength and it is the only group among all groups that did not show a decrease in strength after thermocycling.

In addition, Scheffe's test procedure was conducted to compare and show differences between means of research variables among groups for flexural strength after thermocycling and the summary is exhibited in Table 4.6.

Flexural strength testing for group E exposes a significantly higher value than the control groups (A and B) but no significant difference when compared to 2% MCC group (Group C) after thermocycling.

From the line chart presented in Figure 4.4 which compare flexural strength values in all test groups with and without thermocycling it is apparent that all values decreased, however groups with MCC are still higher than the controls. Addition of MCC had increased flexural strength of the conventional PMMA with and without thermocycling procedure.

Table 4-5: Flexural strength of the specimens after thermocycling

Groups	N	Mean(MPa)	(SD)	F statistics (df)	p-value
A	10	86.81	(15.34)	4 (13.01)	0.000
B	10	99.03	(12.10)		
C	10	123.93	(23.30)		
D	10	117.54	(16.94)		
E	10	133.31	(16.75)		

Table 4-6: Multiple comparisons* of mean flexural strength of the specimens after thermocycling

Group	A	B	C	D
B	.647			
C	**.001	.052		
D	.827	.998	**.023	
E	**.000	**.002	.817	**.001

**by post hoc test (Scheffe's procedure) ; ** Statistically significant (p<.0001).*

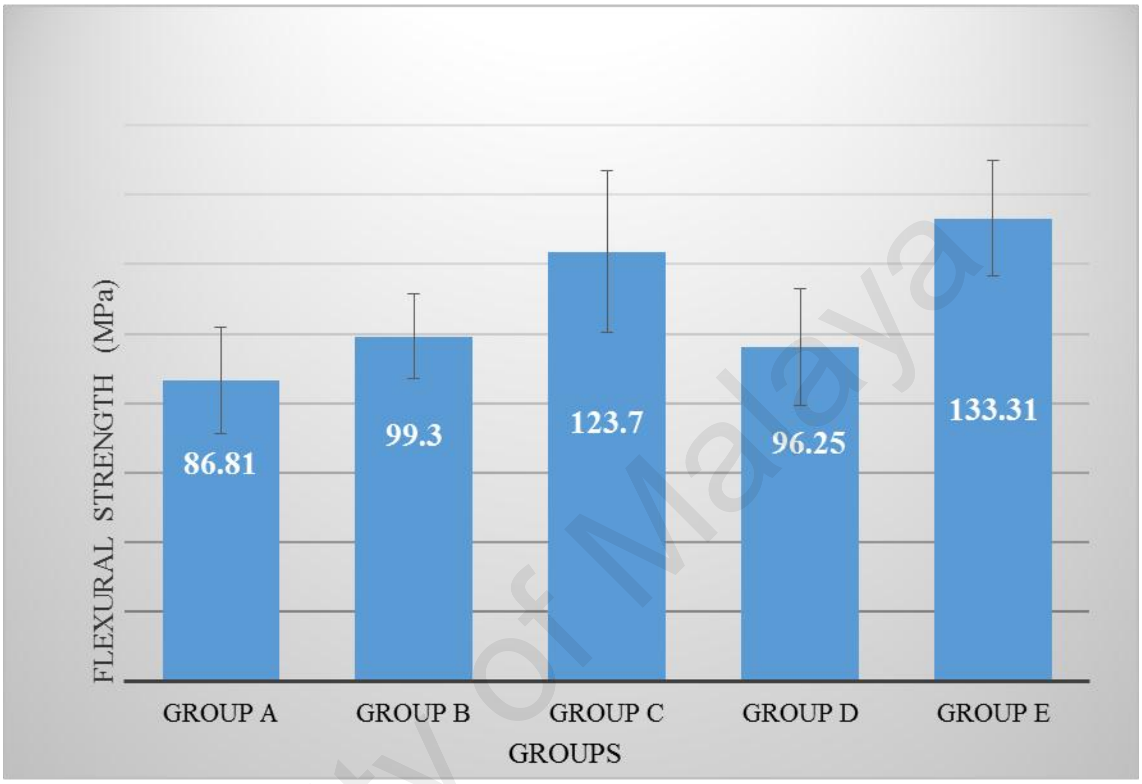

Figure 4-3: Flexural Strength after thermocycling

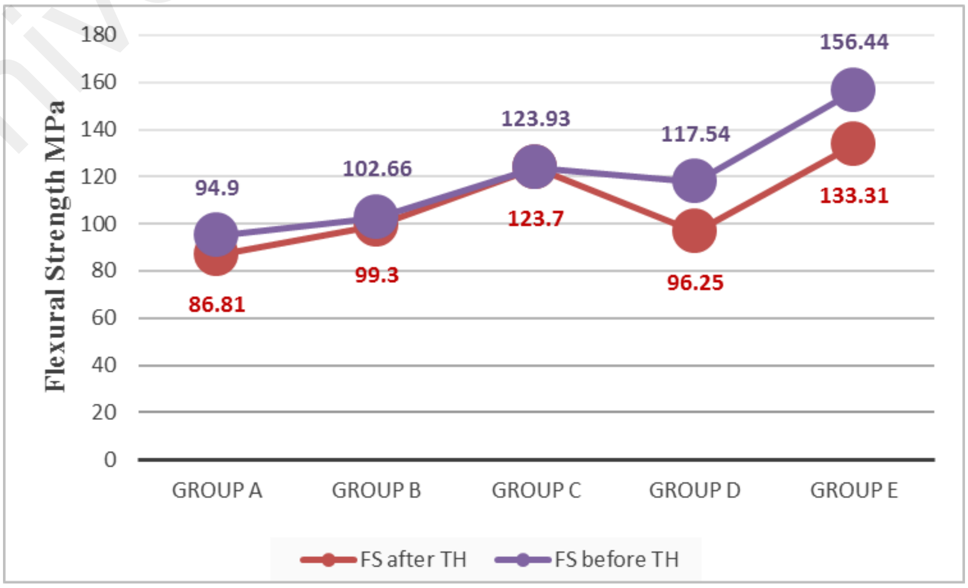

Figure 4-4: Comparison of flexural strength between all groups before and after thermocycling

4.2.2 Flexural modulus

A significant difference in flexural modulus was found between MCC groups and the controls (p>0.05) table 4-7. Multiple comparison between groups has shown significant difference in flexural modulus between group C and the conventional resin (group A) as appeared in table 4-8 .Interestingly, group C has same value of flexural modulus with and without thermocycling (8.11 and 8.22) GPa respectively.

Table 4-7: Flexural modulus of the specimens after thermocycling

Groups	N	Mean (GPa)	(SD)	F statistics (df)	p-value
A	**10**	**5.59**	**(0.10)**	**4 (3.332)**	**0.018**
B	**10**	**6.69**	**(0.19)**		
C	**10**	**8.11**	**(0.14)**		
D	**10**	**6.98**	**(0.18)**		
E	**10**	**7.75**	**(0.17)**		

Table 4-8: Multiple comparisons* of mean flexural modulus of the specimens after thermocycling

Group	A	B	C	D
B	**.700**			
C	****.032**	**.457**		
D	**.483**	**.997**	**.675**	
E	**.146**	**.836**	**.970**	**.957**

*by post hoc test (Scheffe's procedure) ; ** Statistically significant (p<.0001).

4.3 Microstructure photography

SEM micrographs of characteristic cellulose specimens with different MCC amounts content, showed that the cellulose is evenly distributed in the resin matrix and the level of impregnation of the MCC with the polymer framework was generally good, and that the vicinity of voids has not being seen as appeared in Figure 4-5.

On failure mode investigation, SEM micrographs of the fractured site showed the control, and cellulose specimens have similar modes of failure as illustrated in Figure4-6. No overwhelming mode of failure was seen in cellulose test samples; which may be explained by the fact that the presence of cellulose in the matrix does not disturb the consistency and compatibility of the matrix resin as in control groups. Cellulose remained adhered to the matrix resin after fracture, indicating good MCC-matrix bonding.

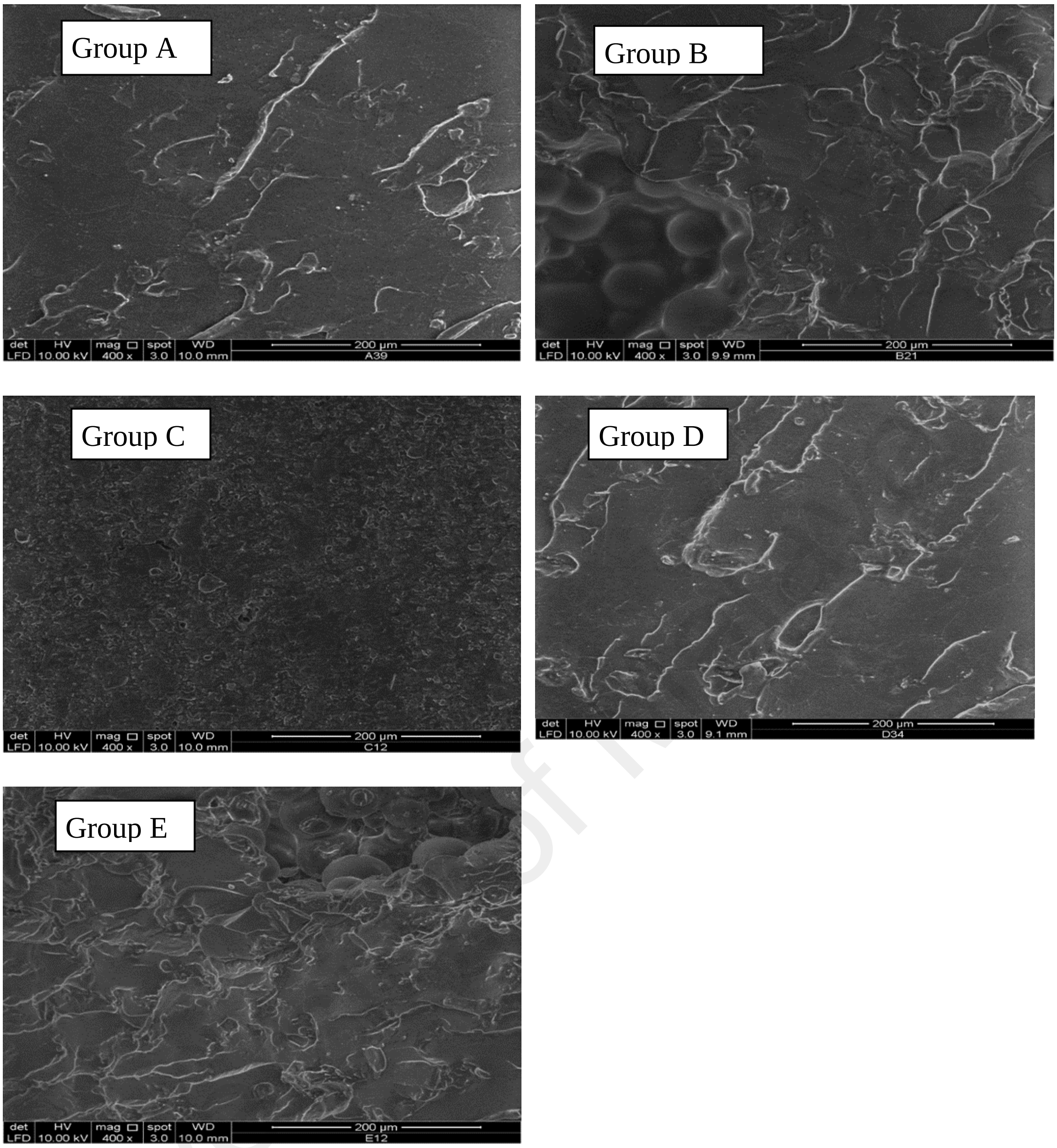

Figure 4-5: **SEM micrographs (400× magnification) of the specimen surface.**

The specimens produced from reinforced resin treated with MCC addition showed that there were no micro-gaps observed between the filler particles and the acrylic resin matrix.

All reinforced specimens had a relatively smooth fracture section with absence of voids in the matrix. PMMA matrix had better compatibility with cellulose particles as illustrated by the even dispersion .It also explains why the mechanical properties were enhanced by the addition of MCC.

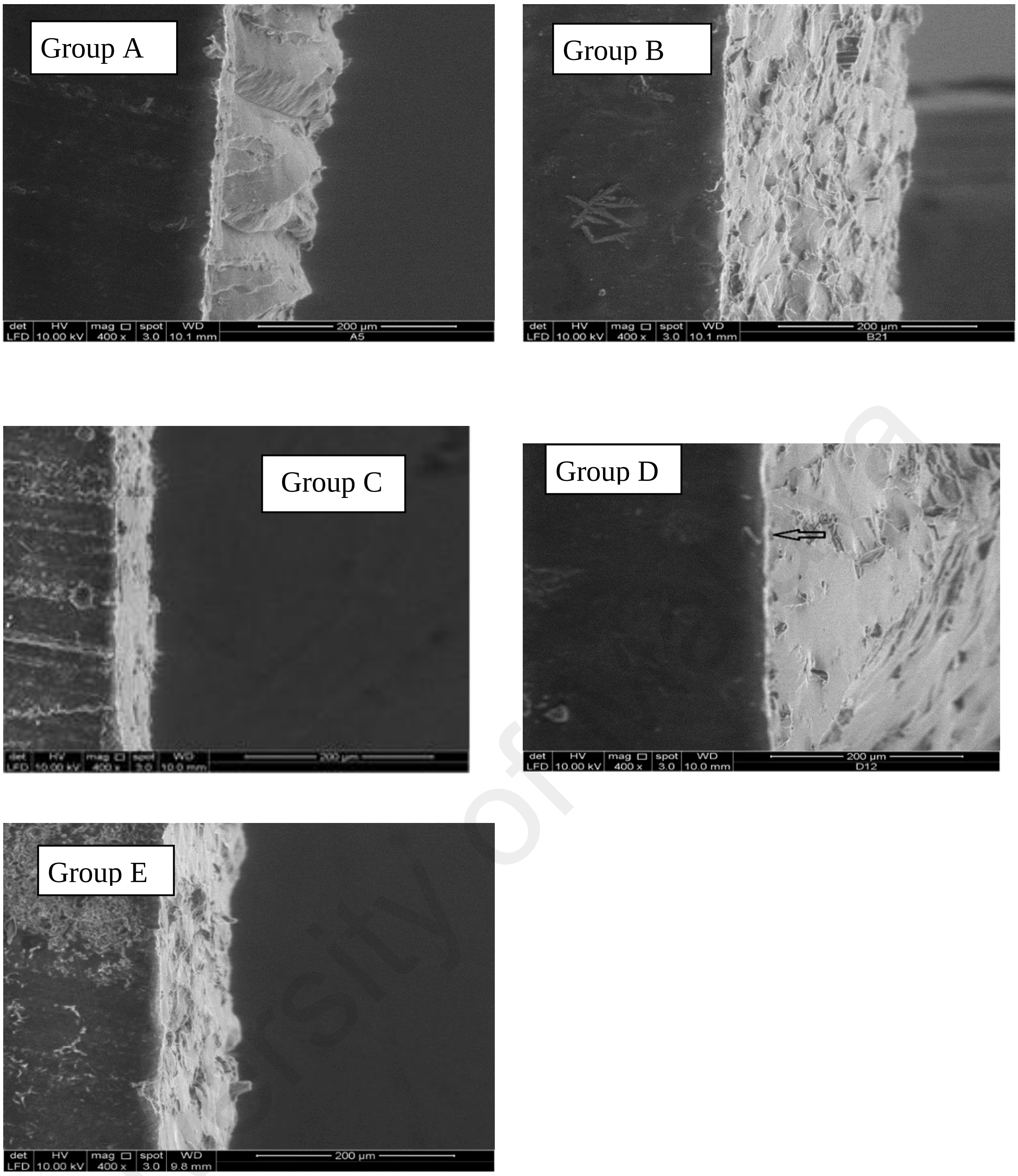

Figure 4-6: Fractured borders at 400x magnification

4.4 Materials integrity with fourier transform infrared spectroscopy screening

The Fourier transform infrared spectroscopy (FTIR) analysis was used to predict the integrity between MCC and PMMA. The results of FTIR spectra of cellulose reinforced groups, conventional and high impact PMMA are shown in Table B-11 and accordingly data can be interpreted by dividing it into two major groups, namely Group 1 and 2.

4.4.1 Group 1 (MCC + group C, D and E)

This group consisted of MCC and test groups that have MCC addition, group C, D and E. The two charts of conventional PMMA + MCC 5% (Group E) and MCC showed an additional bands around 3318 and 3418 cm^{-1} of -OH (H-bonded) group but MCC represented another band at 1641 cm^{-1} for -OH groups of cellulose. The bands of C-O-C and C=O are absent in MCC.

The other bands are almost similar in band values and intensity except for the intensity of the bands for cellulose-treated (2%, 5%) and untreated samples. The dominant peaks in the region between 3400-3500 cm^{-1} are due to OH bending while the peaks between 2800-2900 cm^{-1} are associated with -CH aliphatic (Yu *et al.*, 2011). The prominent peak between 1638 and 1641 cm^{-1} is attributed to C=O. The peaks at 1435 to 1438 cm^{-1} represent -CH_2- group bending while -CH_2- of cellulose appeared at 1386 $cm^{-1.}$ Absorbencies at 1141 to 1158 cm^{-1} are related to C-O- C group (Wilkinson, 2001).

4.4.2 Group 2 (PMMA, MAA, group A and group B)

This group consisted of monomer, polymer, conventional and high impact PMMA. It was noticed that the absence of C≡C bands in both (MMA) and (group B). The other bands are almost the same but the intensity of C=O and C-O increased to be group B > group A > MMA.

The differences between group 1 and group 2; there are not C≡C bands in group 1 and -OH bands are absent in group 2. The highest intensity of C=O is found in the chart of group B.

Figure 4-7: FTIR spectra of all groups

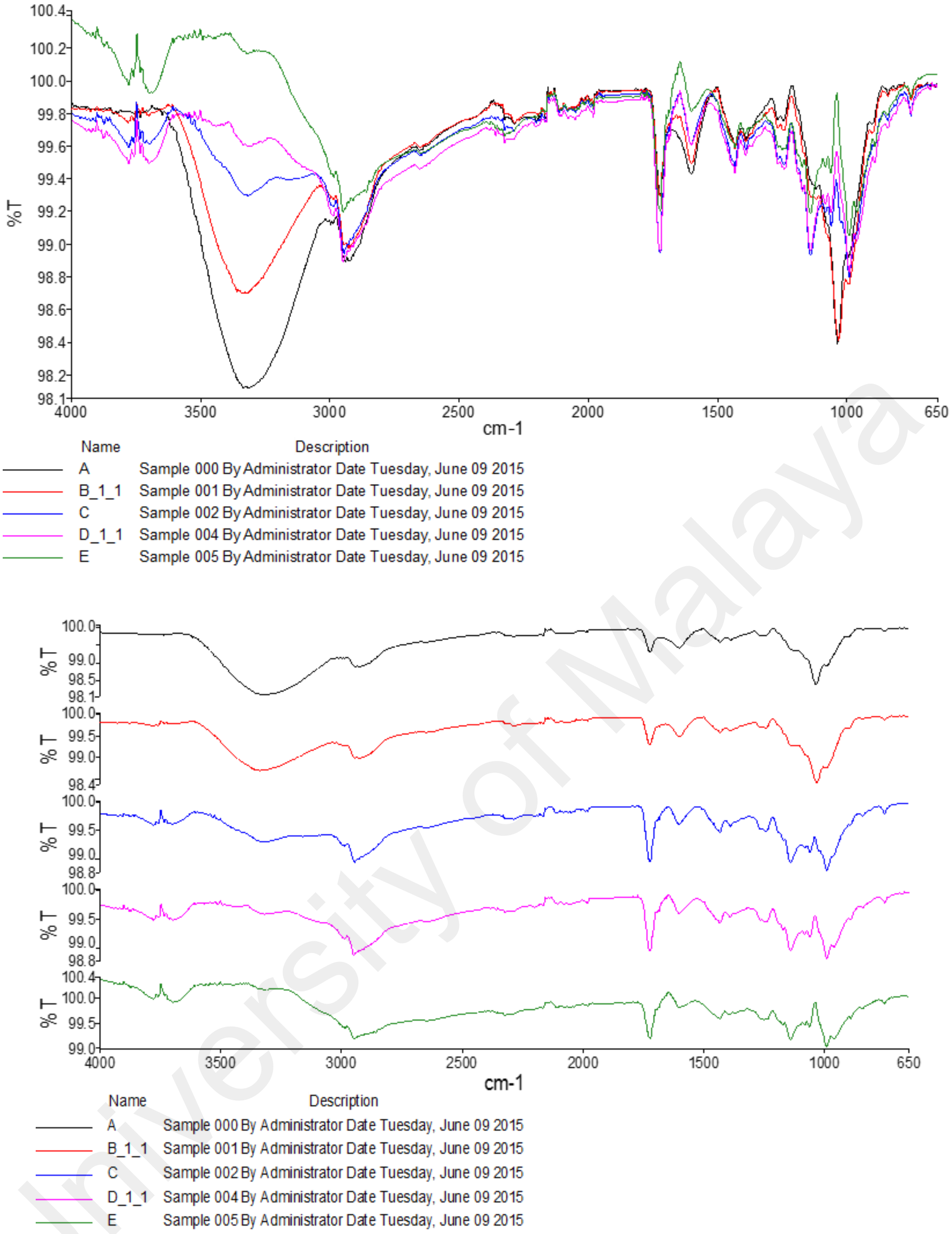

Figure 4-8: E Overlay of the spectra of all groups

4.5 Cytotoxicity test

Cytotoxicity was evaluated by MTT test assay according to ISO 7405:2008 that specifies test methods for the evaluation of biological effects of medical devices used in dentistry. It includes testing of pharmacological agents that are an integral part of the device under test of newly made and thermocycled test specimens. Extracts were prepared according to (ISO 10993-12:2012 - Part 5) the literature reported that acrylic bases releases residual toxic materials (Vallittu *et al.*, 1995). After immersion of samples in DMEM medium for 24h, the hydro soluble constituents which had been released was analysed and their cytotoxic effect were evaluated and compared with aid of MTT assay.

For this *in vitro* experiment, cytotoxicity test was repeated twice. MTT assay/cell viability percentages of all experimental groups are presented in Figure 4-9. Viability of all groups including before and after thermal cycling was more than 90%. There was no statistically significant difference among groups and all groups were classified as non-cytotoxic. Cytotoxicity was rated based on cell viability relative to controls as reported by (Sun *et al.*, 2014).

Cell viability percentage, colorimetric readings and (SD) of all test groups of the first and second experiments are presented in Table C-1 and Table C-2.

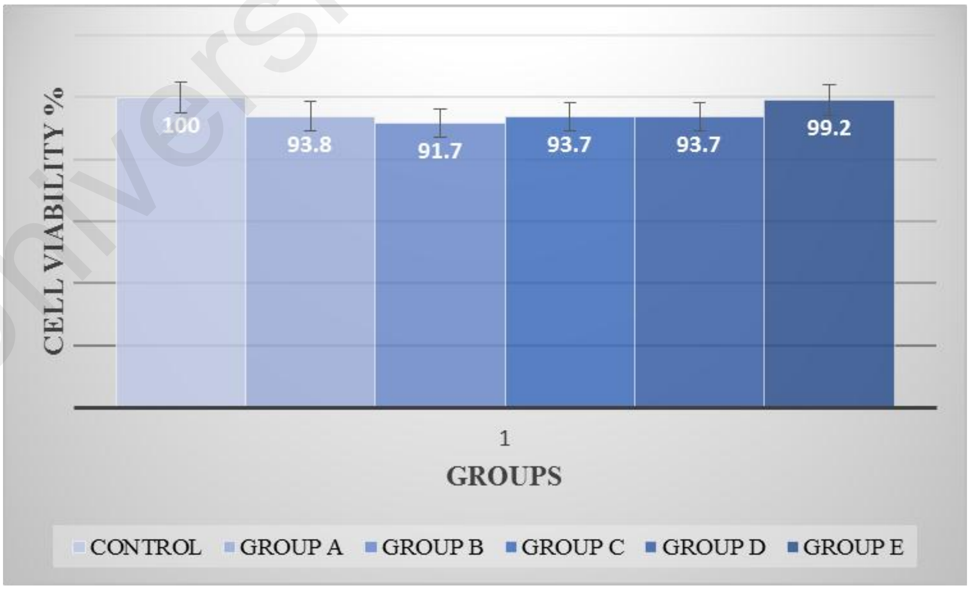

Figure 4-9: Cell viability of all test groups

CHAPTER 5: DISCUSSION

As mentioned in the literature review, a wide range of studies on denture base reinforcement with different additives such as fibres, nanoparticles (Wang *et al.*, 2016) or chemical modification to improve its mechanical behavior (Soygun *et al.*, 2013; Vallittu, 1993) have been reported. In clinical practice, poly methyl methacrylate resin is the material of choice for the fabrication of conventional removable dentures (Paranhos *et al.*, 2013; Soygun *et al.*, 2013) in the rehabilitation of edentulous patients. The material is favored in prosthetic dental practice because of its simplicity in processing and equipment usage, cost-effectiveness as well as its similarity in shade to oral tissues (Goldstein *et al.*, 2012). However, the acrylic denture base has moderate mechanical properties (Mowade *et al.*, 2012) and many studies state that the fatigue failure mechanism as the main causative factor in denture fracture (Darbar *et al.*, 1994) or failure (Jagger *et al.*, 1999). Usually, the denture base is subjected to many different influences inside and outside the oral cavity, such as biting forces, thermal changes, daily exposure to saliva, food, water and implication of a sudden impact due to a fall that may result in denture failure and/or fracture. Denture fracture will bring about disturbance and inconvenience for patients including functional and aesthetical problems, additional costs and time consumption resulting from denture repair or replacement. It is encouraging to find many studies on denture base reinforcements showing an improvement in strength but do not go on to be applied clinically. One of the reasons for this is the difficulty in the proper placement of the reinforcement material in its desired position during the processing of the acrylic resin (Silikas *et al.*, 2005). Previous studies have also reported undesirable aesthetic results, where adding aramid or carbon fibres to denture base resin as strengtheners resulted in an unfavourable appearance (Vallittu, 1996).

In light of recent developments in the science and engineering of materials, green composites (polymer and filler particles originating from renewable, natural sources) are said to be the future of the materials. The application of MCC as green fillers in some polymeric composites is associated with an improvement in some mechanical characteristics of those polymers such as tensile and flexural strengths (Thakur *et al.*, 2014; Van Krevelen *et al.*, 2009; Wambua *et al.*, 2003). Very few previous studies evaluating the utilization of natural fibres as an acrylic denture base reinforcement were found in the literature. These fibres reported include sisal (Xu *et al.*, 2013) , Oil Palm Empty Fruit Bench (OPEFB) fibres and ramie vegetable (Xu *et al.*, 2011).

In the present study, we investigated the effects of applying MCC obtained from OPEFB fibre in the modification of traditionally used heat-cured PMMA denture base in order to develop and improve its mechanical characteristics. In addition, we also studied the microstructure, chemical composition and biocompatibility of the resultant composite. Cellulosic materials are much stiffer than polymer matrix and as a result it adds stiffness to the composites (Lee *et al.*, 2014). In addition, MCC has the advantage of high specific surface area compared to other conventional cellulose fibres. We find from this study that the degree of bonding between MCC filler and PMMA polymer has improved the grafting capability between the two and consequently improving the flexural properties of this PMA-MCC composite. Canche-Escamilla *et al* (2002) reported that by increasing the amount of MMA in the mixture, the add-on property of the grafted polymer improved; this may explain the improved flexural modulus and strength properties seen in Group C.

The results indicated a significant increase in the flexural and modulus strengths of the conventional PMMA in virtually all test groups containing the MCC in various concentrations. These results are supported by similar studies (Elshereksi *et al.*, 2009;

Kiziltas *et al.*, 2014; Pöllänen *et al.*, 2013) indicating that the good mechanical properties of MCC consequently play an important role in improving mechanical properties when mixed with other polymers.

Several studies have reported that excessive fibre concentration, does not always improve the mechanical properties of composites (Bentur *et al.*, 2006) on the contrary, it can act as a critical point in the final strength of composites (Zhang *et al.*, 2003). However, findings in the current study showed a significant difference in the improvement of mechanical properties when more MCC was added to the conventional resin. This result may be explained by the fact that MCC displays exceptional mechanical properties such as high modulus and the high surface area of the micro crystals (Sung *et al.*, 2013). Accordingly, MCC has a greater surface area for bonding with the matrix in comparison to fibres. Furthermore, as mentioned in the literature, the strengthening through filler reinforcement is based on the principle that a relatively soft ductile matrix is fully capable of transferring an applied load to filler particles (Fonseca *et al.*, 2016).

In contrast to the challenges of placing reinforcement fibres, in the current study, we found that MCC was easily and homogeneously added and mixed with the PMMA and there were no concerns regarding fibre projection in the case of degradation, displacement during processing or fracture (Tandon *et al.*, 2010).

In this study, MCC, PMMA liquid and powder showed the usual course of fabrication of acrylic denture to reach the dough stage, and no increase in viscosity of the mixture was observed as opposed to the findings in some other studies (Chu *et al.*, 2004).

Another interesting finding was that the reinforcement of the denture base using MCC powder did not show any undesirable appearance. MCC is an ideal material to use as reinforcement in transparent polymeric matrix because the PMMA denture base material modifies its mechanical properties positively and assists in preventing crack propagation (Abuzar *et al.*, 2010). However, the usage of fibre in denture base polymer made of PMMA powder and MMA powder mixture sets some specific requirements; for instance, they should be impregnable with the highly viscous denture base resin (Abuzar *et al.*, 2010). As a result, most previously mentioned methods of fibre reinforcements suffer from some serious limitations due to the anisotropic and physical characters of the added fibre from the polymeric matrix of the denture base material (Dyson, 1991).

The morphological structure of polymeric materials is a very important feature because it determines many of its properties (Van Krevelen *et al.*, 2009). The study of consistency attribute of the used materials has been assisted by the use of SEM analysis (Goldstein *et al.*, 2012). SEM is a tool that can be used to obtain thorough information about the state of the interface between MCC particles and the PMMA denture base resins (Bertassoni *et al.*, 2008). This information is vital to understand the potential of MCC in the reinforcement of composites (Mowade *et al.*, 2012) (Soygun *et al.*, 2013). All test groups with MCC addition showed a homogeneous appearance without the presence of any voids, no granular structure was present in the continuous phase of the samples. The observed uniform dispersed of MCC in the PMMA matrix was most likely a result of the good mixing of the formulation components during the preparation process (Vallittu, 1996). The microstructure study at the fractured sides of the samples showed a homogenous distribution of the MCC particles within the resin. No presence of gaps (clefts) was observed between the filler and PMMA resin matrix. This observation may indicate a presence of bonding between the resin and cellulose which could resist the loading stresses and result in a long life span of acrylic base dentures reinforced with MCC (Elshereksi *et al.*, 2009; Yu *et al.*, 2012; Yu *et al.*, 2014).

FTIR was used to collect information on the chemical structure, functional groups and bonds between MCC and PMMA materials. It determined the quality and consistency of the reinforced samples. In this study, we compared it to the control groups and initial materials used in the fabrication of samples. As mentioned, MCC is an organic compound and it is extracted from cellulose which is considered to be one of the most abundant renewable organic material produced in the biosphere (Wang *et al.*, 2014). Table B-11 shows the differences in IR spectra for cellulose-treated (2%, 5%) and untreated samples.

The IR spectrum of MCC-free groups (Group A and Group B), showed C=C stretching at 2300 and 2330 cm^{-1}. Carbon (C) =oxygen (O) was observed for both cellulose-treated (group C, D and E) and untreated samples at 1719 to 1723 cm^{-1} but the intensity was higher in the untreated ones. In general, the intensity of the functional group was better in the case of cellulose and this is good from the chemical point. On the other hand, a comparison of the IR spectra between the two groups of cellulose (2%, 5%) showed the absence of OH- peak in the case of 2% cellulose, but the intensity of the other peaks was higher in the case of 2% than the 5%; from the chemical point, 2% is considered better than 5%. Our IR results indicated that the absence of OH- of cellulose at a frequency of 1641 cm^{-1} in group C, D and E must have been due to bonding between MCC and PMMA. That can be explained under the highlights of the unique character of MCC and ability of grafting with other polymers through the hydroxyl group in their surfaces (Hubbe *et al.*, 2008). When spectral peaks changed shape, or the relative intensities changed, then it would suggest that the additive is adding spectral features or modified (Ling *et al.*, 2016).

A primary biocompatibility screening for materials is important in the branch of dental materials (O'Brien, 2002; Schmalz *et al.*, 2009). Cytotoxicity testing is a known procedure for the above mentioned purpose (Demirci *et al.*, 2008), where the cytotoxic influence is determined by assessing the cellular mitochondrial function. A minimum one-day period of soaking newly fabricated dentures in water prior to insertion into the patient's mouth is recommended (Vojdani *et al.*, 2006). This justifies the usage of eluates in cytotoxicity test with 24-hour periods of immersion (Saravi *et al.*, 2012). The eluates obtained from soaking the prepared samples in the media are used to find out their effects on culture growth and they are subsequently compared to control cultures containing only cells and medium. In some previous studies conducted in the same area, it was found that two factors may influence the amount of residual monomer content namely the mixture ratio between powder and liquid and the processing procedure (Alla *et al.*, 2013). In this study, the addition of cellulose changed the powder to liquid ratio in all test groups, but the processing procedure remained the same. Therefore, cytotoxic effect is related to the composition of materials and the amounts of polymer and monomer. Cell viability, evaluated with MTT assay, was more than 90% in all groups. Test and control groups were non-cytotoxic. It can be concluded that microcrystalline cellulose-reinforced polymer induces no cytotoxicity.

Although the objectives of the research have been achieved; however, there remain limitations that need further investigations. Additions of 2 and 5% only of MCC have been tested. Therefore further study is recommended to determine the ultimate and ideal amount of MCC that would make PMMA stronger. The desired balance of mechanical properties highly recommends the study of other mechanical properties such as impact, tensile and compressive strengths are recommended to be investigated in future studies. An *in vivo* study of the MCC reinforced PMMA is highly suggested based on findings from cytotoxicity test.

CHAPTER 6: CONCLUSION

The present study was designed to determine the effects of MCC on the denture base resin. Results obtained from the investigation of flexural strength and modulus of elasticity demonstrated a remarkable improvement compared to the plain conventional and high impact denture bases. Moreover, reinforced samples containing different concentrations of MCC demonstrated an apparent improvement in mechanical properties. Experimentally, considerable improvements were observed without the usage of a silane coupling agent, which offers the prospect of more improved characteristics. The absence of cytotoxic effects of the MCC supports its near-to-perfect substance that could reinforce the PMMA and substitute the commercially available high impact acrylic resin denture base. Micro structure investigation of the addition of MCC in different concentration indicated extremely good dispersion of the MCC in the PMMA matrix, consequently good interfacial adhesion and improved mechanical properties have been reported. Possibly this would have been caused by good chemical compatibility between PMMA and MCC which is supported by the FT-IR results indicated that good interaction between the MCC filler and PMMA matrix occurred due to presence of the new peaks. Although this study focuses on the properties of PMMA after the incorporation of MCC, several practical points have emerged in terms of ease and simplicity of addition, mixing and processing of specimens. This research will serve as one of the basic foundations for future studies on the usage of natural products with nano-sized particles as denture base reinforcement.

One of the open issues in research is in observing the effects of MCC inclusion on other properties of reinforced denture base materials. The light weight and white colour of MCC are less likely to alter the density and finished appearance of the denture base material.

The value impacts illustrated in the results of the mechanical and microstructural properties of PMMA through the involvement of MCC, augmented by accepted biological properties, are expected to have the potential to increase the clinical service of the principal material. Consequently, this will support the practitioner in delivering high quality bases for partial or complete dentures. In addition, it will make the most of the success rate and longevity for the benefit of denture wearers. It is likely that research findings in this study, with new materials not yet used in biomedical applications, will further extend the possibility of using MCC in other applications in the area of dental materials.

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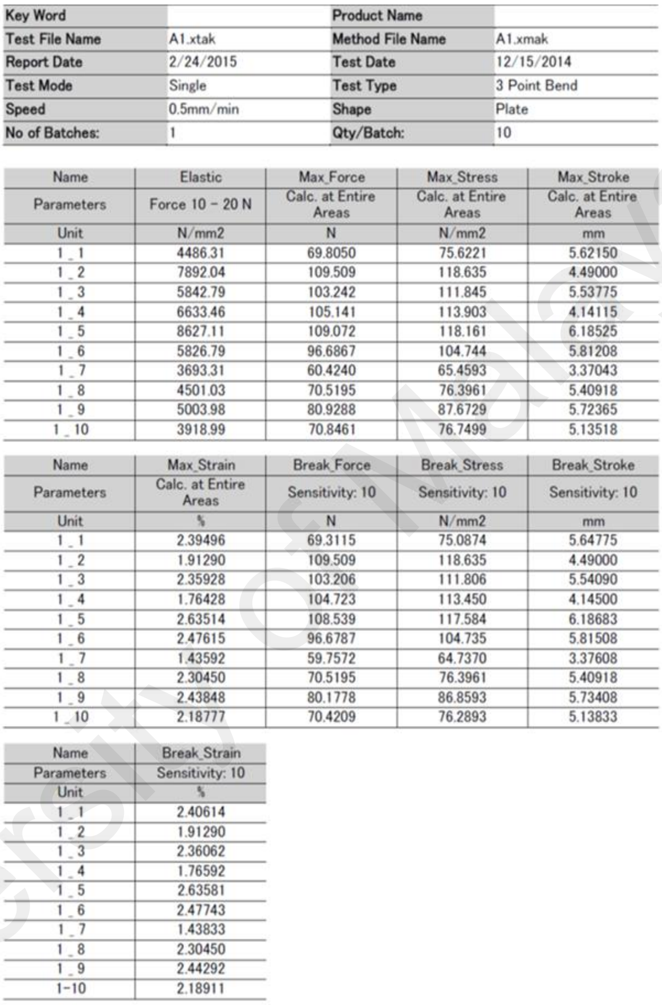
APPENDICES

APPENDIX A RAW DATA OF FLEXURAL STRENGTH AND FLEXURAL MODULUS OBTAINED FROM 3-POINTS BENDING TEST

Table A-1: Values of flexural strength and flexural modulus of GROUP A



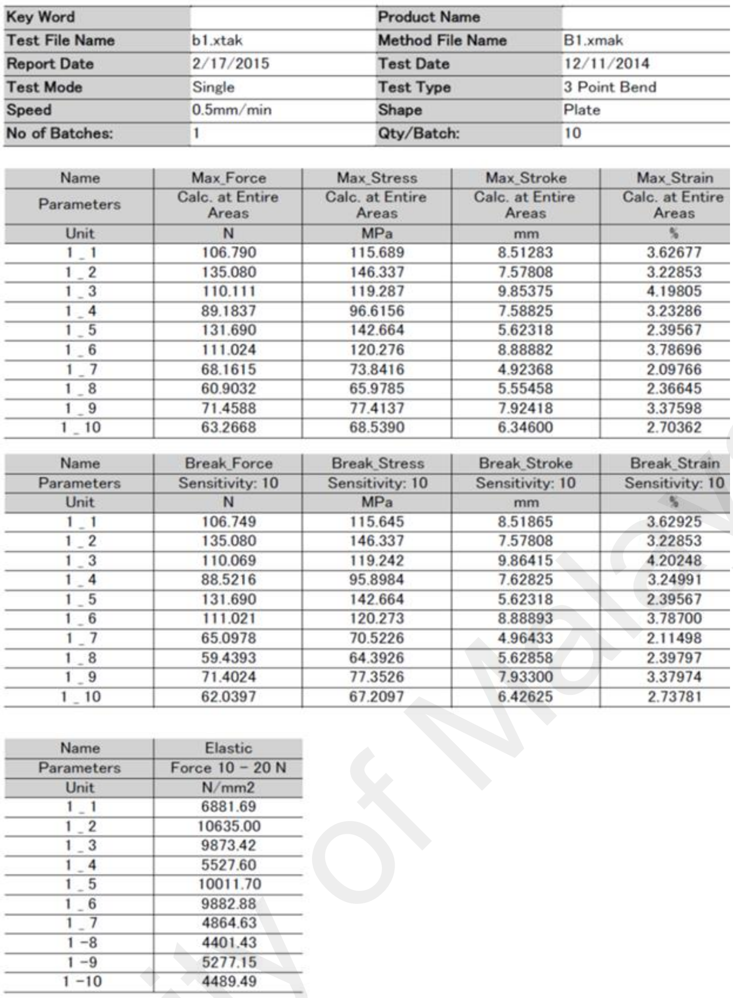
Key Word		Product Name	
Test File Name	A1.xtak	Method File Name	A1.xmak
Report Date	2/24/2015	Test Date	12/15/2014
Test Mode	Single	Test Type	3 Point Bend
Speed	0.5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 – 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	N/mm2	mm
1 _ 1	4486.31	69.8050	75.6221	5.62150
1 _ 2	7892.04	109.509	118.635	4.49000
1 _ 3	5842.79	103.242	111.845	5.53775
1 _ 4	6633.46	105.141	113.903	4.14115
1 _ 5	8627.11	109.072	118.161	6.18525
1 _ 6	5826.79	96.6867	104.744	5.81208
1 _ 7	3693.31	60.4240	65.4593	3.37043
1 _ 8	4501.03	70.5195	76.3961	5.40918
1 _ 9	5003.98	80.9288	87.6729	5.72365
1 _ 10	3918.99	70.8461	76.7499	5.13518

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	N/mm2	mm
1 _ 1	2.39496	69.3115	75.0874	5.64775
1 _ 2	1.91290	109.509	118.635	4.49000
1 _ 3	2.35928	103.206	111.806	5.54090
1 _ 4	1.76428	104.723	113.450	4.14500
1 _ 5	2.63514	108.539	117.584	6.18683
1 _ 6	2.47615	96.6787	104.735	5.81508
1 _ 7	1.43592	59.7572	64.7370	3.37608
1 _ 8	2.30450	70.5195	76.3961	5.40918
1 _ 9	2.43848	80.1778	86.8593	5.73408
1 _ 10	2.18777	70.4209	76.2893	5.13833

Name	Break_Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	2.40614
1 _ 2	1.91290
1 _ 3	2.36062
1 _ 4	1.76592
1 _ 5	2.63581
1 _ 6	2.47743
1 _ 7	1.43833
1 _ 8	2.30450
1 _ 9	2.44292
1-10	2.18911

Table A-2: Values of flexural strength and flexural modulus of GROUP B



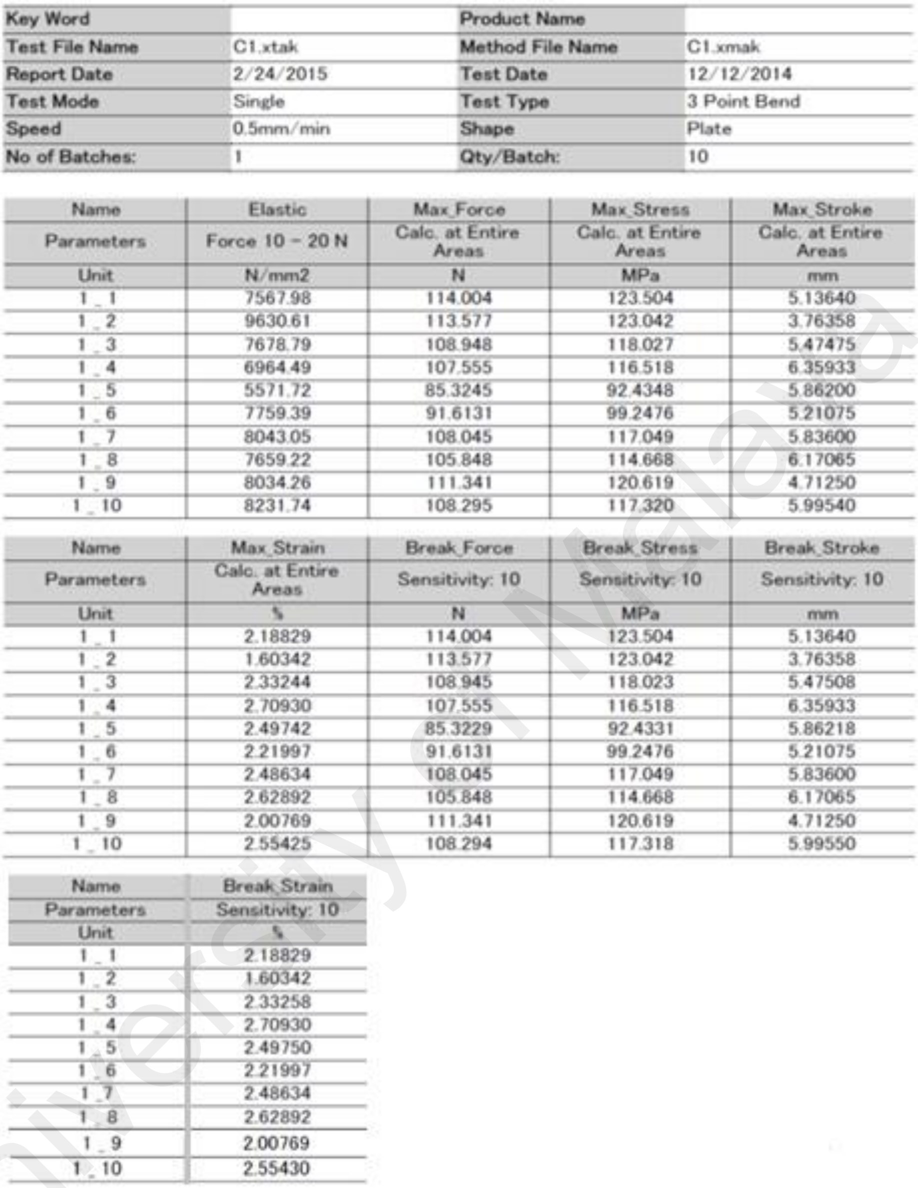
Key Word		**Product Name**	
Test File Name	b1.xtak	**Method File Name**	B1.xmak
Report Date	2/17/2015	**Test Date**	12/11/2014
Test Mode	Single	**Test Type**	3 Point Bend
Speed	0.5mm/min	**Shape**	Plate
No of Batches:	1	**Qty/Batch:**	10

Name	Max_Force	Max_Stress	Max_Stroke	Max_Strain
Parameters	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N	MPa	mm	%
1 _ 1	106.790	115.689	8.51283	3.62677
1 _ 2	135.080	146.337	7.57808	3.22853
1 _ 3	110.111	119.287	9.85375	4.19805
1 _ 4	89.1837	96.6156	7.58825	3.23286
1 _ 5	131.690	142.664	5.62318	2.39567
1 _ 6	111.024	120.276	8.88882	3.78696
1 _ 7	68.1615	73.8416	4.92368	2.09766
1 _ 8	60.9032	65.9785	5.55458	2.36645
1 _ 9	71.4588	77.4137	7.92418	3.37598
1 _ 10	63.2668	68.5390	6.34600	2.70362

Name	Break_Force	Break_Stress	Break_Stroke	Break_Strain
Parameters	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	N	MPa	mm	%
1 _ 1	106.749	115.645	8.51865	3.62925
1 _ 2	135.080	146.337	7.57808	3.22853
1 _ 3	110.069	119.242	9.86415	4.20248
1 _ 4	88.5216	95.8984	7.62825	3.24991
1 _ 5	131.690	142.664	5.62318	2.39567
1 _ 6	111.021	120.273	8.88893	3.78700
1 _ 7	65.0978	70.5226	4.96433	2.11498
1 _ 8	59.4393	64.3926	5.62858	2.39797
1 _ 9	71.4024	77.3526	7.93300	3.37974
1 _ 10	62.0397	67.2097	6.42625	2.73781

Name	Elastic
Parameters	Force 10 - 20 N
Unit	N/mm2
1 _ 1	6881.69
1 _ 2	10635.00
1 _ 3	9873.42
1 _ 4	5527.60
1 _ 5	10011.70
1 _ 6	9882.88
1 _ 7	4864.63
1 -8	4401.43
1 -9	5277.15
1 -10	4489.49

Table A-3: Values of flexural strength and flexural modulus of GROUP C



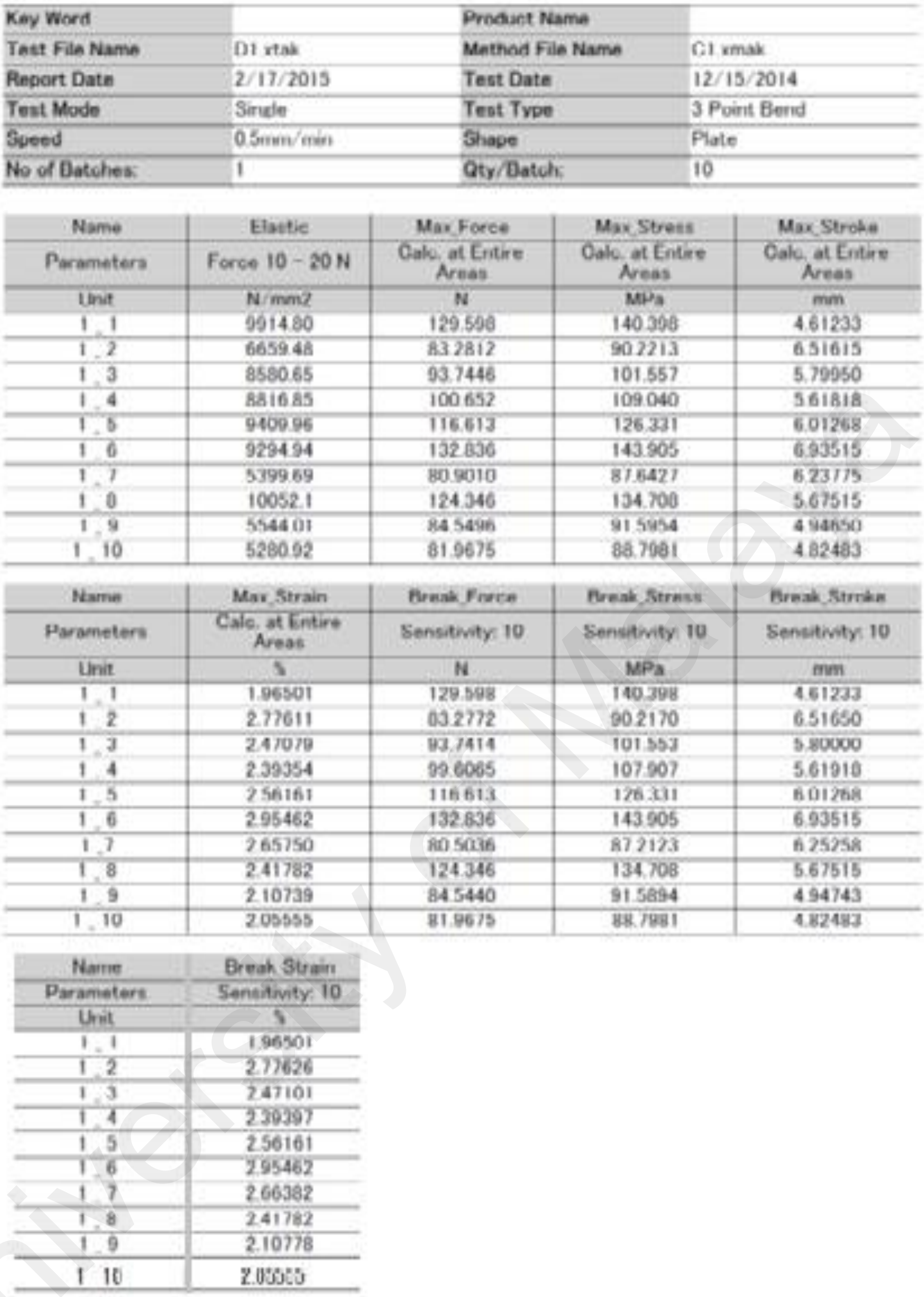
Key Word		**Product Name**	
Test File Name	C1.xtak	**Method File Name**	C1.xmak
Report Date	2/24/2015	**Test Date**	12/12/2014
Test Mode	Single	**Test Type**	3 Point Bend
Speed	0.5mm/min	**Shape**	Plate
No of Batches:	1	**Qty/Batch:**	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	MPa	mm
1 _ 1	7567.98	114.004	123.504	5.13640
1 _ 2	9630.61	113.577	123.042	3.76358
1 _ 3	7678.79	108.948	118.027	5.47475
1 _ 4	6964.49	107.555	116.518	6.35933
1 _ 5	5571.72	85.3245	92.4348	5.86200
1 _ 6	7759.39	91.6131	99.2476	5.21075
1 _ 7	8043.05	108.045	117.049	5.83600
1 _ 8	7659.22	105.848	114.668	6.17065
1 _ 9	8034.26	111.341	120.619	4.71250
1 _ 10	8231.74	108.295	117.320	5.99540

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	MPa	mm
1 _ 1	2.18829	114.004	123.504	5.13640
1 _ 2	1.60342	113.577	123.042	3.76358
1 _ 3	2.33244	108.945	118.023	5.47508
1 _ 4	2.70930	107.555	116.518	6.35933
1 _ 5	2.49742	85.3229	92.4331	5.86218
1 _ 6	2.21997	91.6131	99.2476	5.21075
1 _ 7	2.48634	108.045	117.049	5.83600
1 _ 8	2.62892	105.848	114.668	6.17065
1 _ 9	2.00769	111.341	120.619	4.71250
1 _ 10	2.55425	108.294	117.318	5.99550

Name	Break_Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	2.18829
1 _ 2	1.60342
1 _ 3	2.33258
1 _ 4	2.70930
1 _ 5	2.49750
1 _ 6	2.21997
1 _ 7	2.48634
1 _ 8	2.62892
1 _ 9	2.00769
1 _ 10	2.55430

Table A-4: Values of flexural strength and flexural modulus of GROUP D



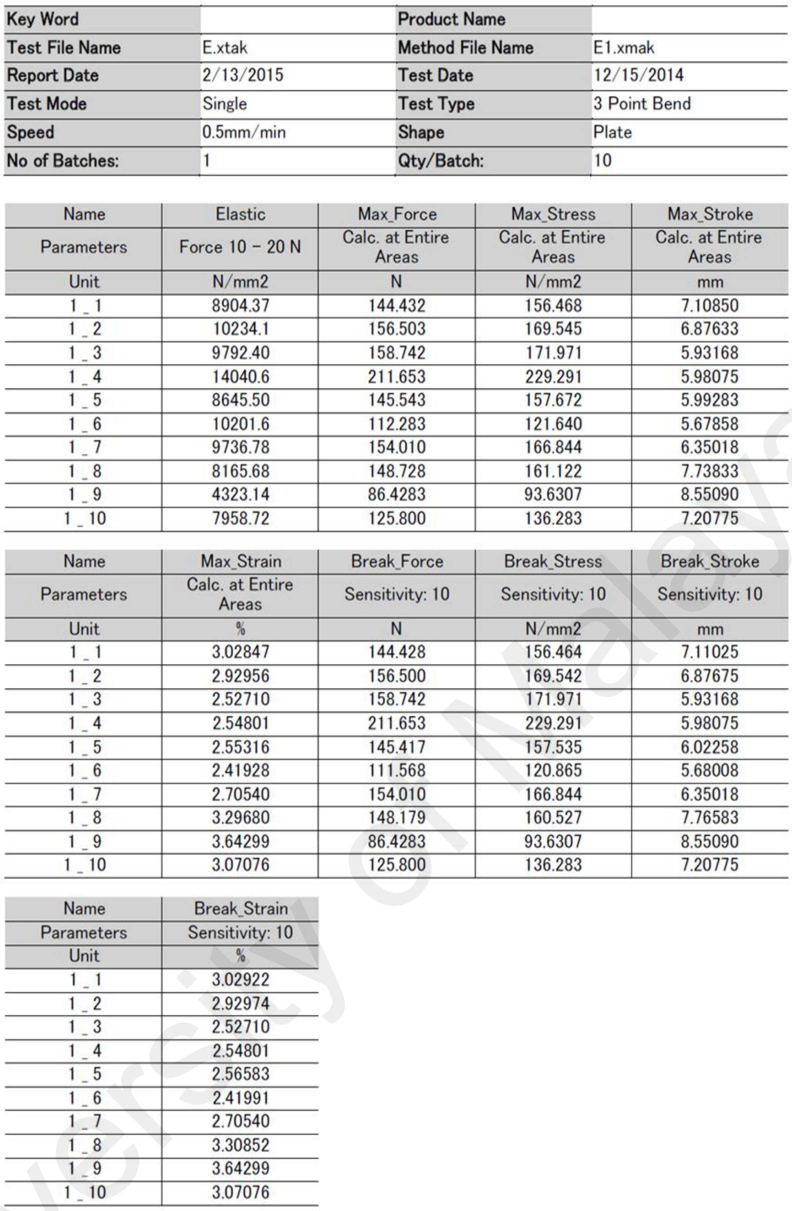
Key Word		**Product Name**	
Test File Name	D1.xtak	**Method File Name**	C1.xmak
Report Date	2/17/2015	**Test Date**	12/15/2014
Test Mode	Single	**Test Type**	3 Point Bend
Speed	0.5mm/min	**Shape**	Plate
No of Batches:	1	**Qty/Batch:**	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	MPa	mm
1 _ 1	9914.80	129.598	140.398	4.61233
1 _ 2	6659.48	83.2812	90.2213	6.51615
1 _ 3	8580.65	93.7446	101.557	5.79950
1 _ 4	8816.85	100.652	109.040	5.61818
1 _ 5	9409.96	116.613	126.331	6.01268
1 _ 6	9294.94	132.836	143.905	6.93515
1 _ 7	5399.69	80.9010	87.6427	6.23775
1 _ 8	10052.1	124.346	134.708	5.67515
1 _ 9	5544.01	84.5496	91.5954	4.94850
1 _ 10	5280.92	81.9675	88.7981	4.82483

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	MPa	mm
1 _ 1	1.96501	129.598	140.398	4.61233
1 _ 2	2.77611	83.2772	90.2170	6.51650
1 _ 3	2.47079	93.7414	101.553	5.80000
1 _ 4	2.39354	99.6065	107.907	5.61910
1 _ 5	2.56161	116.613	126.331	6.01268
1 _ 6	2.95462	132.836	143.905	6.93515
1 _ 7	2.65750	80.5036	87.2123	6.25258
1 _ 8	2.41782	124.346	134.708	5.67515
1 _ 9	2.10739	84.5440	91.5894	4.94743
1 _ 10	2.05555	81.9675	88.7981	4.82483

Name	Break Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	1.96501
1 _ 2	2.77626
1 _ 3	2.47101
1 _ 4	2.39397
1 _ 5	2.56161
1 _ 6	2.95462
1 _ 7	2.66382
1 _ 8	2.41782
1 _ 9	2.10778
1 _ 10	2.05555

Table A-5: Values of flexural strength and flexural modulus of GROUP E



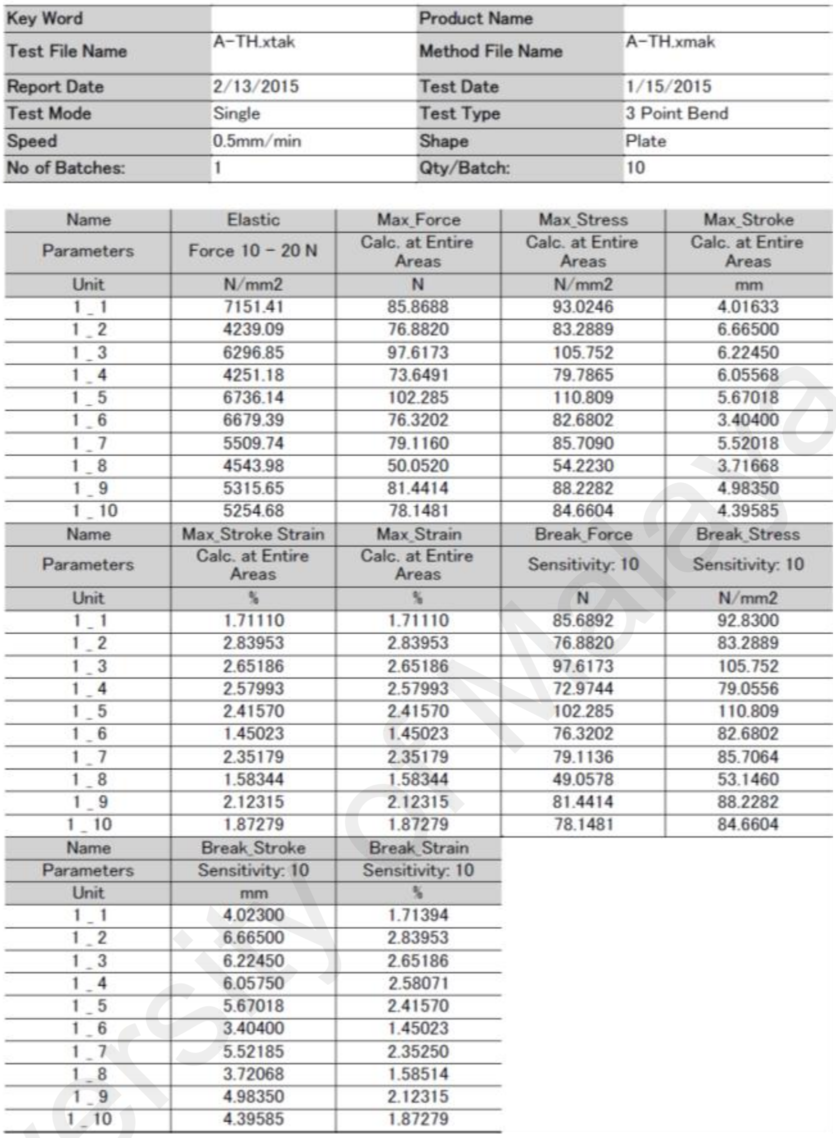
Key Word		**Product Name**	
Test File Name	E.xtak	**Method File Name**	E1.xmak
Report Date	2/13/2015	**Test Date**	12/15/2014
Test Mode	Single	**Test Type**	3 Point Bend
Speed	0.5mm/min	**Shape**	Plate
No of Batches:	1	**Qty/Batch:**	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	N/mm2	mm
1 _ 1	8904.37	144.432	156.468	7.10850
1 _ 2	10234.1	156.503	169.545	6.87633
1 _ 3	9792.40	158.742	171.971	5.93168
1 _ 4	14040.6	211.653	229.291	5.98075
1 _ 5	8645.50	145.543	157.672	5.99283
1 _ 6	10201.6	112.283	121.640	5.67858
1 _ 7	9736.78	154.010	166.844	6.35018
1 _ 8	8165.68	148.728	161.122	7.73833
1 _ 9	4323.14	86.4283	93.6307	8.55090
1 _ 10	7958.72	125.800	136.283	7.20775

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	N/mm2	mm
1 _ 1	3.02847	144.428	156.464	7.11025
1 _ 2	2.92956	156.500	169.542	6.87675
1 _ 3	2.52710	158.742	171.971	5.93168
1 _ 4	2.54801	211.653	229.291	5.98075
1 _ 5	2.55316	145.417	157.535	6.02258
1 _ 6	2.41928	111.568	120.865	5.68008
1 _ 7	2.70540	154.010	166.844	6.35018
1 _ 8	3.29680	148.179	160.527	7.76583
1 _ 9	3.64299	86.4283	93.6307	8.55090
1 _ 10	3.07076	125.800	136.283	7.20775

Name	Break_Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	3.02922
1 _ 2	2.92974
1 _ 3	2.52710
1 _ 4	2.54801
1 _ 5	2.56583
1 _ 6	2.41991
1 _ 7	2.70540
1 _ 8	3.30852
1 _ 9	3.64299
1 _ 10	3.07076

Table A-6: Values of flexural strength and flexural modulus of GROUP A after thermocycling



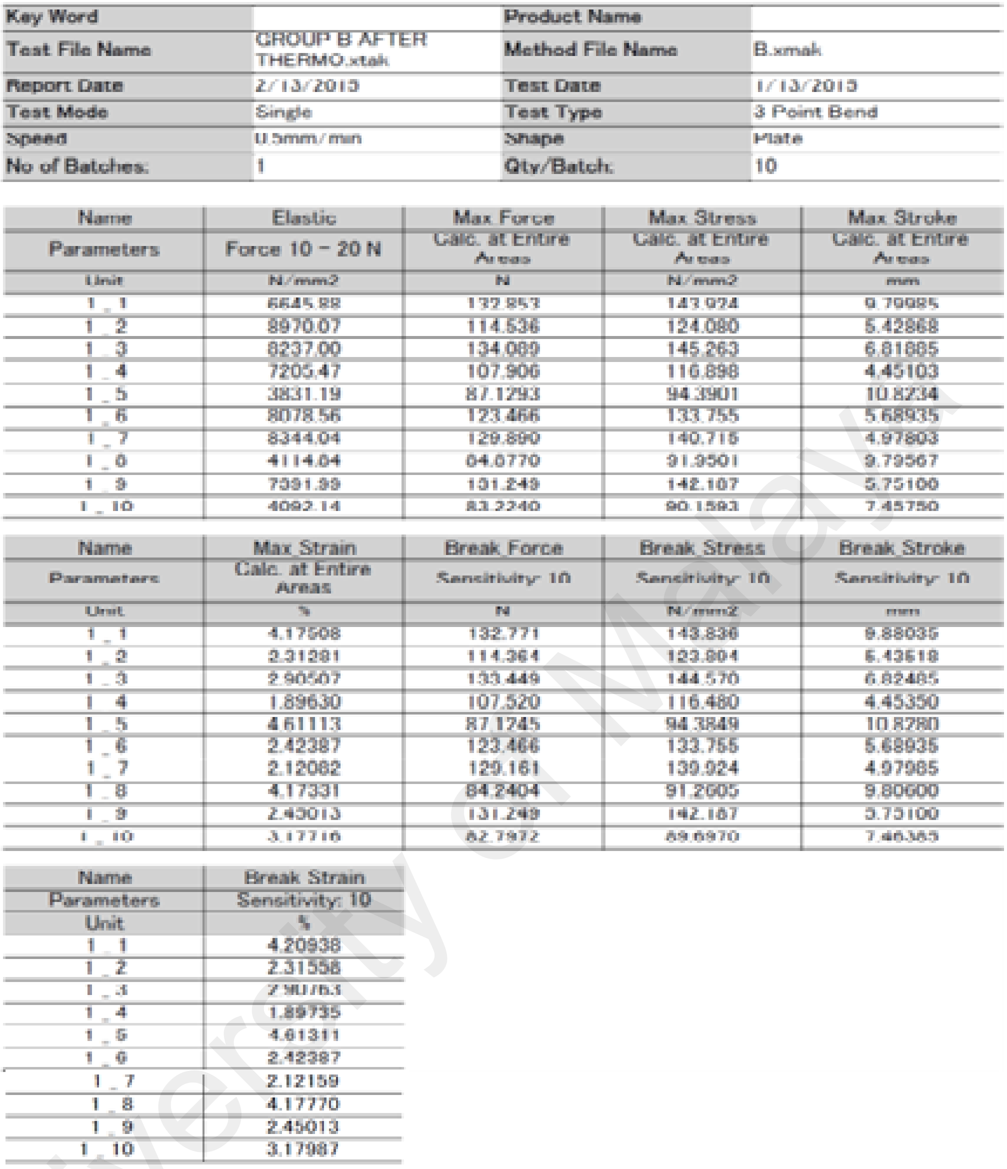
Key Word		Product Name	
Test File Name	A−TH.xtak	Method File Name	A−TH.xmak
Report Date	2/13/2015	Test Date	1/15/2015
Test Mode	Single	Test Type	3 Point Bend
Speed	0.5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 − 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	N/mm2	mm
1 _ 1	7151.41	85.8688	93.0246	4.01633
1 _ 2	4239.09	76.8820	83.2889	6.66500
1 _ 3	6296.85	97.6173	105.752	6.22450
1 _ 4	4251.18	73.6491	79.7865	6.05568
1 _ 5	6736.14	102.285	110.809	5.67018
1 _ 6	6679.39	76.3202	82.6802	3.40400
1 _ 7	5509.74	79.1160	85.7090	5.52018
1 _ 8	4543.98	50.0520	54.2230	3.71668
1 _ 9	5315.65	81.4414	88.2282	4.98350
1 _ 10	5254.68	78.1481	84.6604	4.39585

Name	Max_Stroke Strain	Max_Strain	Break_Force	Break_Stress
Parameters	Calc. at Entire Areas	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10
Unit	%	%	N	N/mm2
1 _ 1	1.71110	1.71110	85.6892	92.8300
1 _ 2	2.83953	2.83953	76.8820	83.2889
1 _ 3	2.65186	2.65186	97.6173	105.752
1 _ 4	2.57993	2.57993	72.9744	79.0556
1 _ 5	2.41570	2.41570	102.285	110.809
1 _ 6	1.45023	1.45023	76.3202	82.6802
1 _ 7	2.35179	2.35179	79.1136	85.7064
1 _ 8	1.58344	1.58344	49.0578	53.1460
1 _ 9	2.12315	2.12315	81.4414	88.2282
1 _ 10	1.87279	1.87279	78.1481	84.6604

Name	Break_Stroke	Break_Strain
Parameters	Sensitivity: 10	Sensitivity: 10
Unit	mm	%
1 _ 1	4.02300	1.71394
1 _ 2	6.66500	2.83953
1 _ 3	6.22450	2.65186
1 _ 4	6.05750	2.58071
1 _ 5	5.67018	2.41570
1 _ 6	3.40400	1.45023
1 _ 7	5.52185	2.35250
1 _ 8	3.72068	1.58514
1 _ 9	4.98350	2.12315
1 _ 10	4.39585	1.87279

Table A-7: Values of flexural strength and flexural modulus of GROUP B after thermocycling



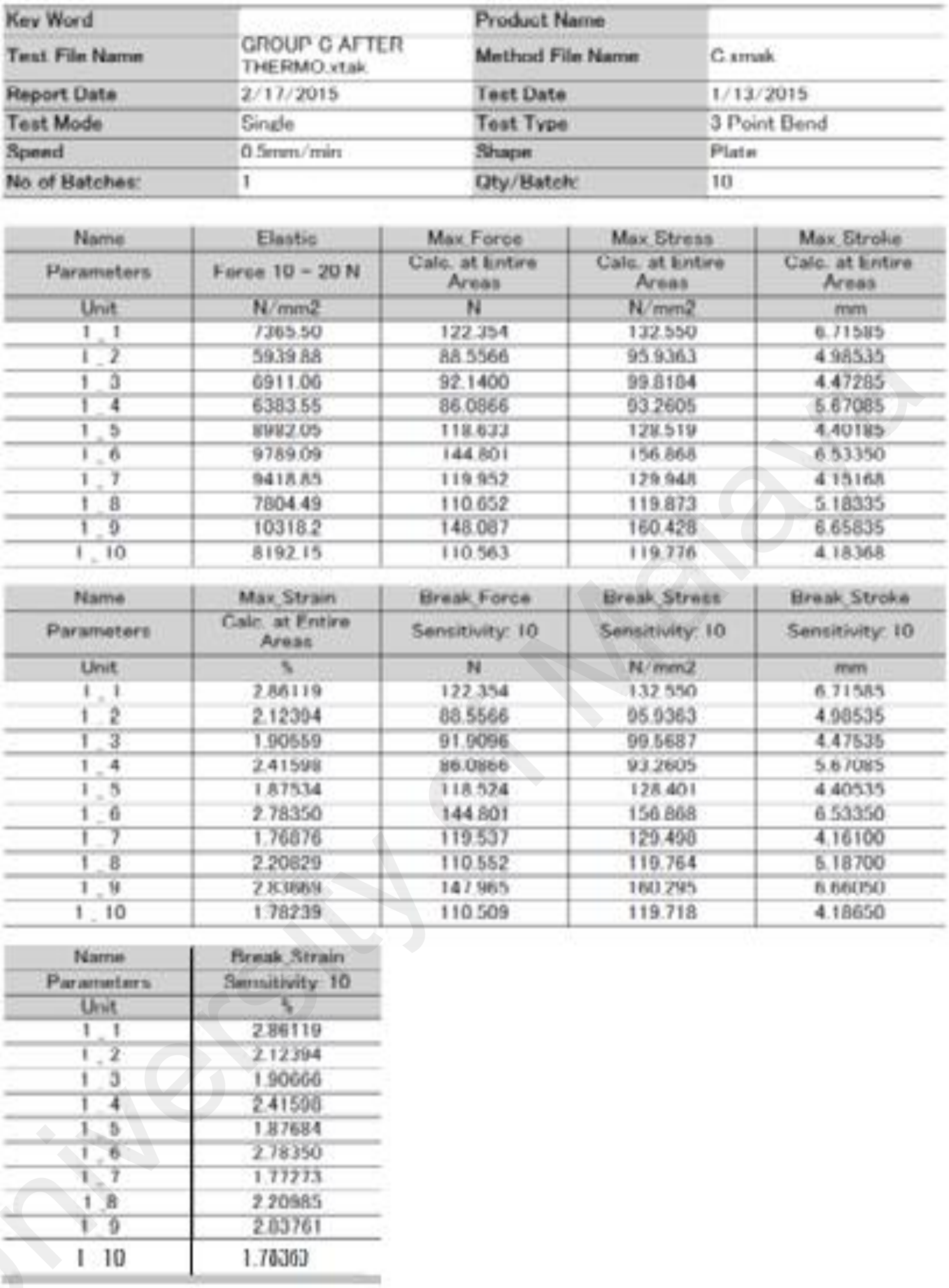
Key Word		**Product Name**	
Test File Name	GROUP B AFTER THERMO.xtak	**Method File Name**	B.xmak
Report Date	2/13/2015	**Test Date**	1/13/2015
Test Mode	Single	**Test Type**	3 Point Bend
Speed	0.5mm/min	**Shape**	Plate
No of Batches:	1	**Qty/Batch:**	10

Name	Elastic	Max Force	Max Stress	Max Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	N/mm2	mm
1 _ 1	6645.88	132.853	143.924	9.79985
1 _ 2	8970.07	114.536	124.080	5.42868
1 _ 3	8237.00	134.089	145.263	6.81885
1 _ 4	7205.47	107.906	116.898	4.45103
1 _ 5	3831.19	87.1293	94.3901	10.8234
1 _ 6	8078.56	123.466	133.755	5.68935
1 _ 7	8344.04	129.890	140.715	4.97803
1 _ 8	4114.04	84.8770	91.9501	9.79567
1 _ 9	7391.39	131.249	142.107	5.75100
1 _ 10	4092.14	83.2240	90.1593	7.45750

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	N/mm2	mm
1 _ 1	4.17508	132.771	143.836	9.88035
1 _ 2	2.31281	114.364	123.804	5.43518
1 _ 3	2.90507	133.449	144.570	6.82485
1 _ 4	1.89630	107.520	116.480	4.45350
1 _ 5	4.61113	87.1745	94.3849	10.8280
1 _ 6	2.42387	123.466	133.755	5.68935
1 _ 7	2.12082	129.161	139.924	4.97985
1 _ 8	4.17331	84.2404	91.2605	9.80600
1 _ 9	2.45013	131.249	142.167	5.75100
1 _ 10	3.17716	82.7972	89.6970	7.46365

Name	Break Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	4.20938
1 _ 2	2.31558
1 _ 3	2.90763
1 _ 4	1.89735
1 _ 5	4.61311
1 _ 6	2.42387
1 _ 7	2.12159
1 _ 8	4.17770
1 _ 9	2.45013
1 _ 10	3.17987

Table A-8: Values of flexural strength and flexural modulus of GROUP C after thermocycling



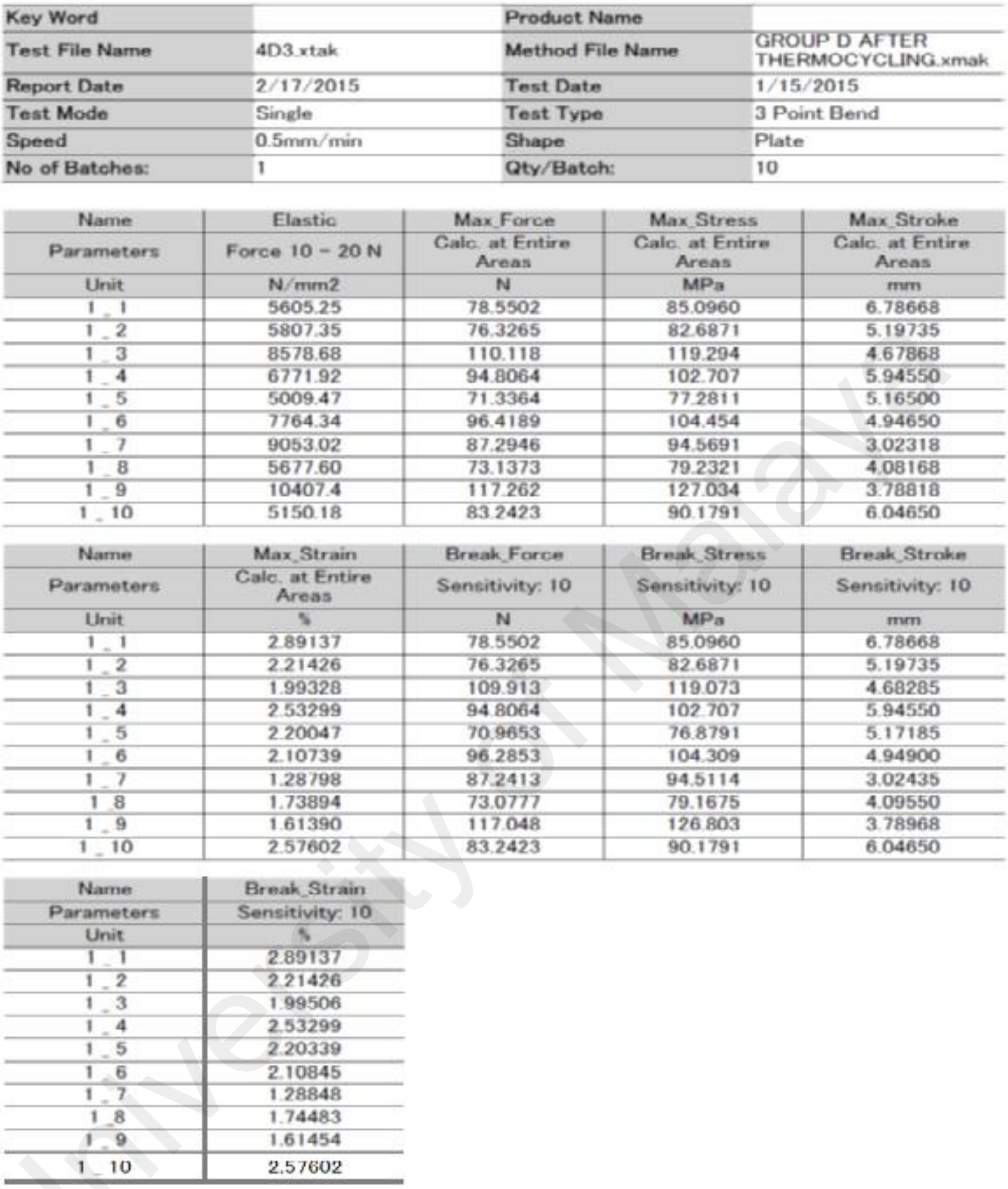
Key Word		Product Name	
Test File Name	GROUP C AFTER THERMO.xtak	Method File Name	C.xmak
Report Date	2/17/2015	Test Date	1/13/2015
Test Mode	Single	Test Type	3 Point Bend
Speed	0.5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	N/mm2	mm
1 _ 1	7365.50	122.354	132.550	6.71585
1 _ 2	5939.88	88.5566	95.9363	4.98535
1 _ 3	6911.06	92.1400	99.8184	4.47285
1 _ 4	6383.55	86.0866	93.2605	5.67085
1 _ 5	8982.05	118.633	128.519	4.40185
1 _ 6	9789.09	144.801	156.868	6.53350
1 _ 7	9418.85	119.952	129.948	4.15168
1 _ 8	7804.49	110.652	119.873	5.18335
1 _ 9	10318.2	148.087	160.428	6.65835
1 _ 10	8192.15	110.563	119.776	4.18368

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	N/mm2	mm
1 _ 1	2.86119	122.354	132.550	6.71585
1 _ 2	2.12394	88.5566	95.9363	4.98535
1 _ 3	1.90559	91.9096	99.5687	4.47535
1 _ 4	2.41598	86.0866	93.2605	5.67085
1 _ 5	1.87534	118.524	128.401	4.40535
1 _ 6	2.78350	144.801	156.868	6.53350
1 _ 7	1.76876	119.537	129.498	4.16100
1 _ 8	2.20829	110.552	119.764	5.18700
1 _ 9	2.83869	147.965	160.295	6.66050
1 _ 10	1.78239	110.509	119.718	4.18650

Name	Break_Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	2.86119
1 _ 2	2.12394
1 _ 3	1.90666
1 _ 4	2.41598
1 _ 5	1.87684
1 _ 6	2.78350
1 _ 7	1.77273
1 _ 8	2.20985
1 _ 9	2.83761
1 _ 10	1.78363

Table A-9: Values of flexural strength and flexural modulus of GROUP D after thermocycling



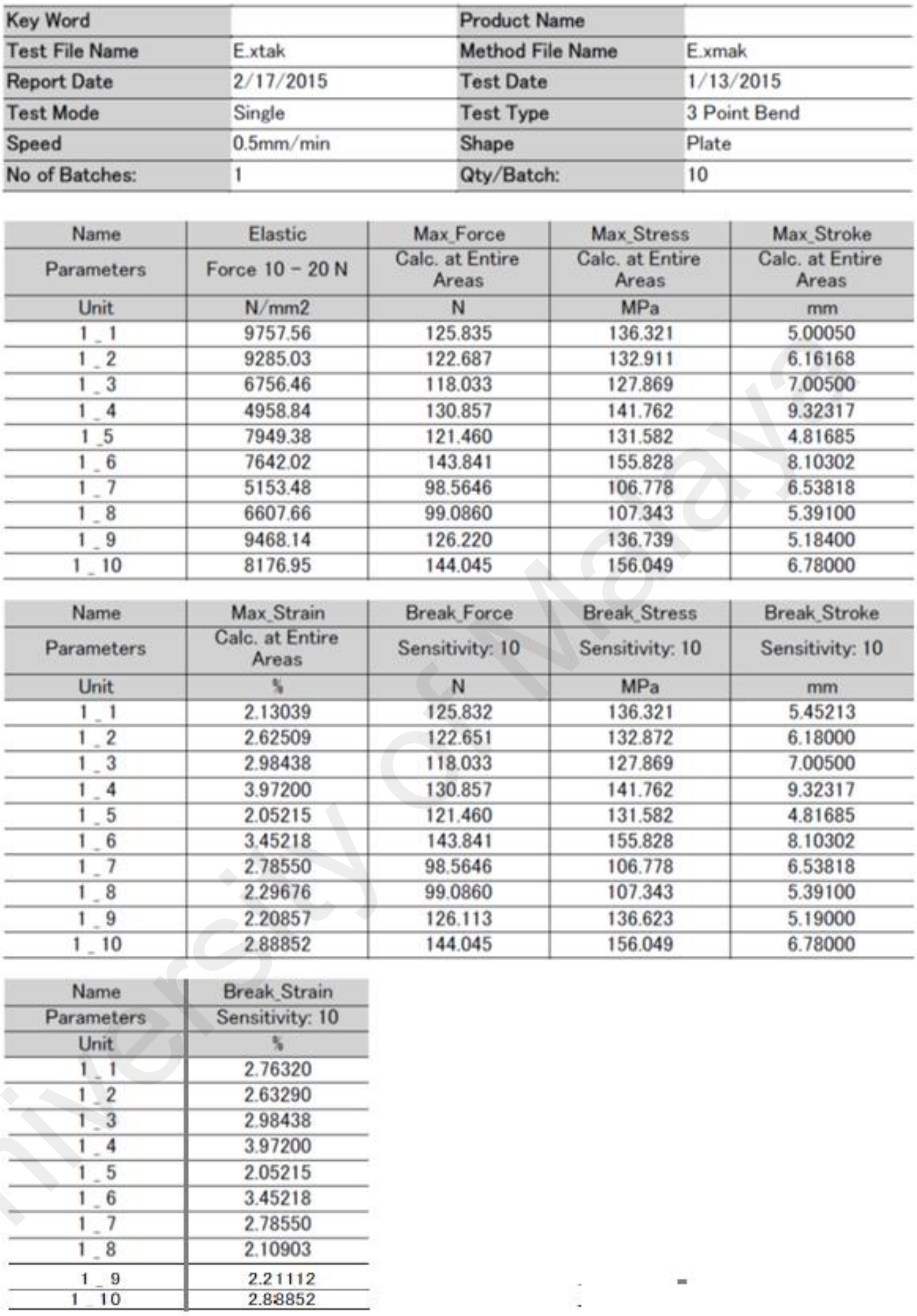
Key Word		**Product Name**	
Test File Name	4D3.xtak	**Method File Name**	GROUP D AFTER THERMOCYCLING.xmak
Report Date	2/17/2015	**Test Date**	1/15/2015
Test Mode	Single	**Test Type**	3 Point Bend
Speed	0.5mm/min	**Shape**	Plate
No of Batches:	1	**Qty/Batch:**	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	MPa	mm
1 _ 1	5605.25	78.5502	85.0960	6.78668
1 _ 2	5807.35	76.3265	82.6871	5.19735
1 _ 3	8578.68	110.118	119.294	4.67868
1 _ 4	6771.92	94.8064	102.707	5.94550
1 _ 5	5009.47	71.3364	77.2811	5.16500
1 _ 6	7764.34	96.4189	104.454	4.94650
1 _ 7	9053.02	87.2946	94.5691	3.02318
1 _ 8	5677.60	73.1373	79.2321	4.08168
1 _ 9	10407.4	117.262	127.034	3.78818
1 _ 10	5150.18	83.2423	90.1791	6.04650

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	MPa	mm
1 _ 1	2.89137	78.5502	85.0960	6.78668
1 _ 2	2.21426	76.3265	82.6871	5.19735
1 _ 3	1.99328	109.913	119.073	4.68285
1 _ 4	2.53299	94.8064	102.707	5.94550
1 _ 5	2.20047	70.9653	76.8791	5.17185
1 _ 6	2.10739	96.2853	104.309	4.94900
1 _ 7	1.28798	87.2413	94.5114	3.02435
1 _ 8	1.73894	73.0777	79.1675	4.09550
1 _ 9	1.61390	117.048	126.803	3.78968
1 _ 10	2.57602	83.2423	90.1791	6.04650

Name	Break_Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	2.89137
1 _ 2	2.21426
1 _ 3	1.99506
1 _ 4	2.53299
1 _ 5	2.20339
1 _ 6	2.10845
1 _ 7	1.28848
1 _ 8	1.74483
1 _ 9	1.61454
1 _ 10	2.57602

Table A-10: Values of flexural strength and flexural modulus of GROUP E after thermocycling



Key Word		Product Name	
Test File Name	E.xtak	Method File Name	E.xmak
Report Date	2/17/2015	Test Date	1/13/2015
Test Mode	Single	Test Type	3 Point Bend
Speed	0.5mm/min	Shape	Plate
No of Batches:	1	Qty/Batch:	10

Name	Elastic	Max_Force	Max_Stress	Max_Stroke
Parameters	Force 10 - 20 N	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas
Unit	N/mm2	N	MPa	mm
1 _ 1	9757.56	125.835	136.321	5.00050
1 _ 2	9285.03	122.687	132.911	6.16168
1 _ 3	6756.46	118.033	127.869	7.00500
1 _ 4	4958.84	130.857	141.762	9.32317
1 _ 5	7949.38	121.460	131.582	4.81685
1 _ 6	7642.02	143.841	155.828	8.10302
1 _ 7	5153.48	98.5646	106.778	6.53818
1 _ 8	6607.66	99.0860	107.343	5.39100
1 _ 9	9468.14	126.220	136.739	5.18400
1 _ 10	8176.95	144.045	156.049	6.78000

Name	Max_Strain	Break_Force	Break_Stress	Break_Stroke
Parameters	Calc. at Entire Areas	Sensitivity: 10	Sensitivity: 10	Sensitivity: 10
Unit	%	N	MPa	mm
1 _ 1	2.13039	125.832	136.321	5.45213
1 _ 2	2.62509	122.651	132.872	6.18000
1 _ 3	2.98438	118.033	127.869	7.00500
1 _ 4	3.97200	130.857	141.762	9.32317
1 _ 5	2.05215	121.460	131.582	4.81685
1 _ 6	3.45218	143.841	155.828	8.10302
1 _ 7	2.78550	98.5646	106.778	6.53818
1 _ 8	2.29676	99.0860	107.343	5.39100
1 _ 9	2.20857	126.113	136.623	5.19000
1 _ 10	2.88852	144.045	156.049	6.78000

Name	Break_Strain
Parameters	Sensitivity: 10
Unit	%
1 _ 1	2.76320
1 _ 2	2.63290
1 _ 3	2.98438
1 _ 4	3.97200
1 _ 5	2.05215
1 _ 6	3.45218
1 _ 7	2.78550
1 _ 8	2.10903
1 _ 9	2.21112
1 _ 10	2.88852

Table B-11: Frequency (cm-1) values and the indicated functional groups of the different samples

Group C		Group D		Group E		MCC		MMA		PMMA		Group A		Group B	
f (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*	*f* (cm^{-1})	Fn.g*
-	-	-	-	3418	-OH b*	3318	-OH b*	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2994	C-H a* s*	2992	C-H a* s*	2991	C-H a* s*	2891	C-H a* s*	2956	C-H a* s*	2994	C-H a* s*	2995	C-H a* s*	2947	C-H a* s*
2950	C-H a* s*	2949	C-H a* s*	2950	C-H a* s*	-	-	-		2951	C-H a* s*	2950	C-H a* s*	-	-
-	-	-	-	-	-	-	-	-	-	2330	C=C	2300	C=C	-	-
2167	C=C	-	-	-	-	-	-	-	-	-	-	-	-	-	-

2052	**C=C**	**2051**	**C=C**	**-**	**-**	**-**	**-**	**-**	**-**	**2085**	**C=C**	**2067**	**C=C**	**-**	**-**
2028		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
-	**-**	**-**	**-**	**2159**	**C=C**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
1723	**C=O of c*s***	**1723**	**C=O of c* s***	**1723**	**C=O of c* s***	**-**	**-**	**1719**	**C=O s***	**1722**	**C=O s***	**1722**	**C=O s***	**1723**	**C=O s***
-	**-**	**-**	**-**	**-**	**-**	**1641**	**O-H s***	**1638**	**O-H s***	**-**	**-**	**-**	**-**	**-**	**-**
-	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1452**	**-CH_2- b***	**-**	**-**	**-**	**-**	**-**	**-**

APPENDIX B FREQUENCY (CM^{-1}) VALUES AND THE INDICATED FUNCTIONAL GROUPS OF THE DIFFERENT SAMPLES

Table B-1 (Continued): Frequency (cm-1) values and the indicated functional groups of the different samples.

1436	**$-CH_2-$ b***	**1435**	**$-CH_2-$ b***	**1436**	**CH_2- b***	**-**	**-**	**1438**	**$-CH_2-$ b***	**1437**	**$-CH_2-$ b***	**1438**	**$-CH_2-$ b***	**1438**	**$-CH_2-$ b***
-	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1403**	**$-CH_2-$**	**-**	**-**	**-**	**-**	**-**	**-**
1386	**$-CH_2$ of c* b***	**1386**	**$-CH_2$ of c* b***	**1386**	**$-CH_2$ of c* b***	**-**	**-**	**1378**	**$-CH_2-$ b***	**1387**	**$-CH_2-$b***	**1387**	**CH_2 b***	**1386**	**$-CH_2-$ b***
-	**-**	**-**	**-**	**-**	**-**	**1367**	**$-CH_2-$b***	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
-	**-**	**-**	**-**	**-**	**-**	**1314**	**$-CH_2-$b***	**1323**	**$-CH_2-$ b***	**-**	**-**	**-**	**-**	**-**	**-**
-	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1298**	**C-O s***	**-**	**-**	**-**	**-**	**-**	**-**
1238	**C-O- C b***	**1239**	**C-O-C b***	**1239**	**C-O- C b***	**-**	**-**	**-**	**-**	**1239**	**C-O-C b***	**1239**	**C-O-C b***	**1238**	**C-O-C b***
1189	**C-O-C s***	**1190**	**C-O-C s***	**1189**	**C-O-C s***	**1199**	**C-O-C s***	**1194**	**C-O-C s***	**1190**	**C-O-C s***	**1189**	**C-O-C s***	**-**	**-**
-	**-**	**-**	**-**	**-**	**-**	**1158**	**C-O-C s***	**1154**	**C-O-C s***	**-**	**-**	**-**	**-**	**-**	**-**
1142	**C-O-C s***	**1142**	**C-O-C s***	**1141**	**C-O-C s***	**-**	**-**	**-**	**-**	**1141**	**C-O-C s***	**1141**	**C-O-C s***	**1142**	**C-O-C s***
1066	**CH s*for C-O of the carbohydrates**	**1063**	**CH s* for C-O of the carbohydrates**	**1061**	**CH s* for C-O of the carbohydrates**	**1094**	**for C-O of the carbohydrates**	**1015**	**CH s***	**1064**	**CH s***	**1064**	**CH s***	**-**	**-**

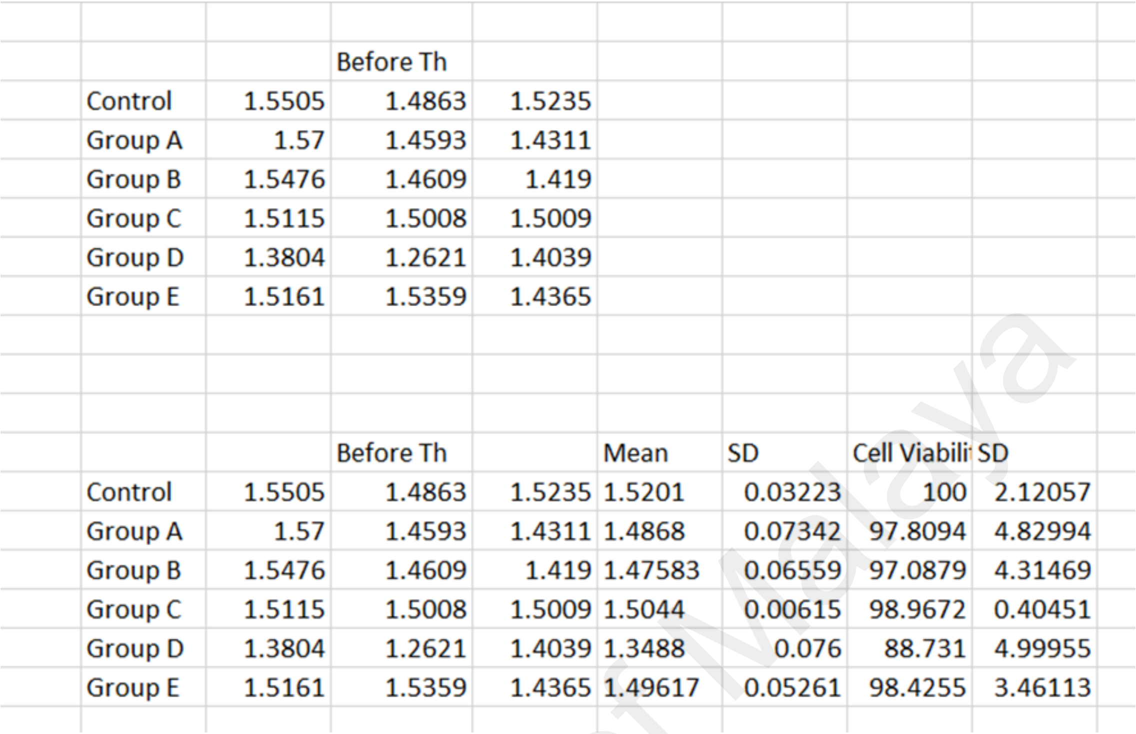
Table B-1 (Continued): Frequency (cm-1) values and the indicated functional groups of the different samples.

-	**-**	**-**	**-**	**-**	**-**	**1027**	**CH s***	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
985	**CH of the carbohydrates**	**985**	**CH of the carbohydrates**	**986**	**CH of the carbohydrates**	**996**	**CH of the carbohydrates**	**985**	**CH**	**986**	**CH**	**986**	**CH of the carbohydrates**	**983**	**CH of the carbohydrates**
965	**CH of the carbohydrates**	**965**	**CH of the carbohydrates**	**966**	**CH of the carbohydrates**	**-**	**-**	**-**	**-**	**965**	**CH**	**965**	**CH**	**965**	**CH**
840	**CH of the carbohydrates**	**840**	**CH of the carbohydrates**	**840**	**CH of the carbohydrates**	**894**	**C-H of the carbohydrates**	**896**	**CH**	**840**	**CH**	**840**	**CH**	**838**	**CH**
-	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
-	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**814**	**CH**	**-**	**-**	**-**	**-**	**-**	**-**
750	**-**	**750**	**-**	**750**	**-**	**-**	**-**	**-**	**-**	**750**	**-**	**751**	**-**	**752**	**-**
-	**-**	**-**	**-**	**-**	**-**	**670**	**-**	**653**	**-**						
-	**-**	**-**	**-**	**-**	**-**	**615**	**-**	**600**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
-	**-**	**-**	**-**	**500**	**-**	**518**	**-**	**505**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
486		**487**	**-**	**487**	**-**	**-**	**-**	**-**	**-**	**482**	**-**	**486**	**-**	**491**	**-**

a* : Aliphatic
b* :Bending
c* : Cellulose
S*: Stretching
f :Frequency (cm^{-1})
Fn.g*: Functional groups.

APPENDIX C CYTOTOXICITY TEST

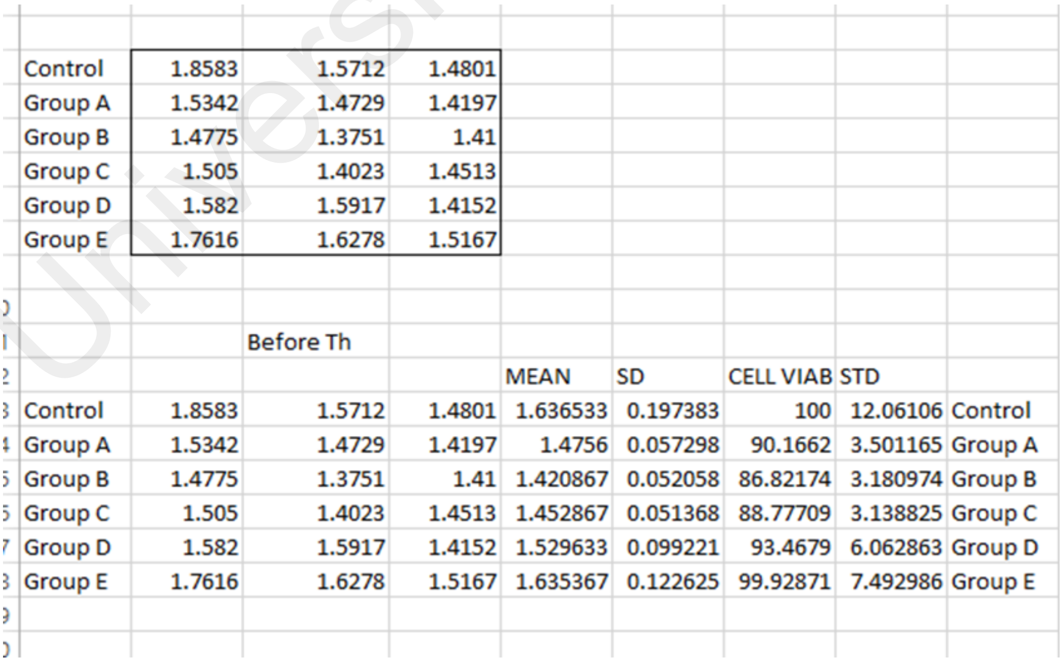
Table C-1: Readings of first experiment of cytotoxicity before thermocycling



		Before Th					
Control	1.5505	1.4863	1.5235				
Group A	1.57	1.4593	1.4311				
Group B	1.5476	1.4609	1.419				
Group C	1.5115	1.5008	1.5009				
Group D	1.3804	1.2621	1.4039				
Group E	1.5161	1.5359	1.4365				

		Before Th		Mean	SD	Cell Viabili	SD
Control	1.5505	1.4863	1.5235	1.5201	0.03223	100	2.12057
Group A	1.57	1.4593	1.4311	1.4868	0.07342	97.8094	4.82994
Group B	1.5476	1.4609	1.419	1.47583	0.06559	97.0879	4.31469
Group C	1.5115	1.5008	1.5009	1.5044	0.00615	98.9672	0.40451
Group D	1.3804	1.2621	1.4039	1.3488	0.076	88.731	4.99955
Group E	1.5161	1.5359	1.4365	1.49617	0.05261	98.4255	3.46113

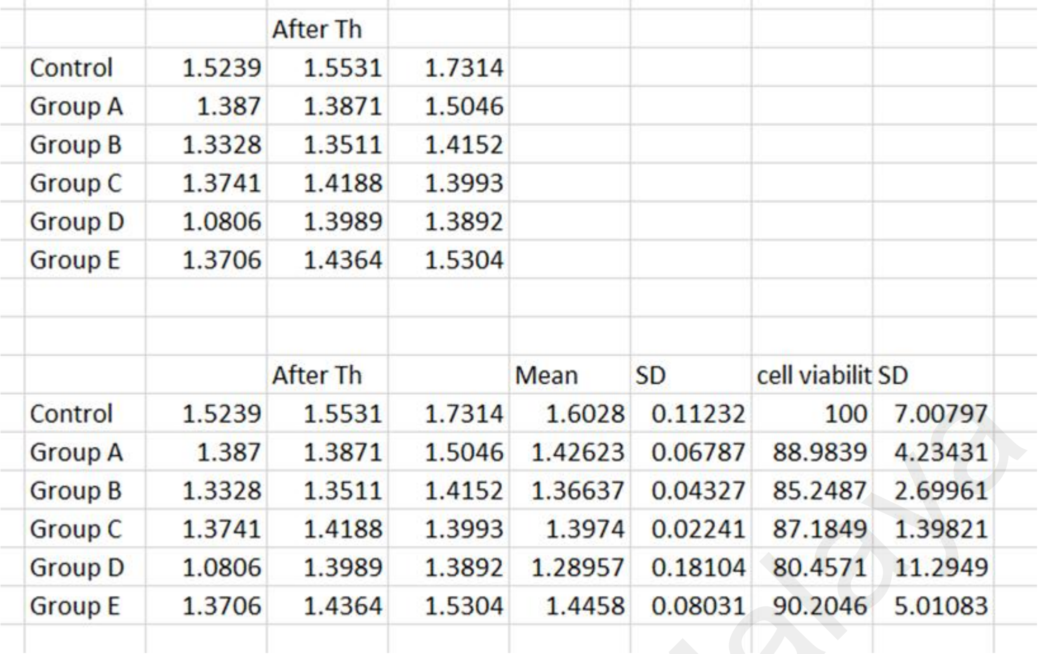
Table C-2: Readings of second experiment of cytotoxicity before thermo cycling



Control	1.8583	1.5712	1.4801
Group A	1.5342	1.4729	1.4197
Group B	1.4775	1.3751	1.41
Group C	1.505	1.4023	1.4513
Group D	1.582	1.5917	1.4152
Group E	1.7616	1.6278	1.5167

		Before Th						
				MEAN	SD	CELL VIAB	STD	
Control	1.8583	1.5712	1.4801	1.636533	0.197383	100	12.06106	Control
Group A	1.5342	1.4729	1.4197	1.4756	0.057298	90.1662	3.501165	Group A
Group B	1.4775	1.3751	1.41	1.420867	0.052058	86.82174	3.180974	Group B
Group C	1.505	1.4023	1.4513	1.452867	0.051368	88.77709	3.138825	Group C
Group D	1.582	1.5917	1.4152	1.529633	0.099221	93.4679	6.062863	Group D
Group E	1.7616	1.6278	1.5167	1.635367	0.122625	99.92871	7.492986	Group E

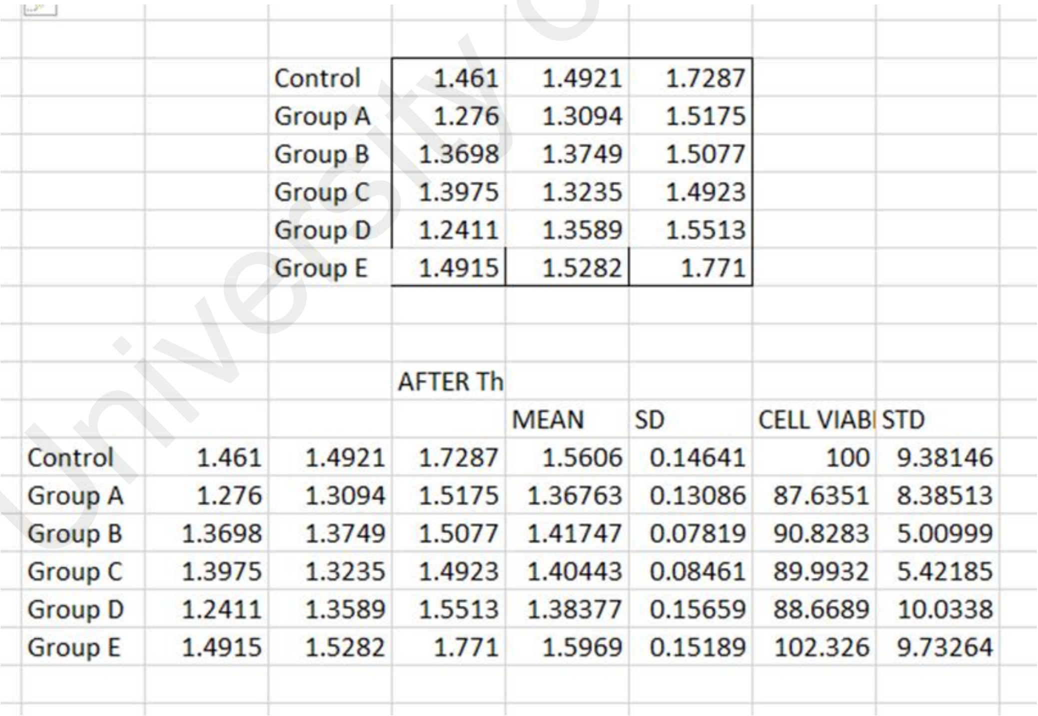
Table C-3: Readings of first experiment(cytotoxicity)after thermocycling



		After Th	
Control	1.5239	1.5531	1.7314
Group A	1.387	1.3871	1.5046
Group B	1.3328	1.3511	1.4152
Group C	1.3741	1.4188	1.3993
Group D	1.0806	1.3989	1.3892
Group E	1.3706	1.4364	1.5304

		After Th		Mean	SD	cell viabilit	SD
Control	1.5239	1.5531	1.7314	1.6028	0.11232	100	7.00797
Group A	1.387	1.3871	1.5046	1.42623	0.06787	88.9839	4.23431
Group B	1.3328	1.3511	1.4152	1.36637	0.04327	85.2487	2.69961
Group C	1.3741	1.4188	1.3993	1.3974	0.02241	87.1849	1.39821
Group D	1.0806	1.3989	1.3892	1.28957	0.18104	80.4571	11.2949
Group E	1.3706	1.4364	1.5304	1.4458	0.08031	90.2046	5.01083

Table C-4: Readings of second experiment(cytotoxicity) after thermocycling



Control	1.461	1.4921	1.7287
Group A	1.276	1.3094	1.5175
Group B	1.3698	1.3749	1.5077
Group C	1.3975	1.3235	1.4923
Group D	1.2411	1.3589	1.5513
Group E	1.4915	1.5282	1.771

			AFTER Th				
				MEAN	SD	CELL VIABI	STD
Control	1.461	1.4921	1.7287	1.5606	0.14641	100	9.38146
Group A	1.276	1.3094	1.5175	1.36763	0.13086	87.6351	8.38513
Group B	1.3698	1.3749	1.5077	1.41747	0.07819	90.8283	5.00999
Group C	1.3975	1.3235	1.4923	1.40443	0.08461	89.9932	5.42185
Group D	1.2411	1.3589	1.5513	1.38377	0.15659	88.6689	10.0338
Group E	1.4915	1.5282	1.771	1.5969	0.15189	102.326	9.73264

Th. Thermocycling

APPENDIX D PMMA

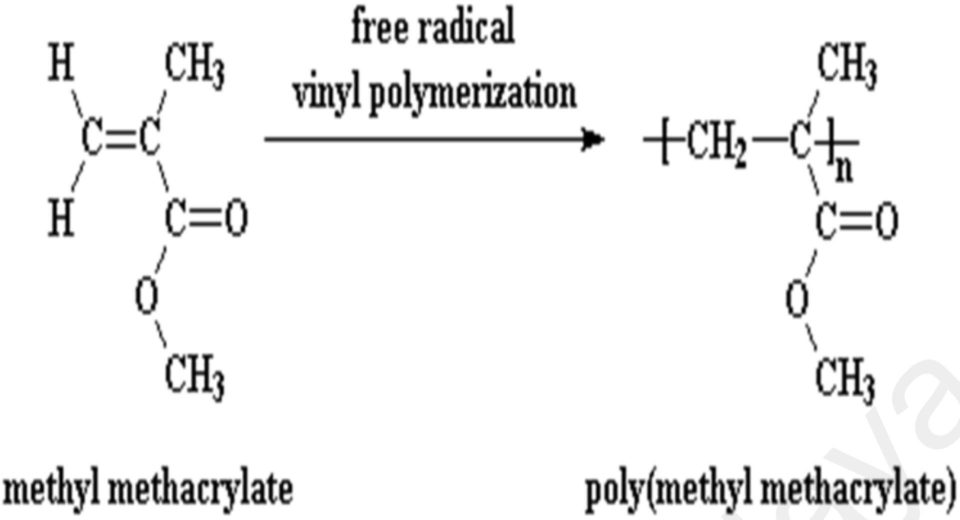


Figure D-1: Chemical structure of PMMA

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Figure D-2: Fractured upper acrylic denture base

http://www.1stchoicedentures.co.uk/resources/IMG-20120314-00160a.jpg

APPENDIX E EXAMPLES OF SOME SYNTHETIC FILLERS USED IN PMMA REINFORCEMENT

Figure E-1: Glass flakes

http://mengyicong88.en.ec21.com/offer_detail/Sell_glass_flakes--8718429.html

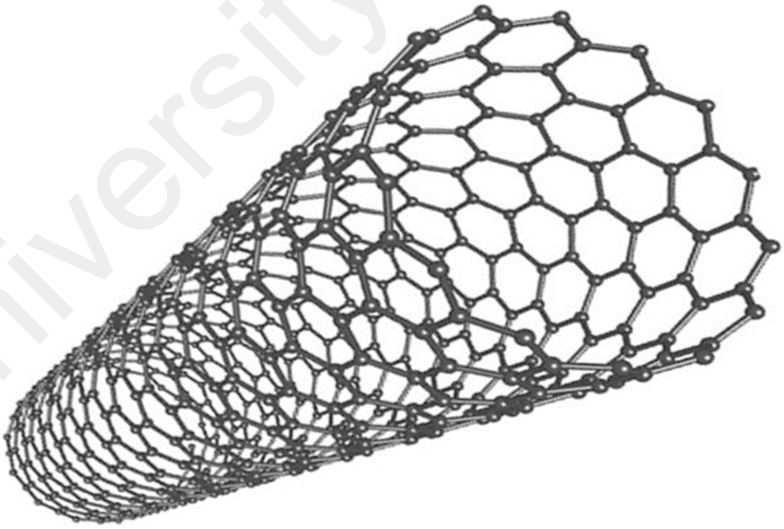


Figure E-2: Carbon nanotubes

http://www.carbonallotropes.com/carbon-nanotubes/39-single-wall-carbon-nanotubes.html

Figure E-3: Glass fibres

http://fiber513591278.en.ec21.com/Alkali_Resistant_Glass_Fiber_Chopped--5663726_7565091.html

Figure E-4: Carbon fibres

https://commons.wikimedia.org/wiki/File:Carbon_fiber.jpg

Figure E-5: Nylon fibres

http://3.imimg.com/data3/GA/IP/MY-13547509/nylon-fiber-250x250.jpg

Figure E-6:Aramid fibres

http://www.pp-fiber.com/images/gxnxw/gxnxw_fl.jpg

APPENDIX F

EXAMPLES OF SOME NATURAL FIBRES USED IN PMMA REINFORCEMENT

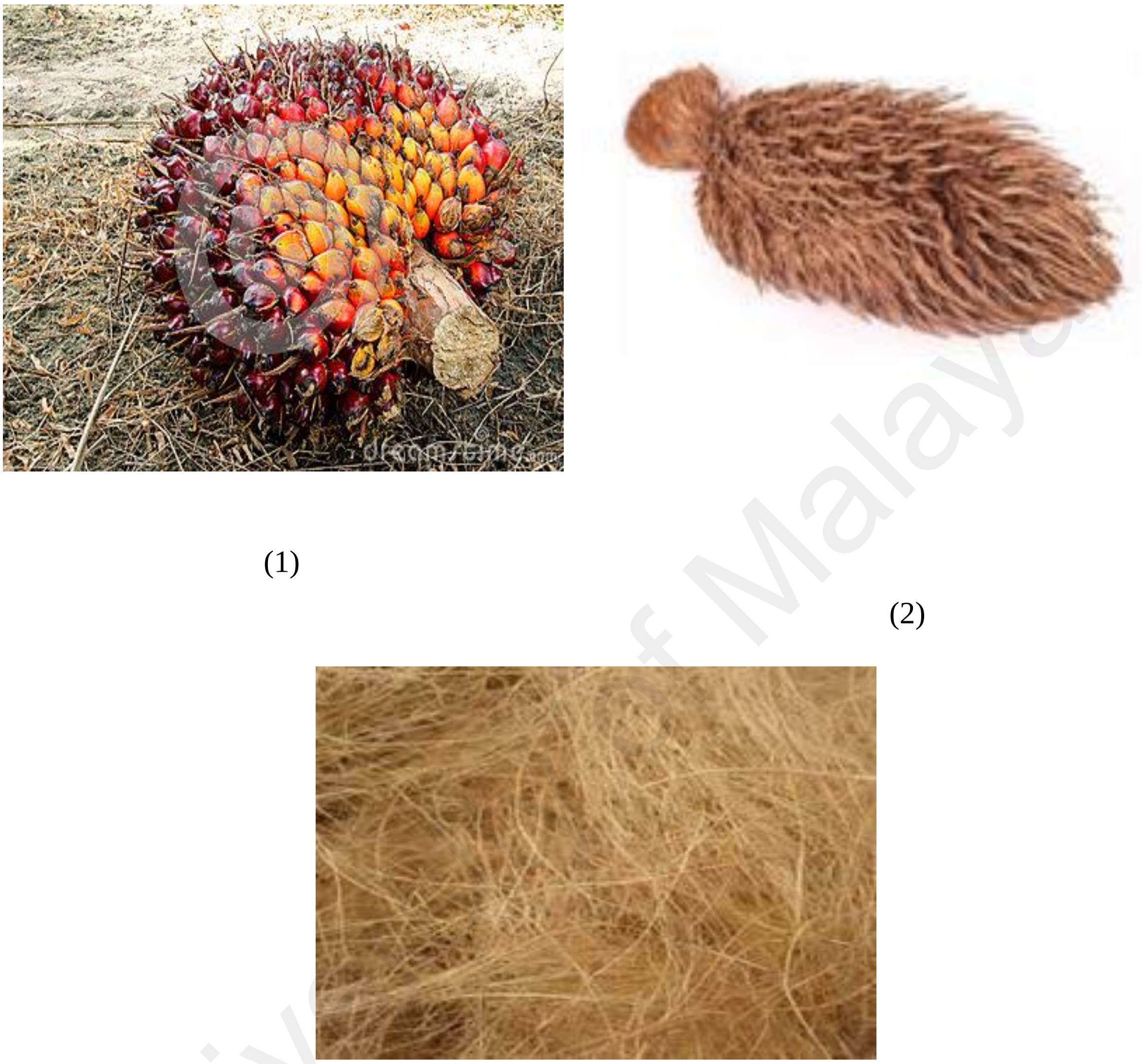
Figure F-1: Sisal fibres

http://www.bhtengda.com/upload/2008624112051249O2.jpg

Figure F-2: Ramie fibres

http://67.media.tumblr.com/91b82b6c40f87267fb57148e268d4db0/tumblr_mmuslgVQyj1sqwnaqo1_500.jpg

APPENDIX G
OIL PALM FRUIT



(1)

(2)

(3)

Figure G-1: (1) Palm oil fruit bunch. (2) Empty fruit bunch. (3) Fibres of empty fruit bunch.

(1) https://thumbs.dreamstime.com/x/oil-palm-fruit-bunches-harvested-resting-ground-57650366.jpg

(2) http://test.bfdic.com/en/d/file/Features/Features/2009-03-12/627f87d20bf331cb86e6f3994351dfdd.jpg

(3) ttp://3.imimg.com/data3/TY/WE/MY-6006381/coir-fiber-250x250.jpg

APPENDIX H

CHEMICAL STRUCTURE OF CELLULOSE

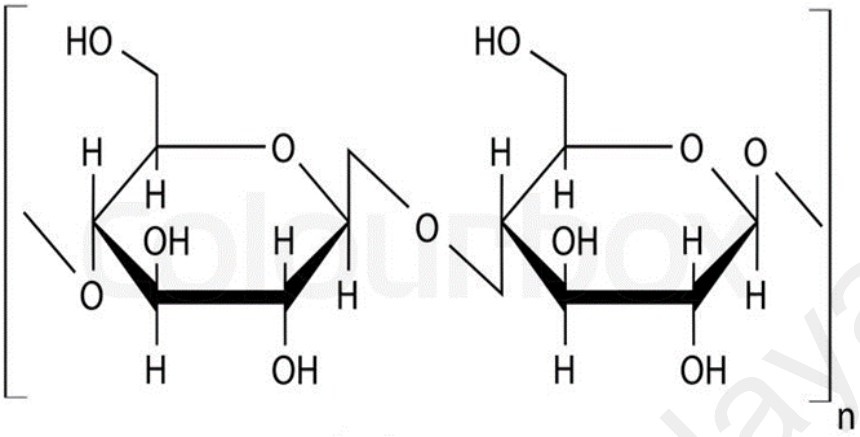


Figure H-1: Chemical structure of cellulose

https://www.colourbox.com/preview/9746487-the-structural-formula-of-cellulose-polymer.jpg

LIST OF PUBLICATIONS AND PAPERS PRESENTED

PUBLICATIONS

Title: Effect of palm oil based microcrystalline cellulose on properties of acrylic denture base resin. Status: *On process*

Title: Assessment of cytotoxicity of oil palm based microcrystalline cellulose reinforce denture base resin. Status: *On process*

ADDITIONAL OUTPUT OF THE PROJECT

AWARDS

First place for oral presentation (3 Minutes Pitching). International Association for Dental Research Malaysian Section (South-East Asian Division).15th Annual Scientific Meeting of Malaysian Section IADR. Faculty of Dentistry, Universiti Kebangsaan Malaysia, Kuala Lumpur on 5th February 2016.

ORAL PRESENTATIONS

Ali, A. A. (2016). *Flexural strength of denture base resin reinforced with microcrystalline cellulose of oil palm empty fruit bunch.* Paper session presented at the meeting of International Association for Dental Research Malaysian Section (South-East Asian Division). Universiti Kebangsaan Malaysia, Kuala Lumpur on 5th February 2016.

Ali, A. A. (2016). *Assessment of cytotoxicity of oil palm based microcrystalline cellulose reinforced acrylic denture base resin.* Dental congregation2016, Royale Chulan, Damansara. Faculty of Dentistry, University of Malaya, Kuala Lumpur