A Comparison of Polymeric Denture Base Materials

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SUMMARY

Since its introduction in 1937, poly(methyl methacrylate) (PMMA) has become the most commonly used material for denture bases. This is largely due to its favourable although not ideal characteristics. One of the main problems associated with PMMA is the polymerisation shrinkage exhibited by the material. Injection moulding systems have been developed to compensate for this by continuously injecting PMMA resin at pressure throughout a carefully controlled polymerisation procedure.

This study aimed to compare acrylic specimens processed by injection moulding and conventional pressure packing in relation to dimensional accuracy. Subsequent experiments evaluated adherence of *Candida albicans* to denture base materials. A cobalt chrome control denture base was used to fabricate stone moulds in which 20 injection moulded and 20 conventional pressure packed PMMA resin denture bases were produced. These denture bases incorporated 6 reference points between which sequential measurements were taken using digital callipers. Base plate adaptation was additionally measured by weighing a vinyl polysiloxane film to reproduce any discrepancy between the denture base and master model.

Linear dimensional measurements revealed that changes in dimension did not occur evenly over the entire denture base for either sample group. Injection moulded samples exhibited statistically significant differences when compared to control in two of the six measured linear dimensions. Conventional

pressure packed materials exhibited a statistically significant difference in one of the measured linear dimensions compared to control. Statistically significant linear dimensional differences were determined between injection moulded and conventional pressure packed materials in three measured dimensions. For injection moulded materials, the location of the injection moulding inlet may have influenced the dimensional accuracy

For the weighed vinyl polysiloxane data, a greater weight of material was recovered from conventional pressure packed material samples than injection moulded samples. These data demonstrate that injection moulded denture bases have superior internal surface adaptation compared to conventional pressure packed acrylic resin.

Squares of denture base material were produced by injection moulding and conventional pressure packing techniques. Self cured PMMA resin was additionally included in candidal adherence and surface morphology analysis. Profilometer testing determined self-cure resin surfaces had more irregular surface characteristics than surfaces of conventional or injection moulded samples. Conventionally processed samples exhibited the smoothest material surface. However, conventional and injection moulded sample groups were similar.

Scanning electron microscopy of the three material sample groups was performed to determine surface morphology and patterns of candidal adherence and subsequent biofilm formation. SEM examination revealed

variations in surface morphology and following 1 hour, *Candida albicans* cells were observed to adhere and aggregate within the various surface irregularities of all three materials. Examination after 24 hours demonstrated the complex intertwining hyphae evident on all the material samples, irrespective of initial candidal adherence patterns.

No significant differences were observed between attachment of the 9 *C. albicans* clinical strains when tested independently against each sample group. However, comparison of the mean attachment of all strains to the 3 sample groups revealed a statistically significant difference in attachment capacity between conventional and self cured sample groups. Self cured PMMA resin samples exhibited significantly less candidal attachment than conventionally processed samples, indicating that material surface factors may play a greater role in promoting or preventing candidal adhesion that the organism per se.

As the denture bearing mucosa is compressible and the achieved palatal seal largely dependent on the prepared post dam, small dimensional changes demonstrated in this study may be of limited clinical relevance to the success or failure of the material as a denture base. *C. albicans* were found to adhere to all three types of PMMA resin and if left undisturbed, *Candida* cells proliferated to form a biofilm upon all resin materials. Therefore, the observed differences in attachment are likely to be of limited clinical importance in the prevention of candidal infection without consideration to denture and oral hygiene.

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LIST OF ABBREVIATIONS

ml	millilitres
µl	micro litre
wt	weight
kg	kilogram
g	grams
µg	microgram
m	meter
mm	millimetre
µm	micrometer
h	hour
min	minutes
M	Moles
UV	Ultraviolet
HCl	Hydrogen Chloride
TiO_2	Titanium Dioxide
RPMI	Roswell Park Memorial Institute medium
PBS	Phosphate buffered saline
SAB	Sabouraud's dextrose
psi	pounds per square inch
KPa	kilopascal
rpm	revolutions per minute
CAD/CAM	Computer Aided Design/Computer Aided Manufacture
kHz	Kilo Hertz

LIST OF PUBLICATIONS

Attachment of *Candida albicans* to Denture Base Acrylic Resin Processed by Three Different Methods. Young, B., Jose, A., Cameron, D., McCord, F., Murray, C., Bagg, J., Ramage, G. International Journal of Prosthodontics 2009;22:499-489 (Appendix 1)

Reducing the Incidence of Denture Stomatitis: Are Denture Cleansers Sufficient? Jose, A., Coco, B., Milligan, S., Young, B., Lappin, D.F., Bagg, J., Murray, C., Ramage, G. Journal of Prosthodontics 2010;19:252-267 (Appendix 2)

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DEDICATION

Dedicated to David and my parents Hugh and Linda.

Thank you for your love and support.

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Chapter 1:

INTRODUCTION

1.1 General Introduction

Over the last three decades, developments in dentistry have largely been instigated as a result of scientific research. Of particular note, are developments in the field of dental materials and a drive towards the practice of evidence based dentistry. Increased media coverage has resulted in improved patient knowledge and awareness of the treatments dental practitioners provide and also, as a direct result, increased patient expectations.

Many aspects of Prosthodontic treatment; be that clinical or laboratory based, may impact on overall patient satisfaction and the clinical success of treatment. This thesis will focus on denture base materials, in particular the resin based poly(methymethacrylate) (PMMA) materials. These materials are the most widely used non-metallic denture base materials (Jagger, 2002; Price, 1994). They do, however, have a number of well documented problems, which will be discussed in the following text.

Before embarking on an overview of the many synthetic prosthodontic materials available, it may be of interest to investigate the history of Prosthodontics and development of the various materials considered as precursors to modern day materials.

1.2 The history of Prosthodontics and denture materials

Tooth loss and the use of materials to replace them is not a new idea. Prostheses to replace missing teeth have been described throughout history (Hargreaves, 1980). Unlike the polymers used today, dentures up until the 1700s were usually made from wood, ivory, whale or hippopotamus bone and carved to fit edentulous spaces. The skill required and difficulty in constructing these prostheses made them prohibitively expensive to all but the wealthiest in Society (Hargreaves, 1981). George Washington, perhaps one of the most celebrated persons of the eighteenth century, wore dentures throughout his presidency (1789-1797). These comprised some of his own teeth, and replacement teeth made of bovine or hippopotamus teeth or lead. Washington was reported to have initially worn partial dentures, which were fastened to his remaining natural teeth. After the loss of his remaining dentition, his first set of complete dentures were constructed by John Greenwood (Phinney, 2003). The dentures were apparently secured in his mouth with a set of springs and swivels (Phinney, 2003). One must question the efficacy of such an intraoral appliance.

1.2.1 Porcelain

The first set of porcelain dentures was reportedly developed by Duchateau and de Chemant in 1774. It is from this process that de Chemant patented porcelain teeth and dentures in 1789 (Murray & Darvell, 1993). In the 1700s, Pfaff first described a method of introducing warm wax to the mouth and, allowing it to set before its removal, produced a negative representation of the

patient's oral tissues. From this record, a plaster of Paris cast of the patient's mouth was constructed (Peluso 2004).

1.2.2 Vulcanite

With the invention of vulcanised rubber by Charles Goodyear in 1839 the cost of producing dentures reduced significantly enough to make them accessible to a large number of consumers. The invention of vulcanite saw a considerable increase in demand for accurately fitting prosthesis at reasonable cost (Khindria *et al.*, 2009).

Vulcanite was the only satisfactory non-metallic denture base material available for some time. Vulcanite is a material formed by the addition reaction of natural rubber and sulphur. The resultant material is thermoset. The production of vulcanite was carried out in a steam pressure vessel, referred to as a vulcaniser, at 160 to 170^{o}C (Price, 1994). Sulphur bonding allows cross linking between the rubber polymer chains to form a rigid, opaque and stable solid (Price, 1994; Rueggeberg, 2002).

The vulcanite denture base was fitted with porcelain teeth and marked an important advance in dental polymer research. Although vulcanite represented a significant improvement on ivory, the previously favoured material, it still left much to be desired. Due to a lack of chemical bonding between porcelain teeth and vulcanite denture base, mechanical retention was required. This was in the form of diatorics, undercut holes made in posterior porcelain teeth,

which vulcanite would then flow into during processing. Alternatively, pins were placed in anterior teeth (Engelmeier, 2003). The main disadvantage of vulcanite was its poor aesthetics, largely attributed to its lack of translucency. Vulcanite was also porous and potentially caused accumulation of plaque and oral fluids that resulted in an unhygienic denture base (De Vanscott & Boucher, 1965).

1.2.3 Celluloid

Celluloid, a polymer based on natural cellulose, was introduced in circa 1870. This was produced by plasticising cellulose nitrate with camphor. The resulting material could be pigmented to the desired pink colour. A denture base was then constructed by pressing the celluloid blank into a dry, heated mould (Gorgas, 1891; Rueggeberg, 2002).

Initially, celluloid appeared to be a promising alternative to the widely used vulcanite. However, it was found to rapidly discolour over time, absorbing water and stains from food, drinks and tobacco (Rueggeberg, 2002). Patients also commonly complained of a residual camphor taste from the denture base and it proved a difficult material to repair (Ferracane, 2001). Owing to these adverse factors, popularity of celluloid soon waned. Other than a brief re-appearance in the 1920s, it was largely discarded as a denture material (Greener, 1972; Khindria *et al.*, 2009)

1.2.4 Phenol-formaldehyde

Dr. Leo Bakeland discovered phenol formaldehyde resin in 1909. This was termed ‘Bakelite’ and first produced for commercial use in dentistry in 1924 (Khindria *et al.*, 2009; Murray & Darvell, 1993; Rueggeberg, 2002). Immediately after processing, aesthetics were judged to be excellent. However, staining quickly became a problem in addition to the remaining taste of phenol. Furthermore, phenol-formaldehyde denture bases were very brittle and prone to fracture (Khindria *et al.*, 2009). They also proved very difficult to repair and therefore lost favour. The shelf life of the material was short and properties varied between manufactured batches (Greener, 1972; Murray & Darvell, 1993)

1.*2.5 Polyvinyl chloride (PVC)*

A co-polymer of vinyl chloride (80%) and vinyl acetate (20%) was introduced as a denture base material in the 1930s. This was processed in a similar manner to celluloid, by pressing a heated blank of the material into a mould (Greener, 1972). One of the problems associated with this technique was the presence of residual stresses in the material subsequent to processing. This is due to calcination of the gypsum mould which would occur if the material was heated to a sufficient temperature to allow stress relief. These residual stresses resulted in gradual deformation of the denture base and commonly, fracture during functional wear (Greener, 1972). Aesthetics were also compromised by heating the material, which caused discolouration (Drury, 1935).

These weaknesses in the co-polymer denture base resulted in its reduced use as a denture base material. PVC plasticized with either dibutyl or dioctyl phthalate is still used as a denture lining material or for construction of sports mouth guards today. When used to construct protective mouth guards, the material is manufactured in a pre-plasticised sheet. This is then heated and moulded to the desired contour with the use of a vacuum to seal the sheet of material over a cast of the patient's teeth (Patrick, 2006). Although still used for the purpose of denture lining, the material's properties are far from ideal. They harden over time as the plasticiser leaches out during wear (Munksgaard, 2004). Like silicone lining materials, they are also difficult to polish. Owing to their poor adhesive properties, PVC materials tend to detach from the denture base. This results in poor denture hygiene and acts as an irritant to the oral mucosal tissues (Greener, 1972).

1.3 Ideal denture material properties

In order for a material to succeed as a denture base, it must be acceptable for use by the dental technician, the dental surgeon and most importantly, the patient. To fulfil the requirements of all the above, the material ought to have the mechanical, stable, physical, biocompatible and aesthetic qualities outlined in Table 1.1 (Grant, 1992).

Table 1.1 Ideal properties of a denture base material (Grant, 1992)

Mechanical, stable, physical, biocompatible, aesthetic and other properties required by a denture base material in order to be deemed 'successful' for use

Mechanical	**Stable**	**Physical**	**Biocompatible**	**Aesthetic**	**Other**
Adequate transverse strength	Abrasion resistant	Same thermal expansion co-efficient as denture tooth material	Non-toxic	Pigmentable	Radioopaque
High modulus of elasticity	Capable of maintaining high polish	Conduct heat	Non-irritant	Translucent	Easy to manufacture
High proportional limit	Easily cleaned by patient (hygienic)	Low density (light weight)	In-soluble in oral fluids	Highly polishable	Low cost
High impact strength	Radioopaque	Melting point higher than ingested food/drinks	Non-absorbent		
Abrasion resistant		Dimensionally stable (during processing and function)	Inert		
Capable of maintaining high polish					

1.4 Poly(methyl methacrylate) (PMMA)

No denture material has yet been invented which fully satisfies the ideal criteria contained in Table 1. Since its introduction in 1937, poly(methyl methacrylate) (PMMA) has become the most commonly used material for denture bases. It remains most popular of all the polymeric denture base materials. This is largely due to its favourable, although not ideal, characteristics as outlined in Table 1.2 (Johnson, 1994).

PMMA is far from a perfect denture base material. It exhibits volumetric shrinkage during polymerisation that leads to dimensional changes in the denture base produced from the primary wax pattern (Anusavice, 2003; Combe, 1992). Further distortion and inaccuracies are introduced due to the high coefficient of thermal expansion exhibited by PMMA (approximately 80 × $10^{-6}/^{o}C$) (Combe, 1992; Greener, 1972).

Furthermore, denture bases constructed from PMMA are unfortunately not radiopaque. Therefore, they are not detectable on radiographs. This means that should a denture constructed from PMMA fracture and be accidentally inhaled or swallowed, it cannot be detected by radiographic means (Chandler, 1971; Kasim, 1998; Murray *et al.*, 2007)

Table 1.2 PMMA characteristics (Greener, 1972)

PMMA's favourable and unfavourable characteristics for use as a denture base material.

Favourable	**Unfavourable**
Ease of processing	Large polymerisation shrinkage
Pigmentable	High thermal expansion co-efficient
High polish attainable	Radiolucent
Adequate strength	Allergy possible
Easy to repair after fracture	
Low water sorption	
Low solubility	
Relatively low toxicity	
Odourless	
Tasteless	

1.4.1 PMMA developments and alternatives

Since PMMA was introduced, most dental material research has focused upon developing materials with higher strength, lower levels of residual methacrylate monomer after processing, improved dimensional stability, increased radiopacity and improved resistance to candidal infiltration (Dhir, 2007). Polymers such as polyamides, epoxy resin, polystyrene, vinyl acrylic, rubber graft copolymers and polycarbonate have been developed and tested as potential alternative denture base materials (Stafford, 1980; Stafford, 1986). However, these have not generally proved successful.

A study by Hedzelek *et al*. (2006) compared Ivocap® and Zhermacryl® acrylic resins with Microbase® polyurethane denture base material. This study determined that samples of the alternative denture base material had poorer mechanical strength than samples produced from poly(methyl methacrylate), processed following manufacturers instructions for all materials studied (Hedzelek, 2006). Thus far, a suitable alternative material has yet to be discovered.

1.4.2 PMMA chemistry

The polymerisation of PMMA involves a number of chemical reactions. These are initiation, propagation, termination, chain transfer and tacticity (Darvell, 2002). Each of these will now be considered in turn.

PMMA is formed by addition polymerisation of multiple methylmethacrylate molecules in the presence of an initiator, typically benzyl peroxide (Combe, 1992). Benzyl peroxide, in the presence of heat or chemical activation, breaks down to free radicals. These act upon the vinyl group of methyl methacrylate, opening the double bond causing formation of a new single carbon bond. This is known as a free radical addition polymerisation chain reaction (Figure 1.1) (Anusavice, 2003). During the polymerisation process 2 polymer chains or more, depending on the quantity of glycol dimethacrylate included in the mixture, may be united (Harrison *et al.*, 1978).

The opening of each double bond results in production of another free radical, which may in turn attack and join another double bond. This results in production of another free radical and continuation of the reaction. This repeated reaction is referred to as chain propagation. It is thought that all free radical attacks at this point link the methyl methacrylate residues together by methylene bridges ($–CH_2$-). These chains, carrying active free radical, are referred to as ‘growing’ or ‘live chains’ (Combe, 1992).

Chain termination can occur at any time and is dependent upon the concentration of available free radicals (Greener, 1972). Chain termination results from the mutual reaction of two free radicals. These free radicals may be from a chain, or from the initiator (Greener, 1972). The transfer of a hydrogen atom (hydrogen abstraction) from anywhere in the system, to the attacking free radical, results in termination of one chain reaction and

simultaneous stimulation of another. This new chain reaction may or may not be on an existing polymer chain (Anusavice, 2003) (Figure 1.2).

The free radical formed from the methyl methacrylate double bond is not symmetrical. This results in a carbon atom that also has an asymmetrical environment after reaction. The resulting polymer is atactic (Darvell, 2002).

Figure 1.1 Addition polymerisation of methylmethacrylate monomer

This reaction occurs in the presence of a radicalised initiator, typically benzoyl peroxide

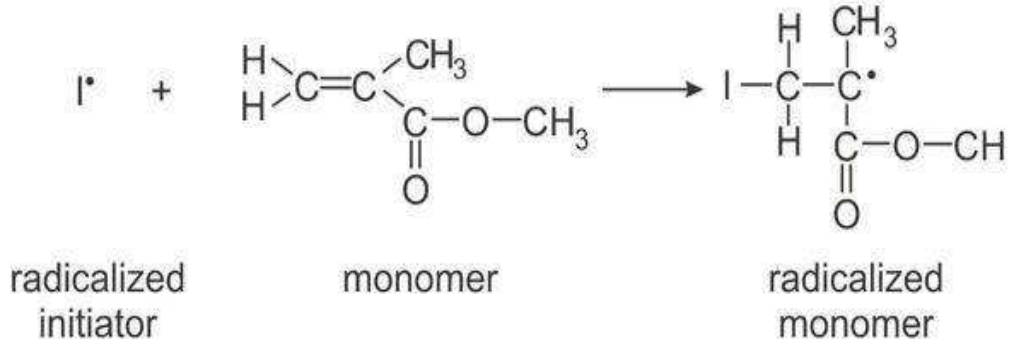
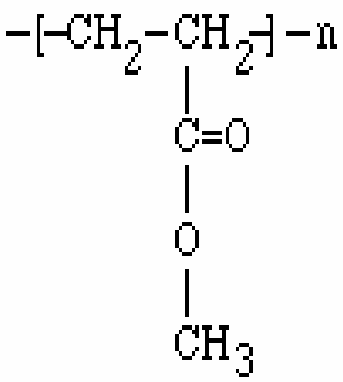


Figure 1.2 Repeating poly(methylmethacrylate) unit

This forms a growing polymer chain. The number of repeating units formed (n) and hence the size of polymer chain, is dependent on the number of available free radicals (Brewer, 2010).



1.4.3 Processing PMMA

1.4.3.1 PMMA handling

PMMA is an acrylic resin, the generic term for any polymer based upon acrylic acid (Greener, 1972). It is a colourless, transparent mass appropriately tinted for its use in dental applications. The relative ease with which PMMA can be processed is one decided advantage over other materials. When provided for use in prosthodontics, PMMA is usually dispensed as a powder-liquid system (Anusavice, 2003). The constituents of the powder and liquid components are detailed in Table 1.3.

Table 1.3 PMMA constituents

Powder and liquid contents for mixing to produce PMMA

Powder	**Liquid**
Pre-polymerised PMMA spheres Benzoyl peroxide (initiator) (1-2%) Pigment (1%)	Methylmethacrylate monomer Hydroquinone (inhibitor) (<1%) Glycol dimethacrylate (crosslinking agent) (1-2%)

When mixed together in appropriate quantities, a malleable soft mass is formed as the material transitions from sandy to web and then dough consistency (McCabe *et al.*, 1975). When it reaches the dough stage, the material can be packed into the mould for flasking and processing (polymerising) to produce a denture base (McCabe *et al.*, 1975). This is subsequently removed from the flask and finished for delivery to the patient.

Methylmethacrylate is readily polymerised by exposure to ultraviolet light, oxy-radical yielding initiators or heat (Greener, 1972). For this reason, the un-polymerised monomer must be stored in an airtight brown bottle in a cool environment. A small amount of anti-oxidant, usually hydroquinone (less than 1%) is commonly added to help prevent spontaneous polymerisation (Skinner, 1967).

Heat, chemical and light activated acrylic resins are available (Greener, 1972). Production of nearly all denture bases is carried out with the heat activated material and this will therefore be considered first.

1.4.3.2 Heat activated (heat cured) PMMA

The energy required for polymerisation of heat activated PMMA is most commonly provided in the form of a water bath, or less frequently, a microwave oven.

To avoid faults during denture processing, it is important that the correct powder-liquid ratio is followed. This is usually 3-3.5/1 by volume or 2.5/1 by

weight (Combe, 1992). If too little liquid is mixed with the polymer granules, then not all the polymer will be wetted by monomer and the processed acrylic will have a granular texture. If too much monomer is incorporated into the mixture, a higher level of polymerisation shrinkage will occur. With the correct polymer/monomer mixture, polymerisation shrinkage of approximately 7% can be expected (0.5% linear shrinkage) (Combe, 1992). Polymerisation shrinkage of pure monomer can however be expected at a level of approximately 21% by volume (Grant, 1992).

1.4.3.3 Compression moulding technique

Heat activated denture bases are most commonly processed by the compression moulding, or conventional pressure packing technique, as will be referred to throughout. To allow processing of the acrylic, the mould must first be prepared. This involves initially selecting acrylic teeth and ensuring that they are in the correct occlusal scheme. This is accomplished with accurate clinical impression making, jaw registration, articulator mounting and tooth trial stages. This involves cooperation with the laboratory and appropriate discussion with technician and patient. The completed wax trial denture is then sealed into the master cast and flasked.

After completely removing the wax from the flask investment, using boiling water and detergent, the master cast is coated with a thin layer of separating medium, usually a solution of sodium alginate or ammonium alginate. This separating medium is painted on and left to dry. A thin layer of calcium alginate is formed upon reaction with the calcium contained in the dental

plaster or stone mould material. These separating materials have generally been shown to be effective (Combe, 1992). However, they are not entirely successful at preventing any residual water left in the plaster/stone from entering the acrylic. This may lead to surface crazing and an unsatisfactory denture finish. The use of a separating medium also helps prevent adherence of the plaster/stone investment material, to the finished acrylic denture base (Grant, 1992). The separating medium should not come into contact with the exposed surface of the acrylic denture teeth, as this would prevent bonding of these with the denture base acrylic (Anusavice, 2003).

The acrylic dough is prepared by mixing the correct proportion of polymer/monomer, and leaving to stand in a closed container until the dough stage is reached ready for packing (McCabe *et al.*, 1975). If left too long, the dough will become stiff and packing into the flask will be difficult, if not impossible (Grant, 1992).

1.4.3.3.1 Dough stage polymerisation and flasking

The dough time of the acrylic depends on a number of factors, including particle size and molecular weight of the polymer, the presence of plasticizer, temperature and polymer/monomer ratio. Smaller particles and a lower molecular weight of polymer increase the speed of dough formation (McCabe *et al.*, 1975). The presence of plasticizer decreases the dough time, as does warming the mix and increasing the polymer/monomer ratio. The time required for the PMMA mix to reach the dough stage is referred to as the dough-forming time. The American National Standards Institute/American

Dental Association (ANSI/ADA) Specification No. 12 for denture base resins requires that the dough stage is reached in less than 40 minutes from the start of the mixing process. Most of the resins in clinical use have a dough forming time of approximately 10 minutes or less. In order to increase the working time of material in the dough stage, refrigeration may be used. However, if this is the case, care must be taken to avoid moisture condensation on the material surface as it reaches room temperature (McCabe *et al.*, 1975). The condensation of moisture on the resin may adversely affect both the physical and aesthetic properties of the acrylic after polymerisation (Tuckfield, 1943). This can be avoided by storing the material in an airtight container, and only opening this once the material has reached room temperature (Anusavice, 2003).

The time the acrylic material remains in the dough stage is critical for allowing compression moulding to take place. ANSI/ADA Specification No. 12 states that the material must remain in the dough stage for at least 5 minutes. This allows enough time for the dough to be packed into the prepared mould after thorough removal of the wax trial denture, and application of the separating medium. It is important that the packing stage is carried out as accurately as possible. If the material is over-packed, then the resulting denture base will be too thick, and inaccuracies with tooth positioning and occlusal vertical dimension may be introduced. Conversely, if the mould is underpacked then the resulting denture base material will be too porous (Ruyter & Svendsen, 1980). To avoid faults during packing, this should be carried out in a number of stages with gradual incremental pressure application, application of a

polythene sheet, trial closure, removal of flash, and repetition of trial closure until no more flash is evident upon opening the denture flask (Taylor, 1941). When no more flash is identified, then the denture flask is definitively closed without the polythene separating sheet in place. A flask carrier is used to maintain continuous pressure on the denture flask during the polymerisation process (Anusavice, 2003; Taylor, 1941).

The packed denture flask is then heated in an oven or water bath, with accurate control of both the time and temperature. It is important that the acrylic is fully cured, in order to avoid a high level of excess irritant monomer (Ruyter & Svendsen, 1980; Smith & Bains, 1956). The rate at which the material reaches its maximal temperature must be carefully controlled in order to avoid gaseous porosity. This may result if the packed flask is plunged into boiling water when there is still a high level of uncured material present. Monomer boils at 100.3^{o}C, and if the material is allowed to reach this temperature before polymerisation has occurred, then the monomer will vaporise and cause porosity in the base (Anusavice, 2003).

PMMA porosity has been reported to be associated with poor aesthetics, due to the update of stains and oral fluids. This may in turn lead to the harbouring of oral micro-organisms, and subsequent oral candidal infection (Davenport, 1970). Porosity levels of above 11% are observed to adversely affect the mechanical properties, including strength of PMMA denture base materials (Keller & Lautenschlager, 1985).

1.4.3.3.2 Heating regimes

There are 2 possible heat regimes used for curing heat activated PMMA denture bases. The first of those involves heating at 72^{o}C for at least 16 hours. The second technique involves heating at 72^{o}C for 2 hours, at which point an unacceptably high level of excess monomer is still present, then increasing the temperature to 100^{o}C and heating for a further 2 hours (Harrison *et al.*, 1978). This technique allows the denture base to be produced more quickly, but there is a higher risk of warpage on deflasking, due to the introduction of internal stresses (Grant, 1992).

1.4.3.3.3 Cooling

After the denture flask has been heated by either of the above heating regimes, it must be slowly cooled. This may be carried out by leaving the flask on the bench, in the cooled oven or water bath. Rapid cooling must be avoided in order to allow relief of any internal stresses incorporated due to the differing contraction co-efficients of the acrylic and mold materials. The presence of internal stresses in the denture base material may lead to the formation of micro-cracks and lead to distortion or fracture of the base (Grant, 1992; Komiyama & Kawara, 1998).

1.4.3.4 Injection moulding

The polymerisation shrinkage exhibited by PMMA may lead to inaccurate adaptation of the base material to the denture bearing tissues, resulting in a poor border seal and lack of stability of the denture base (Lamb *et al.*, 2005).

Changes to the occlusal form of the denture, may result in inaccuracies in the intercuspal position of the prosthesis potentially causing further instability and an unsatisfactory result for the patient (Barsoum *et al.*, 1968; Garfunkel, 1983; Keenan *et al.*, 2003). In an attempt to overcome the dimensional inaccuracies present with conventional compression moulding techniques, Pryor (1942) developed the injection moulding technique. This technique used a specially designed flask into which unpolymerised acrylic resin could be introduced. This flask used a spring mechanism to apply continuous pressure to a reservoir of unpolymerised acrylic resin (Pryor, 1942). This was designed to compensate for polymerisation shrinkage (Keenan *et al.*, 2003). However, an independent trial of the injection moulding technique found no significant advantages over conventional techniques for denture base processing in terms of dimensional accuracy. Therefore, Pryor's technique did not gain favour (Grunewald, 1952).

It was not until the 1970s that Ivoclar Ltd. developed an acrylic resin specifically for use with the injection moulding system (Keenan *et al.*, 2003). As more companies invested in research and development of the injection moulding process and equipment required for this, the method became more commonplace (Ganzarolli *et al.*, 2007; Nogueira *et al.*, 1999). Comparative studies have demonstrated that modern injection moulding techniques result in fewer dimensional inaccuracies than conventional processing techniques (Nogueira *et al.*, 1999; Strohaver, 1989). This is based on the principle that throughout the carefully controlled polymerisation procedure, PMMA resin is continuously injected at pressure to compensate for polymerisation shrinkage.

A comparative study of injection moulded versus conventional pressure packed acrylic resin found a significant reduction in shrinkage of injection moulded (0.65%) compared to conventional resin (0.9%) (Parvizi *et al.*, 2004).

1.4.3.4.1 Injection moulding technique

The injection moulding technique requires a specifically designed flask. One half of this is filled with dental stone into which the master cast along with the wax trial denture is settled. The dental stone is contoured and allowed to set. Wax sprues are then attached to the wax denture, before repositioning the other half of the flask, filled with dental stone, to complete the investment. After the stone has set, the 2 halves of the flask are separated and the wax pattern, including sprues, is boiled out. The stone investment must then be cleaned with detergent in order to avoid contamination of the denture base material with wax remnants. The flask is then reassembled to allow introduction of the acrylic resin. Throughout the injection moulding procedure, a carrier is used to maintain pressure on the assembly during resin introduction and polymerisation (Anusavice, 2003).

When using a powder-liquid mixture, it is important that the apparatus and the polymer mixture are kept at room temperature during introduction of the material to the prepared flask. This avoids the introduction of internal stresses, which may decrease strength and adversely affect surface characteristics of the finished denture base (Anusavice, 2003). The flask is then placed into a water bath for polymerisation. During the polymerisation process, the injection moulding apparatus continually introduces additional unpolymerised resin into

the mould to offset polymerisation shrinkage (Parvizi *et al.*, 2004). On completion of the polymerisation process, the denture is retrieved from the flask and finished with the same technique as dentures produced by conventional compression moulding technique (Strohaver, 1989).

1.4.3.5 Chemically cured PMMA

Chemically cured, or self cured, PMMA is auto polymerised. This means that the polymerisation reaction starts as soon as the powder and liquid components are mixed together (Table 1.4). These are therefore kept separately until required (Sweeney, 1958).

The benzoyl peroxide initiator present in the pre-polymerised poly (methylmethacrylate) spheres may also be activated by chemicals. In this case, no heat is required for the polymerisation reaction to occur. Dimethyl-p-toluidine, a tertiary amine, is used to activate the polymerisation reaction in chemically cured PMMA. After polymerisation has commenced, the reaction is the same as for heat cured materials (Greener, 1972).

1.4.3.5.1 Chemically cured (or 'Self' cured) PMMA properties

Chemically cured materials rarely exhibit the same degree of polymerisation as heat cured materials. For this reason, their strength and hardness values are lower (Grant, 1978). It is possible to exhibit a degree of control over the rate of material hardening by altering the size of polymer particles and the volume of dimethyl-p-toluidine added. As no heating is required, fewer stresses are introduced into the chemically cured materials. Furthermore,

there is less polymerisation shrinkage so these materials may be considered more dimensionally accurate than heat cured types (Sweeney, 1958). However, aesthetics are somewhat compromised with chemically cured acrylic resins. Yellowing of the materials tends to occur over time owing to oxidation of the amine initiator. For these reasons, and the incomplete polymerisation of the material, a higher level of excess monomer tends to be present in the finished denture base (Grant, 1978). Chemically cured acrylic resins are most commonly used only for denture repairs or additions, construction of custom trays or the production of orthodontic removable appliances (Greener, 1972).

Table 1.4 Chemically cured PMMA constituents

Contents of chemically cured PMMA powder and liquid

Powder	**Liquid**
Pre-polymerised PMMA spheres Benzoyl peroxide (initiator) (1-2%) Pigment (1%)	Methylmethacrylate monomer Hydroquinone (inhibitor) (<1%) Glycol dimethacrylate (crosslinking agent) (1-2%) Dimethyl-p-toluidine (activator)

1.4.3.6 Light activated materials

Visible light is used to activate denture base resins comprising a composite mixture of constituents illustrated in Table 1.5 (Anusavice, 2003).

1.4.3.6.1 Light activated PMMA processing

Light activated PMMA is supplied pre-mixed in light-proof pouches. Conventional investment techniques are not possible with the use of light activated resins, since the investment medium would prevent the passage of light to the denture base, and polymerisation would not occur. Instead the teeth must be set, and the material accurately moulded onto the cast. This is then placed in a visible light supply unit for example Triad 2000™ (DENTSPLY Ltd, Addlestone, Surrey) for the time specified by the manufacturer. This is then removed from the cast and finished as with the other polymerisation techniques previously described in Sections 1.4.3.2-1.4.5.1 (Anusavice, 2003).

Table 1.5 Light activated PMMA constituents (Anusavice, 2003)

Contents of light cured PMMA material

Material Constituents
Urethane dimethacrylate
Microfine silica
High molecular weight acrylic resin monomers
PMMA Acrylic beads (fillers)
Camphorquinone (initiator)

1.4.3.7 Microwave processing

Microwave energy may also be used to polymerise poly methyl methacrylate resin. For this technique, a non-metallic investment flask must be used along with resin specifically formulated for microwave processing. The microwave is used to provide thermal energy which allows the polymerisation reaction within the resin denture base to take place. The main advantage of microwave polymerisation techniques is the speed with which the denture base can be produced. Current evidence suggests that denture bases produced by this method have a dimensional accuracy and physical properties similar to those of conventional materials and resins (Keenan *et al.*, 2003; Memon *et al.*, 2001; Rached *et al.*, 2004)

1.4.3.8 Finishing

Finishing of the denture base is required to improve aesthetics and reduce adherence of extrinsic stains to the denture base. A smooth surface polish has also been demonstrated to reduce adherence of *Candida albicans*, a common cause of denture induced stomatitis (Karaagaclioglu *et al.*, 2008; Nevzatoğlu *et al.*, 2007; Pereira-Cenci *et al.*, 2007).

The produced denture base is finished with a combination of acrylic trimming burs and pumice slurry on a wet soft cloth wheel. Whiting is most commonly used on a dry polishing wheel, with care being taken to avoid overheating the denture base, during the final polishing stages (Combe, 1992).

1.5 Criteria for denture success

The success of a denture, and thereby its base material, depends not only on the criteria already described but several other factors. These include patient and clinical/clinician factors or technician related factors. As this study will focus on in vitro investigation of mechanical and microbiological aspects of denture base resins, other factors involved in the success or failure of denture materials are deemed to be out with the scope of this study.

No denture base material has yet been developed which completely fulfils all the criteria for success and conversely does not posses any of the above noted problems. PMMA remains the most popular choice for removable prosthodontics (Phoenix, 1996), largely because of it's relatively low cost and ease of use both clinically and in the laboratory fabrication process (Meng & Latta, 2005).

1.6 Specific fields of research

PMMA has been the most widely used and accepted denture base material for almost seventy years. There are three potential avenues for investigation which have been studied in the field of denture base materials. These are: the development of a new alternative to PMMA, the chemical alteration of PMMA; or the reinforcement of PMMA with another material such as fibres of material possessing more favourable fracture resistance properties (Jagger *et al.*, 1999; Wiskott *et al.*, 1995).

1.6.1 Fracture resistance

Acrylic resin dentures are susceptible to fracture during wear or if dropped (Vallittu, 1997). Fractures in dentures occur principally due to two different types of forces, flexural fatigue or impact (Jagger *et al.*, 1999). Flexural fatigue results from repeated application of a force. If such force is applied on only one occasion, it would have no detriment to the denture but may cause eventual structural failing, most likely due to the formation of microscopic cracks. These cracks will eventually fuse together with the continued application of flexural force to the denture, causing it to fracture. This type of fracture most commonly occurs across the midline of a denture. Impact resulting in a fracture occurs due to the application of force, or impetus of striking the denture against another object. The strength of this force is great enough to cause fracture of the denture after a single application. Denture fractures caused by impact most commonly occur due to dropping the denture during cleaning, coughing, sneezing or due to a sudden blow (Jagger *et al.*, 1999).

Numerous techniques for reinforcement of PMMA with inclusion of other materials have been described (Bowman & Manley, 1984; Carroll & von Fraunhofer, 1984; Jagger *et al.*, 1999; Vuorinen *et al.*, 2008). One of these is reinforcement of the prosthesis with metal wires embedded in the base. The primary problem with this technique is poor adhesion between resin and metal or metal alloys. Moreover, the resulting denture bases are often aesthetically unsatisfactory (Khaledi, 2006).

Carbon and Kevlar fibre reinforcement techniques have also been investigated. These were found to be more aesthetically satisfactory. However the complicated etching process required to improve their incorporation into PMMA, and preparation and positioning of the fibre layers, was found to be both time consuming and technique sensitive (Uzun *et al.*, 1999). Such factors have thereby, reduced the routine applicability of this method.

The use of glass fibres to reinforce PMMA is probably the most common technique described in the literature (Aydin *et al.*, 2002; Cokeliler *et al.*, 2007; Uzun *et al.*, 1999). Not only have these been found to improve the mechanical properties of PMMA, but they are also highly polishable, aesthetic and easy to manipulate (Cokeliler *et al.*, 2007; Jagger *et al.*, 1999).

Several studies have focussed on attempts to improve the mechanical properties of PMMA. A study by Mohamed *et al* (2004) demonstrated that inclusion of hydroxyapatite filler in heat cured methyl methacrylate increased the fracture toughness when compared to commercial denture base material. The addition of treated and untreated silica to PMMA was investigated by McNally *et al* (2006). Silica is commonly used as a filler to improve strength and wear characteristics of dental materials. The data collected in the study did not support this hypothesis that silica would improve the material's impact or transverse strength. Therefore, the authors were unable to recommend the inclusion of silica fillers in PMMA materials (McNally *et al.*, 2006).

1.6.2 Polymerisation shrinkage

As mentioned previously in section 1.4.3.2, the inert dimensional inaccuracies which exist in denture base acrylics, due largely to shrinkage during polymerisation, are one of their most widely acknowledged problems (Craig, 2002). Dimensional inaccuracies, as a result of polymerisation shrinkage may cause there to be instability of the denture base on the denture bearing tissues (Barsoum *et al.*, 1968). Clinically, this may produce either a poorly fitting denture or pain due to uneven loading of the denture bearing mucosa. Polymerisation shrinkage may also produce occlusal inaccuracies, due to unwanted tooth movement during laboratory processing (Baemmert *et al.*, 1990).

1.6.2.1 Techniques evaluating dimension

Assessment of denture base fit and dimensional inaccuracy arising from laboratory production has been assessed in a number of studies (Ganzarolli *et al.*, 2007; Keenan *et al.*, 2003; Lamb *et al.*, 2005; Parvizi *et al.*, 2004). These have studied both injection moulding and conventional pressure-packed processing techniques. The most common methods of assessing dimensional changes within these studies have included measuring between set points on the denture base (Huggett *et al.*, 1992; Nogueira *et al.*, 1999; Parvizi *et al.*, 2004), post dam discrepancies (Lamb *et al.*, 2005), linear assessment of denture base adaption (Anthony, D.H. & Peyton, F.A., 1959) or assessment of overall denture base adaption, or 'fit' (Ganzarolli *et al.*, 2007). The technique described in the literature for measuring discrepancy in overall base adaptation involves coating the fit surface of the denture base with a

standardized portion of vinyl polysiloxane material. This is then seated on the master cast under 5kg axial loading and allowed to polymerise. The resulting silicone film is then trimmed to the border of the denture and weighed using a precision scale. A similar technique has also been described for measuring the internal fit of indirect restorations (Lee, 2008).

1.6.3 Radiopacity

The radiolucent nature of PMMA is one of its disadvantages as a denture base material. It is debatable however, whether or not this would pose a significant problem if PMMA's mechanical properties could be improved making fracture less likely. The potential for producing a radiopaque resin has been investigated in numerous studies (Chandler, 1971; Kasim, 1998; Lang *et al.*, 2000; Saunsbury, 1964). A number of different materials have also been tried and tested as more radiopaque alternatives to PMMA. However, none have so far proved satisfactory.

The most promising material to date appears to be silanated barium fluoride impregnated powdered glass, which is added to polymethylmethacrylate denture resin material. This does not increase the cost of denture production significantly, or decrease the denture base material mechanical properties to an unsatisfactory level (Kasim, 1998). Barium sulphate ($BaSO_4$) has also been added to denture base resins to improve radiopacity. However, inclusion of these salts was found to adversely affect mechanical properties, including transverse and impact strength as well as colour and translucency when included at concentrations higher than 8% (Saunsbury, 1964). However,

specimens required 29% $BaSO_4$ to be easily identifiable on a chest radiograph (Saunsbury, 1964). Lang *et al*. (2000) investigated the potential for triphenylbismuth incorporated into injection moulded heat cure resins to improve radiopacity. At 30% wt/wt in the polymerised resin, this was found to produce radiolucency similar to 0.2mm copper. At this concentration, incorporation of triphenylbismuth was not found to adversely affect either colour stability or transverse strength of injection moulded resins. However, inclusion of triphenylbismuth in compression moulded resins at 30% wt/wt, was found to cause porosity. This percentage of triphenylbismuth adversely affected the material's mechanical properties and aesthetics. Triphenylbismuth caused a faint yellow discolouration in both material types (Lang *et al.*, 2000). This may provide a potential solution to the difficulty currently experienced with radiographic denture identification, but requires further investigation.

1.6.4 Biocompatibility

A range of different resins have been introduced for use as denture base materials. One of the major limitations of these materials is their biocompatibility, or lack of (Jorge *et al.*, 2007). Poly(methyl)methacrylate is the most commonly used non-metallic denture base material. Adequate polymerisation of the material during its processing is essential to ensure it possesses the most favourable mechanical and physical properties, as well as reducing excess monomer levels, improving biocompatibility (Leggat & Kedjarune, 2003; Lung & Darvell, 2005; Urban *et al.*, 2007). It appears to be inevitable that some degree of residual monomer will always be present in

denture base acrylic resin (Lung & Darvell, 2005). The methyl methacrylate monomer leaches out of the denture base and into the wearer's denture bearing tissues. This may result in irritation or allergy (Lung & Darvell, 2005).

Various post polymerisation treatments have been described in order to reduce levels of excess monomer present in the finished denture base. These include water bath immersion and microwave treatment post-polymerisation. However, a study by Jorge *et al.* (2007) recently demonstrated these to have a negligible effect upon reduction of cytotoxicity.

1.6.5 Hypersensitivity

A study by Goon *et al.* demonstrated a positive reaction to patch testing for acrlylate/methacrylate sensitivity in 1% of Swedish and 1.4% of Singapore populations studied (Goon *et al.*, 2008). For denture wearing patients with hypersensitivity to acrylic resins, the provision of prostheses made from an alternative material may pose problems. Polysulfone (PSF), nylon and polycarbonate (PC) have been suggested as potential alternatives for use in such cases (Stafford, 1986; Stafford, 1967; Sweeney, 1958; Tanoue *et al.*, 2005). The mechanical properties of these materials, such as transverse strength and water sorption, have been shown to be less favourable than for PMMA (Pfeiffer & Rosenbauer, 2004; Pfeiffer *et al.*, 2005).

A number of techniques for provision of fixed restorations in patients with PMMA allergy have been described (Cohen, 2005; Guinta, 2005; Hochman &

Zalkind, 1997). However, there remains a lack of published guidance for removable prostheses.

The incidence of allergic reactions to PMMA based denture base materials has been well documented for a number of years (Alanko *et al.*, 1996; Kanerva *et al.*, 1997). In addition, dental technicians involved in the processing of acrylate-based dentures are also at increased risk of contact dermatitis and allergic type reactions. Of all the denture base resins, methylmethacrylate has been demonstrated to be the most likely to provoke an allergic reaction in dental technicians (Kanerva *et al.*, 1993; Kiec-Swierczynska, 1996). A study by Murer *et al.* (1995) demonstrated that dental technicians were 8 times more likely to develop a skin allergy than the average lay person. Additionally, wearing gloves during the processing procedure has been shown to offer only limited protection, since the monomer contained in these materials can easily leach through both latex and vinyl gloves (Hensten-Pettersen, 1998).

1.6.6 Microbiological resistance

In addition to the potential irritation caused to the soft tissues by residual methacrylate monomer, the impression surface of a maxillary denture is also a common reservoir for microorganisms (Budtz-Jorgensen, 1981; Samaranayake *et al.*, 1980). The presence of *C. albicans* is an important factor in the development of chronic atrophic candidosis (Budtz-Jorgensen, 1981; Karaagaclioglu, 2008). The influence of surface free energy and roughness has been investigated by a number of studies, comparing various

polymerisation methods. Surface roughness in particular has been found to increase adherence of *Candida* species (Pereira-Cenci *et al.*, 2007). Surface roughness in turn may be affected by factors such as polymerisation method, material used, and incorporation of fibres into the material (Karaagaclioglu, 2008; Pereira-Cenci *et al.*, 2007).

The denture hygiene of dependent elderly people has been demonstrated to be poor (MacEntee, 2000; Preston *et al.*, 2006; Sweeney *et al.*, 2007). Therefore, various PMMA surface treatments have been investigated in recent years in attempts to decrease candidal adherence (Manfredi *et al.*, 2007; Shibata *et al.*, 2007; Yoshinari *et al.*, 2006). These have included incorporation of methacrylic acid to alter surface charge (Park *et al.*, 2003) and incorporation of apatite-coated TiO_2 photocatalyst and ammonium compound (Pesci-Bardon *et al.*, 2006). All of these modifications have been shown to effectively reduce candidal adherence. This study will further investigate adherence of *C. albicans* in acrylic denture bases, processed by conventional pressure packing, self curing or injection moulding techniques.

1.6.6.1 Evaluation of candidal adherence

Candidal adherence to acrylic resin samples has been examined in a number of studies and can be performed by a variety of techniques. One such commonly described technique involves the use of crystal violet to stain candidal cells adhering to the resin surfaces. This allows calculation of the proportion of the surface area covered by adherent candidal cells using a magnified computer image (Park *et al.*, 2003) or an optical microscope (Moura

et al., 2006). A similar technique, incorporating the fixing of adherent cells with glutaraldehyde followed by Gram staining and light microscope examination, has also been described in the literature (Waltimo *et al.*, 2001; Yildirim *et al.*, 2005). More recently, scanning electron microscopes have been used to aid the counting of adherent yeast cells. This is a slightly more complex technique as the samples must first be fixed with methanol, dried and then coated with gold-palladium (20nm) to allow SEM examination (Karaagaclioglu *et al.*, 2008).

1.7 Aim

To use *in vitro* experiments to compare acrylic specimens processed by 2 different methods in terms of dimensional accuracy and adherence of *Candida* species.

1.8 Objectives

The objectives of this study were:

To measure dimensional accuracy of PMMA denture bases produced by conventional versus injection moulding techniques, in both sagittal and coronal planes, along the post dam and in terms of overall base plate adaptation.

To assess adherence of *Candida albicans* to three different types of denture base acrylic (compression moulded, injection moulded, and self cured samples) using quantitative and qualitative techniques.

Chapter 2:

MATERIALS &

METHODS

This study aimed to compare PMMA acrylic resin specimens produced by different processing techniques in terms of dimensional accuracy and adherence of Candida species. This section has therefore been divided into dimensional accuracy and microbiology sections.

2.1 Dimensional accuracy

2.1.1 Preparation of master CoCr denture base

A gypsum master cast of a maxillary edentulous arch was poured with a ratio of 100g of gypsum to 30ml of water according to manufacturer's instructions (Dentstone KD®, British Gypsum Products, Newark, UK) and allowed to set. It was necessary to ensure the Cobalt Chrome (CoCr) base could be easily removed from the investment material without damage to the mould and subsequent processed denture bases. The cast was therefore trimmed and surveyed to identify undercuts on the edentulous ridge area. Undercuts were removed using acrylic trimming burs and modified areas smoothed using fine grade sandpaper. An addition vulcanising vinyl polysiloxane (Dublisil-15, Dreve Dentamid, GmbH, Unna, Germany) mould of this modified master cast was then produced to allow replication at a later stage.

To fabricate the initial control denture, a layer of base plate wax (Anutex, Kemdent®, Wiltshire, UK) was adapted onto the master cast at a thickness of 1.5-2mm; (Figure 2.1). This was considered an appropriate thickness for a PMMA denture base. This thickness was of adequate strength to avoid fracture of acrylic samples during removal from the investment. For the purpose of this study, no denture teeth were included in order to exclude these as a source of error.

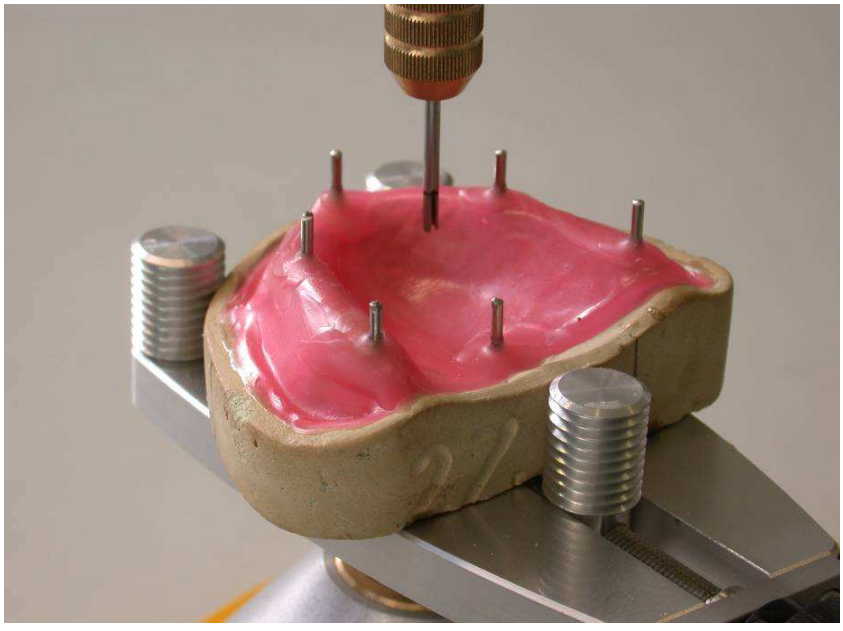
After completing the waxed denture base, 6 set points were selected. These were bilaterally in canine and first molar regions and single points at the incisive papilla and just anterior to the post dam in the midline. These points were selected in order to allow measurement of dimensional changes in both sagittal and coronal planes. 1.5mm diameter (Wiptam®, KC Smith, Monmouth, UK) orthodontic wires of 2mm length were positioned parallel to one another using a surveyor and waxed into position (Figure 2.2). This set-up was invested and a cobalt chromium casting produced. The casting was carefully removed from the investment material and polished. The positioned wires were then trimmed to leave reference points 0.5mm in height. The reference points were polished to give a pointed tip. This was the point from which each measurement was taken.

Figure 2.1 Layer of base plate wax adapted to master cast

Layer of modelling wax (1.5-2mm thick) adapted to master cast and trimmed to land area

Figure 2.2 Surveying reference points to check parallelism

Master cast with layer of wax and reference points on surveyor, being used to check correct alignment of each reference point



2.1.2 Production of moulds

The master CoCr denture base was invested in vacuum mixed improved stone (Velmix, British Gypsum, UK. Cafferata & Co Ltd UK) on a vibrating table (DENSTAR-500, Denstar, Daegu, Korea) to minimise air inclusion. It was ensured that the investment around the base had no thin edges and did not cover base periphery (Figure 2.3). This was then allowed to set completely overnight.

The set-improved stone investment material, containing the master CoCr denture base, was coated with a thin layer of separating medium (GC Multisep®, GC Europe). The opposing flask was half filled with improved stone investment material, mixed in a vacuum mixer and opposing flask halves closed. The remainder of the flask was filled to complete investment process on a vibrating table (Figure 2.4).

Figure 2.3 Invested CoCr control denture base

CoCr denture base invested in improved stone in one flask half. Stone has been cleaned from the periphery of the base

Figure 2.4 Completed investment

Completion of investment of CoCr denture base in improved stone on vibrating table

Two moulds were produced for each processing type in order to increase the number of denture bases able to be concurrently processed. The injection moulding apparatus has been designed to process 2 denture flasks simultaneously. This was also possible with the conventional pressure packing procedure. For injection moulded samples, sprues were attached to the CoCr denture (Figure 2.5) before repositioning the other half of the flask to allow injection moulding to take place, and allowing the investment to set.

Flask halves were carefully separated and checked to ensure no damage had occurred to investment material or CoCr master denture base. Moulds were inspected to ensure no imperfections were present on the investment surface. Denture bases were then fabricated, according to manufacturer's instructions, for either injection moulding or conventional pressure packed acrylic.

Figure 2.5 CoCr denture base prior to investment completion

Sprues attached to CoCr denture base prior to completion of investment

2.1.3 Conventional pressure packed PMMA processing

The stone moulds were thoroughly coated with a thin layer of sodium alginate, which was allowed to dry prior to packing and processing.

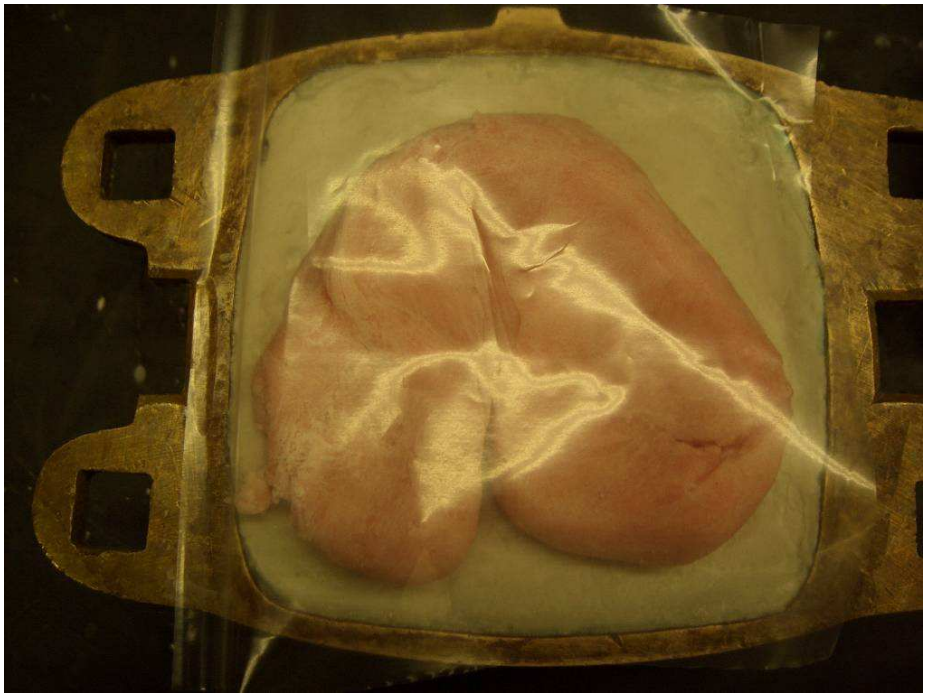
The acrylic dough was then prepared by thoroughly hand mixing polymer powder with monomer liquid (Trevalon®, [Dentsply, India]) in a ratio of 24 grams polymer to 10 millilitres of monomer, according to manufacturer's instructions. The mixture was left to stand in a closed container until the dough stage was reached (approximately 10 minutes) and packed into prepared flask half containing the deepest wax pattern void. A commercially available polythene sheet (Bracon Ltd. Etchingham, UK) was placed over this (Figure 2.6) and the opposing half of flask and investment then positioned and closed. A trial closure of the denture flask was performed with bench hydraulic pressure of 1000psi. This pressure was increased slowly over a period of one minute. The flask was opened, separating sheet discarded and excess flash removed. The separating sheet was reapplied and the trial closure repeated with the pressure held for ten minutes. Upon opening no further flash was evident. A check was made to ensure the mould seal was intact. The mould was then closed without a separating sheet to a pressure of 1000psi. On removal from the bench press, the flask was placed with another flask into a 2-flask spring compress to maintain continuous pressure on the PMMA during the processing procedure.

The acrylic in the packed mould was then polymerised by heating for 7 hours at 72^{o}C and then 2 hours at 100^{o}C in a water bath (Derotor Paco Bath, QD,

UK). This process was controlled by thermostat in order to ensure that PMMA resin samples were polymerised with the same accurate and reproducible heating regime. Samples were bench cooled to room temperature before de-flasking in order to minimise the introduction of stresses to the material.

Figure 2.6 Polythene sheet applied to acrylic dough

Polythene sheet placed over acrylic dough, which has been packed into wax pattern void in investment, prior to trial closure



2.1.4 Injection moulded PMMA processing

Samples were produced using the Palajet Duoflask® system (Heraeus Kulzer, Hanau, Germany). The technique requires a specifically designed flask. After initial investment in one flask half as described for the conventional processing technique, commercially available wax sprues (Heraeus Kulzer, Hanau, Germany) were attached to the CoCr base with a small volume of melted modelling wax to allow injection to take place (Figure 2.5). An 8mm diameter sprue is used for the ingress channel and a 3mm sprue is attached as the exit. A layer of mould seal separating medium was then applied to the investment and allowed to dry.

The other half of the flask was repositioned and locking rings closed in correctly aligned positions. The assembled flask was then filled with dental stone, mixed as per manufacturer's instructions under vacuum, on a vibrating table, to complete the investment. The investment was allowed to set overnight. The two flask halves were separated and the CoCr denture base carefully removed. Sprues were removed by boiling out and the flask halves cleaned with detergent. The moulds were examined for damage and any thin edges of stone present around the sprue channels removed and smoothed with a plaster knife. This was to ensure a smooth even flow of PMMA resin into the mould on injection and to prevent any stone being incorporated into the acrylic. The investment was then coated with a thin layer of mould seal. This was allowed to dry before the flask was reassembled to allow introduction of the acrylic resin (Figure 2.7).

Figure 2.7 Mould Seal Coating

Mould being coated with a thin layer of mould seal prior to reassembly of apparatus for injection

PalaXpress® resin (Heraeus Kulzer, Hanau, Germany) was mixed according to manufacturer's instructions, a measure for powder and liquid is provided in the system kit to a ratio of 2:1 powder: liquid in the dough reservoir. When the dough had reached the appropriate stage at 5 minutes, the reservoir was positioned for injection. The previously prepared flask and investment were placed in the Palajet® System (Heraeus Kulzer, Hanau, Germany) apparatus and secured for injection. Injection of acrylic resin was undertaken with continuous air pressure of 600 KPa on the assembly. The injection was complete when excess dough exuded from the exit sprue hole. The vent in the apparatus was screwed shut in order to maintain pressure throughout the mould during injection.

Once injection was complete, the flask was removed from the injector and placed into a water bath for 30 minutes at 55^{o}C under a pressure of 300 KPa to allow polymerisation. On completion of the polymerisation process, the processed acrylic was retrieved from the flask and finished.

2.1.5 De-investing and storage of denture bases

Flask halves were carefully separated. The denture base samples were then trimmed to remove excess acrylic flash with acrylic trimming burs running at slow speed (20,000 rpm). This allowed the samples to be re-seated onto the master casts with land area visible for dimensional measurement later. Samples were numbered in order of production, for identification purposes with a permanent marker. As water sorption has been shown to cause dimensional changes (Keenan *et al.*, 2003), all samples were stored under

sterile water with a trace of chlorhexidine (Corsodyl™, GlaxoSmithKline, Middlesex, UK) at room temperature for a period of 4 weeks. This solution was changed every 2 days. 20 injection moulded and 20 conventional pressure packed denture bases were produced.

2.1.6 Linear measurement

Distances between the 6 standardised reference points on processed denture bases (Figure 2.8) were measured in triplicate with digital callipers (Digimatic Caliper, Mitutoyo®, Andover, UK) (Figure 2.9) and recorded in a table. Optical magnification (2.5×) was worn by the operator throughout (Keeler Loupes, Keeler Ltd., Windsor, UK). An average value was then calculated for each dimension. Statistical analysis was then performed for each of the 40 specimen denture bases.

Figure 2.8 Denture base sample with reference points

Sample denture base with reference points highlighted and assigned letters to denote dimension measured

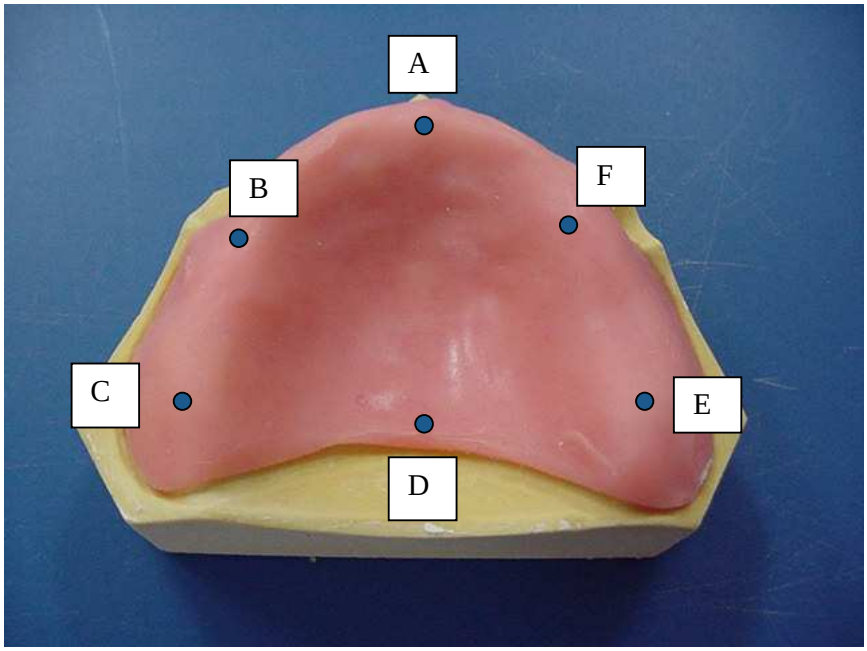


Figure 2.9 Linear Measurement

Measurement between reference points using digital callipers

2.1.7 Overall base plate adaptation

2.1.7.1 Weighed siloxane film

A standardized quantity of light bodied vinyl polysiloxane (1.5g) (Express™ Light body Flow, 3M ESPE, Leicestershire, UK) was used to coat the internal surface of each resin denture base (Figure 2.10). Denture bases were re-seated on the master cast under a 5kg axial load. After 4 minutes setting time, excess impression material was trimmed with a scalpel to the denture border (Figure 2.11). The residual layer of impression material was carefully removed from the surface of the denture base and weighed on a precision scale with an accuracy of 0.001g (GALAXY 400 G400™, Ohaus, Nanikon, Switzerland) (Figure 2.12). The adaptation of each denture base was expressed as the weight of impression material retained between the denture base and the master die. This process was repeated in triplicate for all 40 specimens. The average weight of siloxane film was then calculated for each denture base sample and statistical analysis performed.

Figure 2.10 Weighing vinyl polysiloxane in denture sample

A standardised quantity of light bodied vinyl polysiloxane being weighed and used to coat internal surface of sample resin denture base

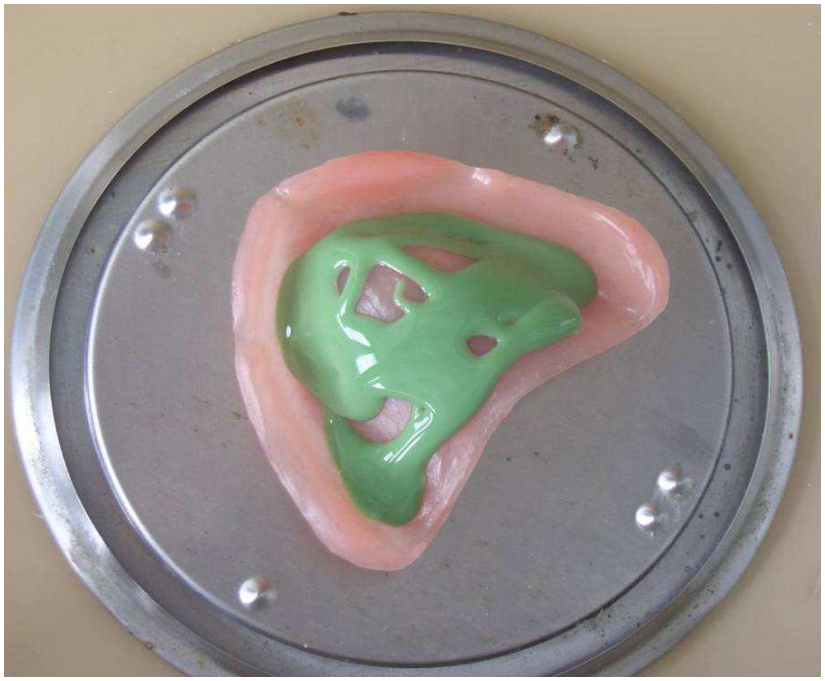


Figure 2.11 Trimming set impression material

A scalpel was used to trim impression material to denture base sample border after setting

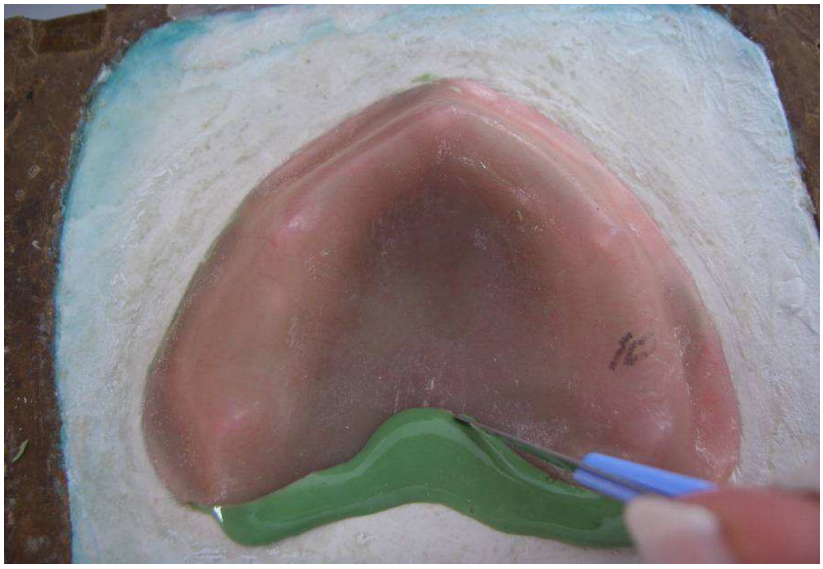


Figure 2.12 Weighing residual impression material

The layer of impression material after removal from internal surface of denture base sample being weighed on precision scales

2.2 Preparation of acrylic samples for surface testing

A metal flask (Bracon Brass) was prepared for processing by coating internal flask surfaces with petroleum jelly (Ecolab Ltd., Kendall, UK). One sheet of commercially available modelling wax (Anutex, Kemdent®, Wiltshire, UK), with a uniform thickness of 1.5mm, was trimmed to fit in the prepared flask. Stone investment (Dentstone KD®, British Gypsum Products, Newark, UK) was mixed according to manufacturer's instructions with water, under vacuum. One half of the prepared flask was placed on a vibrating table and filled with stone mixture. A pre-prepared modelling wax sheet was seated onto this, with care taken to avoid the incorporation of air bubbles (Figure 2.13). The wax surface was then painted with a surface tension solution. Stone was allowed to set with the modelling wax sheet *in situ* and coated with a 50% solution of sodium silicate as a separator. The opposing half of the flask was assembled and filled with stone mix on a vibrating table, as previously described. Stone investment was allowed to set overnight. The wax was then boiled out by submerging the flask in boiling water for 10 min. Flask halves were separated and cleaned thoroughly with commercially available liquid detergent and boiling water. The master cast was coated with a thin layer of sodium alginate (Cold Mould Seal, Quayle Dental, Sussex, England) and the separating medium left to dry (Figure 2.14).

Figure 2.13 Seating modelling wax sheet

Carefully seating pre-prepared modelling wax sheet into investment on vibrating table

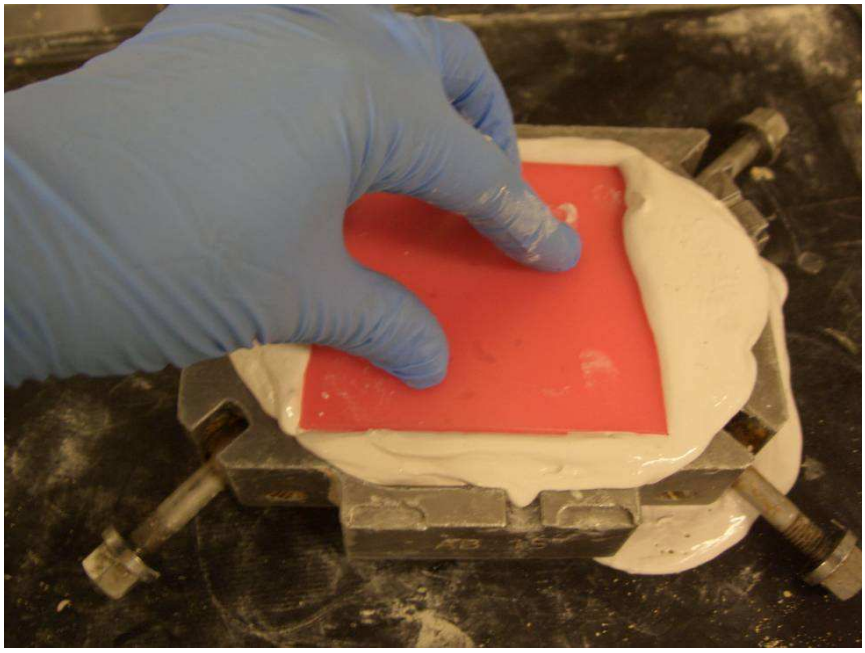


Figure 2.14 Coating with mould seal

A thin layer of mould seal being applied to surface of investment

2.2.1 Conventional pressure packed PMMA resin

PMMA resin denture base samples produced by the conventional pressure packed technique were prepared and processed according to manufacturers instructions, as previously described in Section 2.1.3

2.2.2 Injection moulded technique PMMA resin

Samples were produced using the Palajet Duoflask® system (Heraeus Kulzer, Hanau, Germany) as described in Section 2.1.4.

2.2.3 Self cured PMMA resin

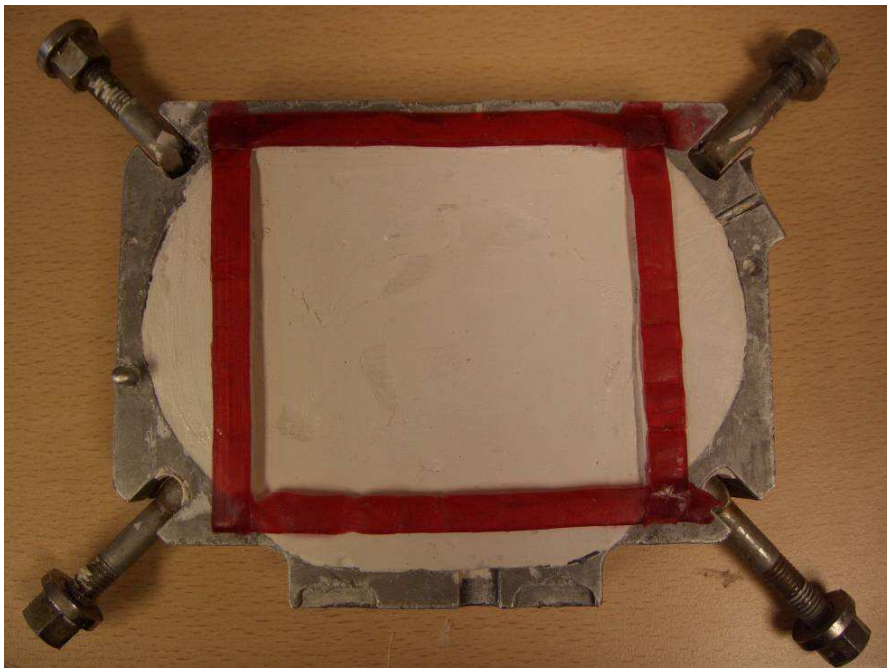
Samples were produced using PalaXpress® autopolymersing acylic resin. A reservoir was produced for introduction of unpolymerised resin mixture using ribbon wax and half of the flask previously prepared for use with conventional PMMA processing technique (stone investment material base) (Figure 2.15). Polymer/monomer was mixed according to manufacturer's instructions in a ratio of 10g powder: 7ml liquid **(**Pala Xpress® autopolymersing acrylic resin) and poured into the prepared reservoir. This was allowed to reach a viscous consistency on the laboratory bench at room temperature. At this stage, the flask half with resin in reservoir was placed in a water bath at 55^{o}C for 15 minutes.

2.2.4 Finishing of acrylic samples

Samples of PMMA resin produced via the three processing methods were adjusted to remove excess material flash. Acrylic sheets were trimmed into 10mm squares using a high speed handpiece and sterile diamond bur. Samples were stored separately in labelled containers under sterile water with a trace of chlorhexidine (Corsodyl™, GlaxoSmithKline, Middlesex, UK) until required. Immediately prior to use, samples were sterilised using an ultraviolet light unit (Philips TUV6 Germicidal lamp, Philips, UK) with UV Radiation emitted at 253.7nm for 10 minutes.

Figure 2.15 Mould for production of self cured acrylic samples

Reservoir prepared in stone investment for introduction of self cure PMMA resin



2.3 Profilometer Study

A profilometer was used to assess the unfinished surface of three randomly selected representative denture acrylic samples for each of the studied processing methods. This procedure was carried out by Dr. Liam Cunningham, Research Associate, Physics and Astronomy Department, University of Glasgow. The instrument used was a white light interferometer (Wyko®, NT1100, Veeco UK, Cambridge, UK). This uses a standard halogen microscope bulb and uses the following roughness parameters and recording profiles:

$$Ra = 1/n \Sigma (X_i - X)$$

Ra: This is the arithmetical mean roughness of the sum of values of the roughness profile within the set measuring length.

and

$$Rq = \sqrt{[1/n \Sigma (X_i - X)^2]}$$

Rq: Also known as the RMS roughness (Roughness Measurement System).

n is the number of pixels
X is the mean height
X_i is the height of an individual pixel

Both of these give a value of the surface roughness.

Profilometer testing was undertaken to establish variations in surface finish between the three studied acrylic types. In order to obtain an accurate evaluation of the surface roughness of the specimens, multiple traces were carried out. A scan of 2450 × 2100 µm was taken for two samples from each

of the three sample groups. Magnification was set to 10.3 ×. Processing options were removed, with no sample tilt or filtering set. A surface data map, indicating peak and valley depth over the sample surface was produced. This was colour coded, representing a spectrum of peak height (10.9µm, red) to minimum trough depth (23.5µm, blue).

2.4 SEM of denture base materials

Denture base materials were prepared for scanning electron microscope (SEM) examination by attachment of samples to stubs with double sided conductive tape and gold-palladium sputter coating. This was carried out at the microscopy suite (University of Glasgow, UK). Gold sputter coating takes place in an argon filled chamber prior to SEM examination (Erlandsen *et al.*, 2004). Samples were viewed under a JEOL JSM-6400 scanning electron microscope (JEOL Ltd., Tokyo, Japan). Preparation described and SEM examinations were performed by Mr. Anto Jose, PhD Student, Microbiology Department, University of Glasgow Dental School.

2.5 Microbiology

2.5.1 Candida albicans strains and identification

9 clinical isolates from a study previously carried out in the same laboratory were studied (Coco *et al.*, 2008). To allow identification during this study, the clinical *Candida* isolates were named DBS1, DBS2, DBS3, DBS4, DBS5, DBS6, DBS7, DBS8 and DBS9. Single morphotypes were identified using the API 32 C biochemical testing panel, according to the manufacturer's instructions (BioMerieux UK Ltd, Basingstoke, UK). The system consists of a single-use disposable plastic strip with 32 wells containing substrates for 29 biochemical assimilation tests (carbohydrates, organic acids, and amino acids), one susceptibility test (cycloheximide), one colorimetric test (esculin) and a negative control. Each well was then scored either positively or negatively, depending on turbidity for each specimen. Results were transformed into numerical biocodes, and the isolates identified through the use of the ID 32C Analytical Profile Index. After identification, isolates were stored on Sabouraud dextrose agar plates (Oxoid, Basingstoke, UK) at 4^{o}C.

2.5.2 Growth conditions and standardisation of Candida albicans

C. albicans clinical isolates were grown on Sabouraud dextrose agar plates at 37^{o}C overnight. The 9 clinical isolates described above were then transferred to 10 mL of yeast peptone dextrose (YPD) in a shaker and grown at 30^{o}C overnight. Cells were centrifuged and washed with 10 mL sterile PBS before resuspending in sterile PBS. Cells were then counted using a Neubauer haemocytometer and adjusted to 1.0×10^{8} in RPMI 1640 medium (Sigma,

Aldrich, UK). All procedures were carried out in a laminar flow cabinet (Microflow Biological Safety Cabinet, LabPlant UK Ltd, Filey, UK).

2.5.3 Candida albicans adhesion

Sterile PMMA samples, as described in Section 2.2, from the three processing method groups (n=4 in each group) were placed into appropriately labelled petri dishes (Sterilin®, Sterilin Ltd., London, UK) with unfinished surfaces facing upwards. For each of the 9 *C. albicans* strains studied, 100 μl of each strain was pipetted onto the surface of each acrylic sample. Plates were then incubated at 37^{o}C for 1 hour. Acrylic samples were removed and washed 3 times in sterile PBS to remove non-adherent cells. Acrylic samples were then placed into separate appropriately labelled bijoux tubes containing 2ml sterile PBS and sonicated to remove adherent *C. albicans* cells for 5 minutes.

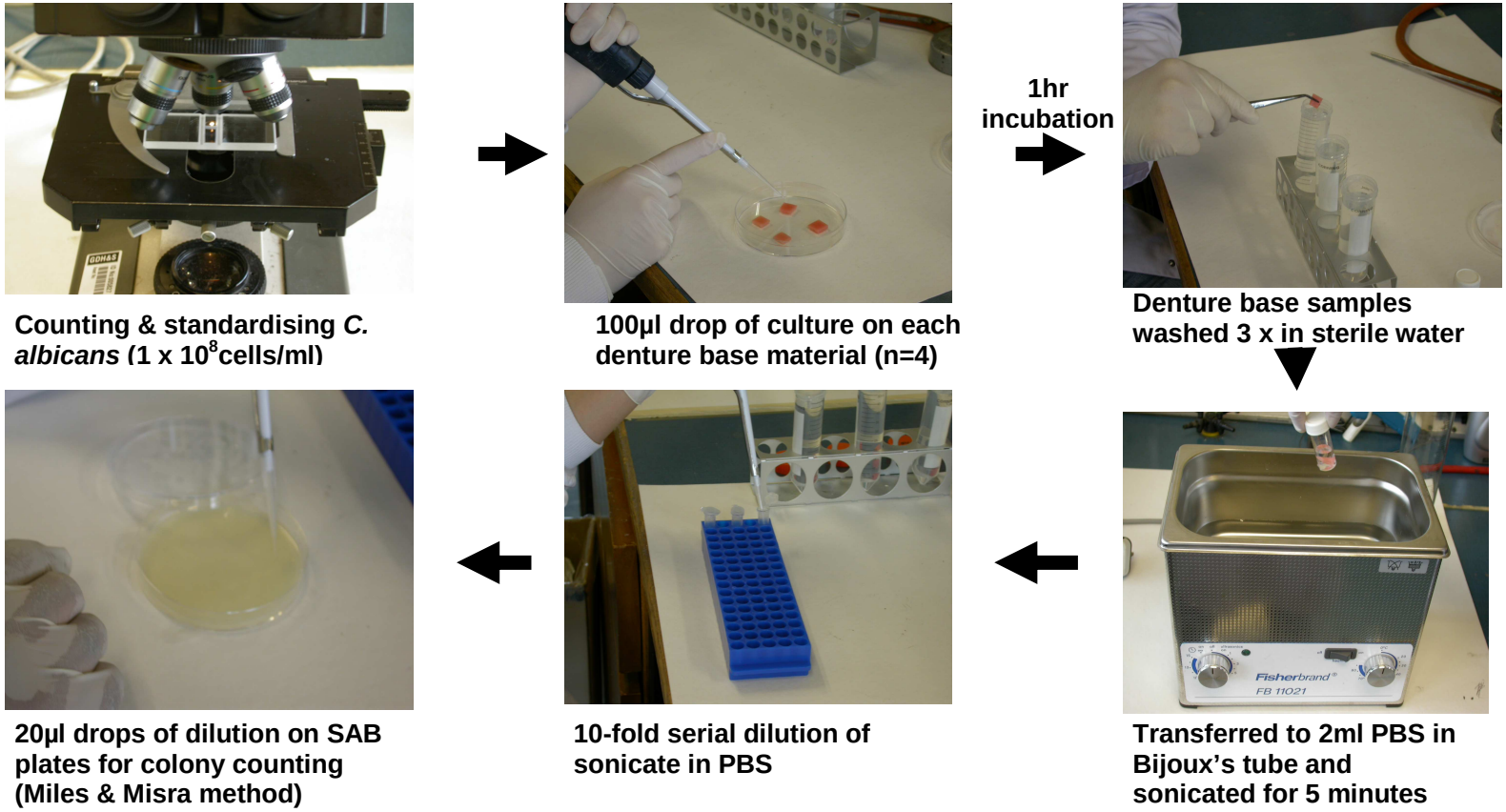
A ten-fold serial dilution was performed until a concentration of 10^{-3} was obtained. Dilutions of 10^{-1}, 10^{-2} and 10^{-3} were plated onto appropriately labelled Sabouraud dextrose agar plates in 5 drops of 20μl and incubated overnight at 30^{o}C. This was to encourage slow growth of viable cell colonies. Colonies were quantified using the Miles and Misra plate count method (Miles, 1938). This process is demonstrated in Figure 2.16.

2.5.4 Candida albicans biofilm formation on denture base materials

One sterile sample of PMMA resin produced by the three different processing techniques, described in Section 2.2, was placed into a 12 well tissue culture plate (Corning, NY, USA). 1mL of standardised cells suspended in RPMI 1640 was added to each well. The plate was incubated at 37^{o}C overnight. Acrylic samples were washed three times in sterile PBS to remove non-adherent cells. This was performed by dipping each acrylic sample into three consecutive tubes of sterile PBS.

Figure 2.16 Adherence testing

Candida albicans adherence testing demonstrated as step-wise process



2.5.5 SEM examination of denture base material and adherent Candida albicans cells

Denture base materials with adherent candida cells were fixed in 2% *para*-formaldehyde, 2% glutaraldehyde, 0.15M sodium cacodylate and 0.15% Alcian Blue (pH 7.4). The fixative was applied to the wells containing Thermanox™ (Nunc Inc, UK) coverslips for 2 hours. Paraformaldehyde was prepared at 60℃ with 80ml distilled water and 8g of paraformaldehyde using a hot plate and magnetic stirrer, sodium hydroxide was added gradually until the solution cleared. PH was then adjusted to 7.2 with 1 M HCl.

Following fixation, the fixative solution was removed using a pipette and 0.15M sodium cacodylate buffer was added to the samples. Samples were then at 4^{o}C until processing.

The 0.15M sodium cacodylate buffer was removed using a pippette. Samples were then washed for 5 minutes with fresh buffer three times, to remove any remaining gluteraldehyde. To prevent exposing the samples to air, a thin layer of buffer solution was left in the wells at all times. A solution of 1% osmium tetroxide (OsO_4) was then added to an equal volume of 0.15M sodium cacodylate buffer. This solution was added to the samples and incubated for 1 hour. Samples were then rinsed three times with distilled water for 10 minutes to remove osmium.

0.5% aqueous uranyl acetate solution was then prepared and kept covered. 0.5% aqueous uranyl acetate was then added to the wells and incubated in

the dark for 30 minutes at room temperature. Spent uranyl acetate was removed and samples rinsed with distilled water.

Samples were then dehydrated in an ascending ethanol series. Dried absolute alcohol was prepared with a 3A molecular sieve (Sigma, Aldrich UK).

Hexamethyldisilazane (HMDS) (TAAB, Berks, UK) was then added to two glass Petri dishes. Samples were transferred from the original 24 well plate into the first Petri dish of HMDS for 5 minutes, and then transferred to a second dish for a further 5 minutes. Samples were removed from the second dish and placed in a new 24 well plate lined with filter paper. The plate was then placed in a dessicator overnight to allow evaporation and drying of samples.

The samples were prepared for SEM examination by attachment to stubs with double sided conductive tape. Gold-palladium sputter coating was then carried out at the microscopy suite (University of Glasgow). This was undertaken in an argon filled chamber prior to scanning electron microscope examination (Erlandsen *et al.*, 2004). Samples were viewed under a JEOL JSM-6400 scanning electron microscope. As in Section 2.4, preparation described and SEM examination was carried out Mr. Anto Jose, PhD Student, Microbiology Department, University of Glasgow Dental School.

2.6 Statistical analysis

Statistical analysis was performed on all the data collected for dimensional and Candidal adherence testing using GraphPad Prism® (GraphPad Software Inc., La Jolla, USA), SPSS® (SPSS Inc., IBM, Chicago, USA) and Microsoft Excel© (Microsoft® Corporation, USA) software.

Quantitative analysis was performed to check for statistical differences between the linear dimension measurements. Percentage change from control was calculated for each specimen. After checking the data was normally distributed, a one-way analysis of variance (ANOVA) was used to compare conventional pressure packed, injection moulded and control groups. An Independent t-test was subsequently used to compare conventional pressure packed and injection moulded sample groups. A significance level of $p<0.05$ was set. The statistical analysis package used only indicates in the output where the largest differences between the data exist, therefore only these have been included in the following results, Table 4.1.

For internal adaptation testing of conventional pressure packed and injection moulded material sample groups, means and standard deviations were calculated. The difference between the means of the two groups and standard deviation was then calculated. For both data groups an unpaired t-test was carried out. Statistical significance was identified at $p<0.05$. An F-test was used to calculate variances within the data.

Quantitative analysis of yeast cell attachment from all nine strains of *C. albicans* to self cured, conventional pressure packed or injection moulded acrylic resins was performed. This was to assess statistical differences present between attachment capacity of each strain tested within each acrylic resin group after 1 hour. In order to allow parametric testing of the data to be carried out, logarithmic transformation was performed. Quantitative analysis of the ability to attach to SC, CPP or IM materials was subsequently carried out on the mean of all nine isolates. Statistical significance was identified at $p<0.05$.

Chapter 3:

RESULTS

3.1 Dimensional accuracy

3.1.1 Linear dimensional measurement

As discussed in Section 1.6.2, the dimensional inaccuracy of poly (methylmethacrylate) is one of the well recognised problems associated with the material. Inaccuracies may exist in the material owing to its polymerisation shrinkage and high co-efficient of thermal expansion. The injection moulding system has been developed to compensate for polymerisation shrinkage by the continuous introduction of PMMA resin during the polymerisation procedure. This study investigated the dimensional accuracy of injection moulding compared to conventional pressure packing.

As was described in Section 2.1.6, dimensional measurements were sequentially recorded between six selected reference points on processed denture bases. Dimensional measurements for the 2 acrylic test groups, compared with a cobalt chrome control, are detailed in Table 3.1.

For the AB dimension, the cobalt chromium denture base control measured 20.20mm. The conventionally processed denture base measured 0.06mm longer than control and the injection moulded samples 0.12mm longer than control. No statistically significant difference existed between CPP or IM materials when compared with control (p=0.15).

The BC dimension in the control measured 20.62mm. The conventionally processed denture base measured 0.1mm less than this and the injection

moulded samples 0.08mm less, than that of control BC measurement. No statistically significant difference existed between CPP or IM materials when compared with control (p=0.6).

For the CD dimension, the control measured 22.55mm. However, the conventionally processed denture base measured 0.21mm less and the injection moulded samples 0.10mm less than control CD measurement. No statistically significant difference was determined between CPP material samples when compared with control (p=0.97).

The DE dimension in the control measured 23.18mm. The conventionally processed denture base measured 0.11mm less and the injection moulded samples 0.01mm longer than control DE measurement. A statistically significant difference was determined between CPP material samples when compared with control (p=0.02).

The EF dimension in the control measured 20.74mm. The conventionally processed denture base measured 0.08mm less and the injection moulded samples 0.65mm longer than control in EF dimension. A statistically significant difference was determined between IM material samples when compared with control ($p<0.001$).

The FA dimension the control measured 21.28mm. The conventionally processed denture base measured 0.04mm longer than this and the injection moulded samples 0.11mm longer than control in FA dimension. A statistically

significant difference was determined between IM material samples when compared with control ($p=0.03$).

Measurements and results of ANOVA comparing Control (C), Injection moulded (IM) and Conventional (CPP) acrylic samples are shown in Table 3.1. Measurements and results of independent t-test comparing IM and CPP acrylics are provided in Table 3.2.

Table 3.1 Dimensional measurements for sample groups and control with ANOVA results. Statistically significant differences were found between IM and CPP materials when compared to control in DE, EF and FA dimensions. These values are highlighted in **bold.**

Reference Measurement	Experimental Group	N	Mean (mm)	SD (mm)	95% Conf interval	Min (mm)	Max (mm)	p-value
AB	C	1	20.20					
AB	CPP	20	20.26	0.09	20.22-20.30	20.1	20.4	
AB	IM	20	20.32	0.12	20.26-20.38	20.2	20.7	0.15
BC	C	1	20.62					
BC	CPP	20	20.52	0.87	20.48-20.56	20.35	20.69	0.60
BC	IM	20	20.54	0.12	20.49-20.60	20.33	20.76	
CD	C	1	22.55					
CD	CPP	20	22.34	0.27	22.21-22.47	21.32	22.62	0.97
CD	IM	20	22.46	0.44	22.25-22.66	20.61	22.70	
DE	C	1	23.18					
DE	CPP	20	23.07	0.14	23.00-23.13	22.81	23.26	**0.02**
DE	IM	20	23.19	0.10	23.13-23.24	22.90	23.40	
EF	C	1	20.74					
EF	CPP	20	20.66	0.72	20.62-20.69	20.55	20.83	
EF	IM	20	21.39	0.10	20.84-20.94	20.74	21.03	**<0.001**
FA	C		21.28					
FA	CPP		21.32	0.09	21.28-21.36	21.15	21.45	
FA	IM		21.39	0.10	21.35-21.44	21.26	21.55	**0.03**

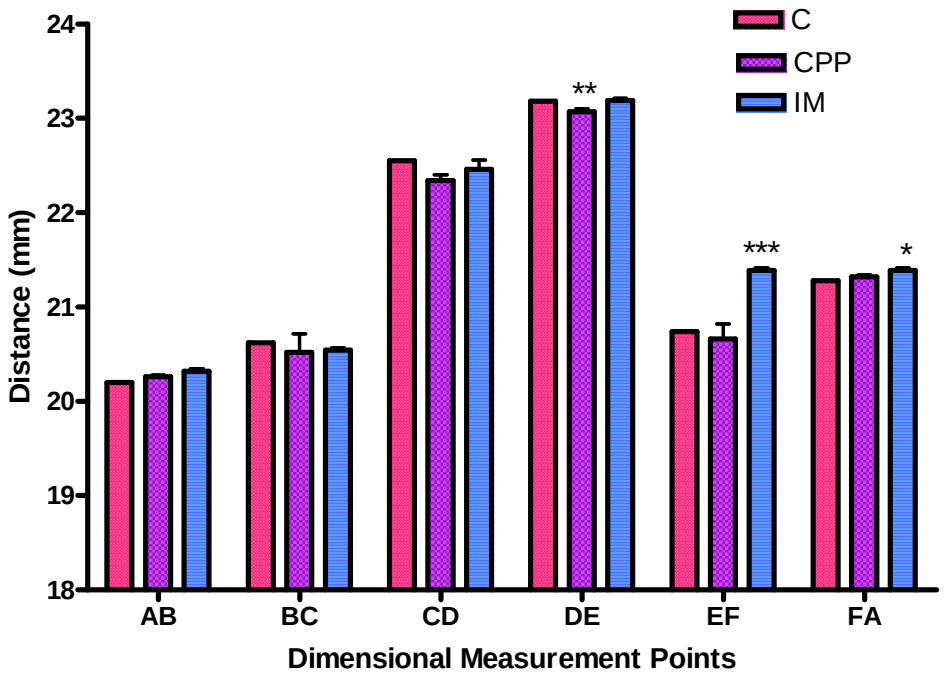
Only most significant difference displayed in output

Table 3.2 Dimensional measurements for acrylic sample groups with independent t-test results

Significant differences were detected between CPP and IM acrylic samples in DE, EF and FA dimensions. These values are highlighted in **bold.**

Reference Measurement	Experimental Group	N	Mean (mm)	SD (mm)	95% Conf interval	Min (mm)	Max (mm)	p-value
AB	CPP	20	20.26	0.09	20.22-20.30	20.1	20.4	
AB	IM	20	20.32	0.12	20.26-20.38	20.2	20.7	0.08
BC	CPP	20	20.52	0.87	20.48-20.56	20.35	20.69	
BC	IM	20	20.54	0.12	20.49-20.60	20.33	20.76	0.56
CD	CPP	20	22.34	0.27	22.21-22.47	21.32	22.62	
CD	IM	20	22.46	0.44	22.25-22.66	20.61	22.70	0.35
DE	CPP	20	23.07	0.14	23.00-23.13	22.81	23.26	
DE	IM	20	23.19	0.10	23.13-23.24	22.90	23.40	**0.01**
EF	CPP	20	20.66	0.72	20.62-20.69	20.55	20.83	
EF	IM	20	21.39	0.10	20.84-20.94	20.74	21.03	**<0.001**
FA	CPP		21.32	0.09	21.28-21.36	21.15	21.45	
FA	IM		21.39	0.10	21.35-21.44	21.26	21.55	**0.02**

Figure 3.1 Analysis of dimensional differences between defined points on denture base samples following conventional pressure packed and injection moulded processing. Statistically significant differences were present between control and conventional samples in DE dimension ($p<0.01$) and control and injection moulding in EF ($p<0.001$) and FA ($p<0.05$) dimensions.



* p<0.05; ** p<0.1; *** p<0.001

3.1.2 Base plate adaptation: weighed siloxane film

This study also investigated the overall sample denture base adaptation using a weighed siloxane film to determine the discrepancy between master cast and impression surface of denture base samples, as is described in Section 2.1.7.1. The mean weight of vinyl polysiloxane (VPS) for each sample material group, with standard deviations, is shown in Table 3.3. The corresponding data are displayed in Figure 3.2. The mean weight of VPS present for the conventional sample group was 1.166g with a standard deviation of 0.23g. The mean weight of VPS present for the injection moulded sample group was 1.02g with a standard deviation of 0.20g. The mean difference between the sample groups was 0.1422g with a standard deviation of 0.06g.

A one way ANOVA was performed on the data, indicating the presence of a statistically significant difference between means of the two sample groups ($P<0.05$).

These data indicate that conventional, pressure packed PMMA samples contained a greater weight of VPS material between denture base and master cast than injection moulded samples.

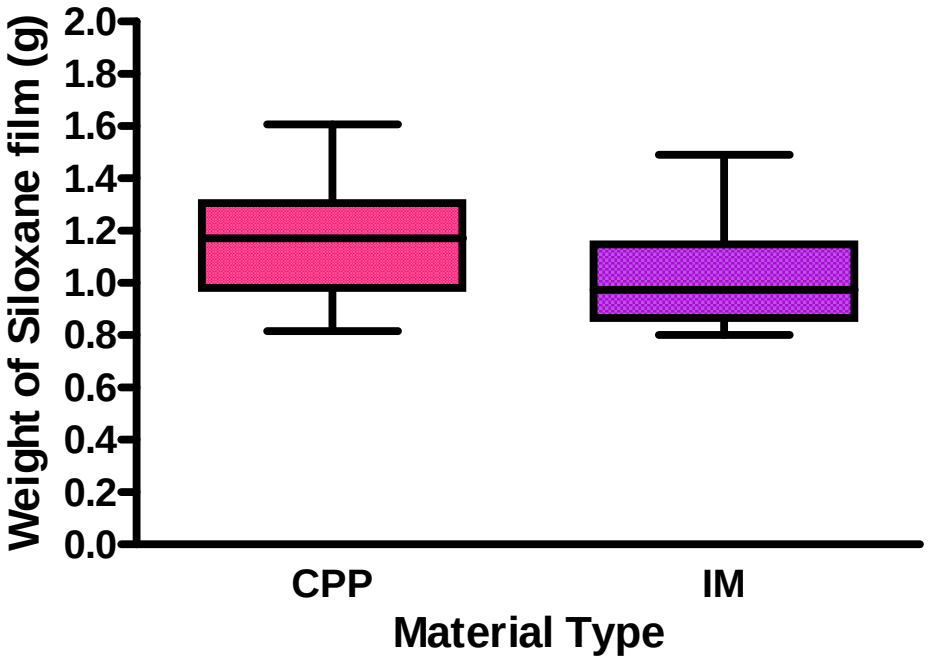
Table 3.3 Mean weight of siloxane film indicating internal adaptation

A greater weight of material was present for conventionally processed samples than for injection moulding. A statistically significant difference existed between means (p=0.042). Injection moulded samples were found to be more dimensionally accurate (p<0.05).

Material	**Mean (g)**	**SD**	**95% CI**
CPP	1.166	0.2267	1.06 -1.27
IM	1.024	0.2005	0.93 -1.12
Mean Difference	0.1422	0.067	0.005 - 0.27

Figure 3.2 Internal adaptation testing for conventional and injection moulded samples

The whisker box plots demonstrate the spread of siloxane film weight for CPP and IM materials. The plots show a lower weight of siloxane film was present between denture base and master cast for injection moulded samples. A statistical difference was detected between the means (*p<0.05).



3.1.3 Discussion

It is widely accepted that, at least in part, the successful function of a complete denture is dependent upon its accuracy of fit (Greener, 1972). Dimensional inaccuracies, as a result of polymerisation shrinkage, have been demonstrated to cause clinical instability of the resulting denture base against the denture bearing tissues (Barsoum *et al.*, 1968). This may in turn lead to pain during function as a result of uneven loading of the denture base. It has been previously stated that "it is axiomatic that the more dimensionally accurate and stable a material is the more retentive and stable will be the denture" (Zissis *et al.*, 1991). Denture base adaptation depends upon a number of factors, both clinical and laboratory in nature, as well as the dimensional accuracy of the material from which it is constructed (Zissis *et al.*, 1991). Injection moulding has been demonstrated in a number of studies to produce better denture base adaptation than the conventional denture processing technique (Ganzarolli *et al.*, 2007; Parvizi *et al.*, 2004). This study investigated 2 components of dimensional accuracy of PMMA denture bases produced by conventional pressure packing or injection moulding techniques. Linear dimension, as described in a number of studies (Huggett *et al.*, 1992; Nogueira *et al.*, 1999; Parvizi *et al.*, 2004) and overall baseplate adaptation (Ganzarolli *et al.*, 2007) were evaluated.

In order to allow accurate comparison of injection moulded and conventional pressure packed acrylic denture bases, a re-useable "master" mould was produced. Similar methodology has been used previously (Huggett *et al.*, 1992). A number of studies have reported that dimensional changes do not

occur evenly over the entire denture base (Anthony, 1959; Huggett *et al.*, 1992; Nogueira *et al.*, 1999; Parvizi *et al.*, 2004; Tezvergil, 2003). Although much data has been published on the dimensional accuracy of injection moulding techniques (Ganzarolli *et al.*, 2007; Garfunkel, 1983; Keenan *et al.*, 2003; Ono *et al.*, 2004; Parvizi *et al.*, 2004), there has so far been no comment, in any of the published studies, on the relationship between the location of the injection moulding inlet and dimensional change. Dimensional changes did not occur uniformly over the sample base. For injection moulded samples, the dimensional changes were dependent on the thickness of the base at the specific area studied and also on distance of the area from the PMMA injection inlet. Dimensional increases in the injection moulded samples compared to control existed between the points furthest from the PMMA injection inlet. It could be theorised that these changes are attributable to expansion of the mould caused by pressure from the injected acrylic resin. By the time the continuously polymerising PMMA resin reaches the most distal point of the denture base, it may have reached a greater viscosity. It is possible that the viscous resin, when pressure is applied to it, may exert enough force to cause some distortion of the mould. This could explain why in the areas of the denture base furthest from the injection inlet, greater dimensional inaccuracies existed than for the conventional technique. Further research is required to investigate the possible causative factors behind these dimensional changes.

The increase in dimensions observed may also be the result of water sorption. Hugget *et al*. (1992) demonstrated an increase in dimensions in injection

moulded PMMA samples after 28 days of storage in water. Similarly, water sorption may account for some of the dimensional increases observed in the samples produced and described herein. However, it is of note, that although not statistically significant, an increase in dimension FA was recorded for the conventional samples in this study. Both conventionally processed and injection moulded samples in studies by Cal *et al.* (2000) and Keenan *et al.* (2003) were found to have absorbed water. Samples in these studies showed an increase in weight, mostly occurring during the first 14 days of immersion. This value varied depending on material filler content. Water sorption was shown to decrease with an increase in filler content (Cal *et al.*, 2000). A similar effect was reported by Tezvergil *et al.* (2003), although they also demonstrated reduced water sorption in injection moulded samples compared to conventional (Tezvergil, 2003).

The use of low viscosity impression material to evaluate accuracy of denture base fit has been used in several studies (Barco *et al.*, 1979; Frejlich *et al.*, 1989; Takamata & Setcos, 1989). The vinyl polysiloxane film records any space between the resin base and the master cast over the entire impression surface area. In contrast to measuring the 'misfit' at selected points on the denture base, this methodology records the 3D distortion of the denture base (Ganzarolli *et al.*, 2007). This technique gives an indication of mass/volume of contained silicone material. As a greater weight of material has a greater volume, density being constant, it is possible to compare surface adaptation of sample material groups. This overall adaptation testing is important, as dimensional changes do not occur in equal volume across the entire denture

base (Shukor *et al.* 2006, Nogueira *et al.* 1999). The results of this study demonstrated that injection moulded denture bases have superior internal surface adaptation in comparison to conventional pressure packed acrylic resin (P=0.042).

This methodology does, however, have limitations. Even for the most accurately fitting specimen, some residual material will always remain between the denture base and the master cast surface. The volume remaining is dependent on the volume and viscosity of the material, the rate and force with which it is displaced, and the opening from which it may escape amongst other factors. The same volume of light bodied vinyl polysiloxane material was used to coat the impression surface of each denture base sample and samples seated with identical 5kg loading on to the master casts. The volume of material remaining owing to the nature of the methodology should therefore have been as similar as possible for each sample. This methodology is identical to that used by Ganzarolli to assess dimensional accuracy of injection moulding, microwave and conventional processing techniques (Ganzarolli *et al.*, 2007).

Although greater dimensional inaccuracies were present in the conventional pressure packed acrylic samples, a discrepancy was still detected between the impression surfaces of injection moulded denture bases and the master cast surface. It is difficult to stipulate how much of this is attributable to errors in the methodology described above. However, the procedure was performed

in triplicate. Thereby, any variability due to operator error in the procedure should have minimised.

For denture base samples produced via the injection moulding procedure, the most dimensional inaccuracies were detected between points on the denture base furthest from the injection moulding inlet. The security of a complete denture depends primarily on close peripheral contact between the denture and its supporting tissues. In particular at the vital palatal border (Lamb *et al.* 2006). Therefore, when using this method regions of the base where dimensional accuracy are of greatest importance, such as the post dam, should perhaps be invested closest to the injection inlet. The thickness of the denture base is also important when considering likely volumetric change (Sadamori *et al.*, 1994; Sadamori *et al.*, 1997). It is necessary to balance the material bulk required to increase strength (Beyli & von Fraunhofer, 1981) with the likely increase in dimensional change. Increasing the thickness of the denture base in the palatal region may also negatively affect patient tolerance by decreasing thermal sensation, and converging on tongue space during function, potentially affecting speech (Harley, 1972).

Linear measurements indicated there to be a statistically significant difference between the two sample groups in three of the measured dimensions (Figure 3.1). The conventional processing method was determined as more accurate in two of these. This is contrary to the results of the overall base plate adaptation testing, which indicated better internal adaptation for the injection moulded samples (Table 3.3, Figure 3.2). Although statistically significant

differences existed, the actual dimensional differences between the two sample groups were extremely small in the case of all linear dimensions (Table 3.1). It is debatable whether the differences observed between conventional pressure packed and injection moulded PMMA samples would actually be clinically significant, as dimensional differences amounted to a maximum of 0.21mm compared to control. As the denture bearing mucosa is compressible and the palatal seal achieved largely dependent on the prepared post dam (Krysinski & Prylinski, 2007; Lamb *et al.*, 2005), small dimensional changes, as demonstrated here, may be of little clinical relevance to the success or failure of the material as a denture base.

3.2 Surface characteristics of denture samples

3.2.1 Profilometry

The surface characteristics of a prosthodontic material, and in particular, surface roughness influences the success of the material as a denture base. Profilometer testing was therefore performed, as described in Section 2.3, to determine surface characteristics of studied materials.

Profilometer testing determined a high level of variation between samples within conventional pressure packed and injection moulded groups. There was less variation in the Ra and Rq values present between samples of self cured resin. However, self-cure resin surfaces were determined to posses more irregular surface characteristics than surfaces of conventional or injection moulded groups. Results are presented in Table 3.4 (Ra) and Figure 3.3 (Rq).

Representative surface maps for the three material sample groups are presented in Figure 3.4. These confirm the rougher surface present in the self cured acrylic resin surface (C). The darker blue-black surface map indicates the highest Ra/Rq values or greatest peak-trough distance for the material surface. The conventionally processed sample (B) appears more orange-yellow on the surface map image, indicating the presence of a smoother surface and lower Ra/Rq values compared to either injection moulded (A) or self cured sample (C). However, on closer examination, the conventional

material sample (A) and injection moulded sample (B) exhibit similar Ra/Rq values overall.

Table 3.4 Ra values for three PMMA sample groups

Greater Ra values were present for samples within the self cured acrylic resin (SC) group. This indicates that self-cured samples had more irregular surface morphology than either conventional or injection moulded samples. The Ra values for conventional pressure packed (CPP) and injection moulded (IM) samples were found to be similar; injection moulded samples possessed slightly higher Ra values than conventional samples, so may be considered to have more irregular surface characteristics.

Material Type	**Sample 1 Ra (µm)**	**Sample 2 Ra (µm)**	**Sample 3 Ra (µm)**	**Average Ra (µm)**
CPP	1.80	3.53	2.50	2.61
IM	1.82	3.99	2.94	2.92
SC	3.51	3.54	2.82	3.29

Figure 3.3 Rq values for 3 samples within each of the 3 studied material groups

Greater Rq values were present for samples within the self cured (SC) acrylic resin group. This indicates that self cured samples were found to be rougher then either conventional pressure packed (CPP) or injection moulded (IM) samples. Injection moulded samples possessed slightly higher Rq values than conventional samples.

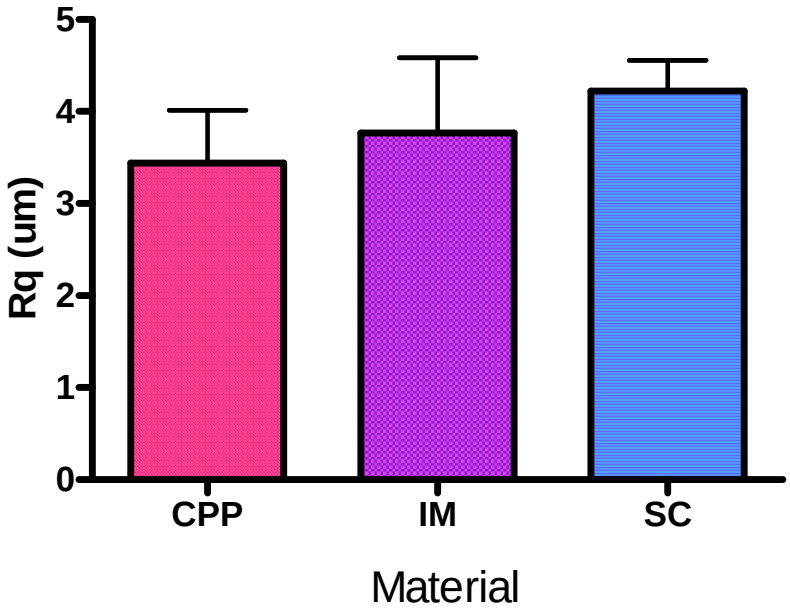
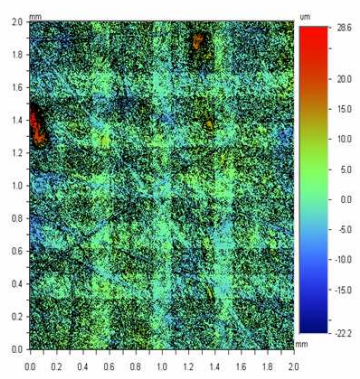


Figure 3.4 Profilometer scans of unfinished sample surface of three denture acrylics

Representative profilometer scans of (A) injection moulded, (B) conventional pressure packed and (C) self cured acrylic resin samples. Higher Ra/Rq values are present for self cured sample surface map indicating rougher surface (increased peak-trough distance).

(A)

*



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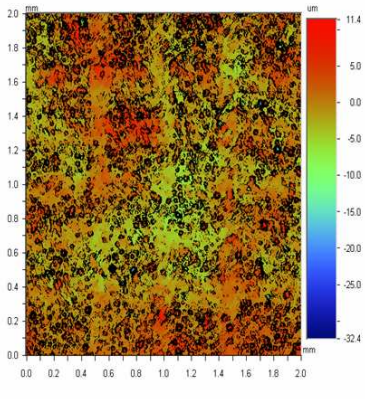
(B)

*



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(C)

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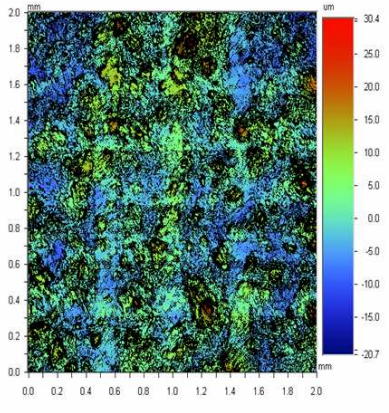


**

*** Area of sample studied (nm)** ****Maximum peak-trough distance (µm)**

3.2.2 Scanning electron microscopy

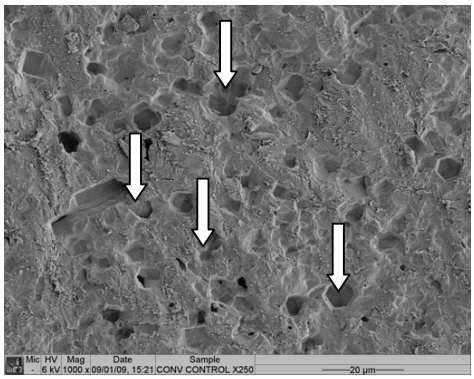
As has been previously described in Section 1.4.8, material surface morphology is known to affect success of the denture base. Along with the profilometer scans, scanning electron microscopy (SEM) was performed on the three sample groups. This was undertaken to examine in more detail surface differences between PMMA types.

Figure 3.5 illustrates the SEM images taken of the different material samples. These revealed variations in the surface morphology of all three sample groups. Samples produced via conventional pressure packing demonstrated more porosity, whereas samples produced via the injection moulding method appeared to show the presence of surface cracks. The SEM images of the self cured acrylic samples appear to show a bubbled surface morphology.

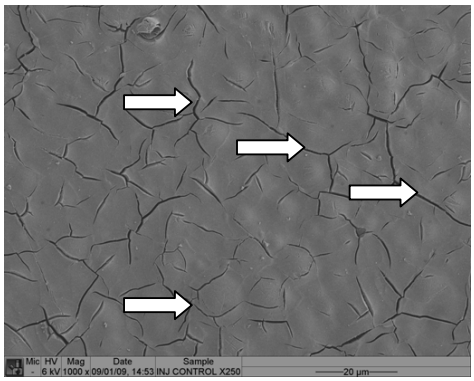
Figure 3.5 Scanning electron microscopy of three denture acrylics

Images of (A) conventional pressure packed (B) injection moulded and (C) self cured acrylic resin samples (×250). Note apparent porosity of conventionally processed sample. Cracks are evident in the surface of the injection moulded sample. Bubbling is evident on the surface of the self cured sample.

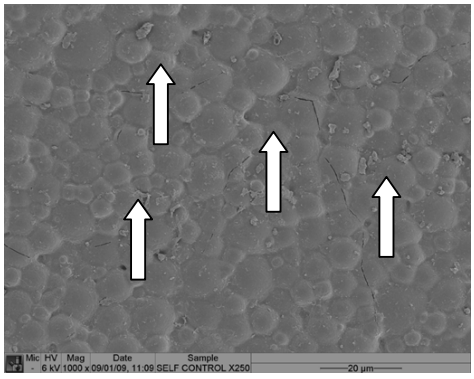
(A)



(B)



(C)



3.2.3 Discussion

Profilometery determined a high level of variation between samples within conventional and injection moulded groups. There was more consistency in the Ra and Rq values obtained for self cured resin samples. However, self-cure resin surfaces were found to be 'rougher' than surfaces of conventional or injection moulded groups. This is in line with previous data pertaining to the surface characteristics of self cured acrylic resin when compared to other processing methods (Kuhar & Funduk, 2005). Anusavice (2003) stated that a reduction in surface roughness may help to reduce the destructive effects of abrasion on the soft tissues by reducing friction. Research indicates that increased surface roughness characteristics result in increased staining and also increased difficulty in removing such contamination (Boyd, 2001). A denture base material with increased abrasion resistance should therefore provide a more aesthetic, stain resistant and microbiologically favourable surface. This is an important factor for clinicians to consider when deciding between polymeric denture base materials. Although only a small number of material samples were examined by profilometry in this study, preliminary results may suggest that injection moulded or conventionally processed PMMA acrylics may be a more suitable choice as a denture base material than currently available self cured materials.

The profilometer scans only a very small surface area of each resin sample (2450 x 2100µm). Variation may therefore be more dependent upon the surface against which that particular area of acrylic was processed than the method by which it was processed.

Although the same type of dental stone was used, and mixed under vacuum conditions, in the same quantity for flasking of each material type, separate batches were mixed to produce the investment for conventional and injection moulded material samples. This may account for some of the surface differences between these sample groups.

Kuhar and Funduk also examined self cured, conventional pressure packed and injection moulded acrylic resin surfaces (Kuhar & Funduk, 2005). Specimens from the 3 acrylic groups were examined both pre-and post finishing and polishing in their study, with profilometry and scanning electron microscopy. The authors determined that “surface roughness was influenced to the greatest extent by finishing and polishing procedures and to a lesser extent by the acrylic resin material.” In line with the results presented in this thesis, they established there to be increased surface roughness values for the SC, when compared to both CPP and IM specimens. However, their results did not indicate the presence of any statistically significant differences between CPP and IM resin samples.

Self cured samples were produced with only one surface in contact with investment. Only this material surface was examined. This may have had some influence on the difference seen in the material produced via this method. As polymerisation of PMMA resin occurs bubbles of polymer vapour are released (Singh & Gupta, 2009). These may result in porosity, and surface irregularities. This may explain the surface roughness seen in the self

cured samples. This ‘bubbled’ effect can be seen more clearly in the SEM images taken of the self cured material surface. In this study it was the unfinished resin surface under examination. After polishing, self cured resin samples have been demonstrated to be porous on SEM analyses elsewhere (Kuhar & Funduk, 2005). This may be due to the fact that finishing the sample may expose the porosity contained within the material. The authors observed large pores in the self cured samples in their study (Kuhar & Funduk, 2005). Therefore, the use of self-curing acrylic techniques is likely to increase microbial adhesion and biofilm formation on the material surface, negatively influencing the properties of the material.

3.3 Interaction of *Candida albicans* with denture materials

3.3.1 Candida albicans attachment to three different denture base acrylics

Adherence of *C. albicans* to denture base acrylics, and subsequent biofilm formation, plays an important role in the development of clinical denture induced stomatitis. Adherence testing was carried out as described in Section 2.5 for all three studied groups; self curing (SC), injection moulding (IM) and conventional pressure packed (CPP). This aimed to determine if significant variation in adherence of different *C. albicans* clinical isolates to the material surface existed between processing methods.

To simulate the clinical situation as closely as possible, PMMA resin sample surfaces were not polished prior to use in the candidal adhesion experiment. Accordingly, some variation was anticipated between the samples. It is widely acknowledged that the smoother a surface is the less likely it is to accumulate plaque and other biological material (Karaagaclioglu *et al.*, 2008; Nevzatoğlu *et al.*, 2007; Pereira-Cenci *et al.*, 2007). Therefore, the conditions present on a smooth surface are less suitable for the proliferation of *Candida* species and other harmful microorganisms. This was the rationale for undertaking surface roughness investigation.

The results of *C. albicans* adhesion for SC, IM and CPP PMMA materials are presented in Figure 3.6. No significant differences were observed between

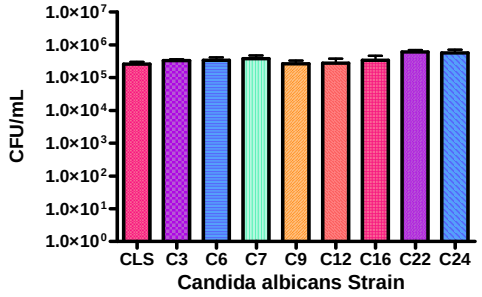
attachment of nine different *C. albicans* clinical strains when tested independently against each individual material ($p>0.05$).

However, when the mean attachment of all nine *C. albicans* strains to the three sample groups were compared, these data indicated the presence of a statistically significant difference in attachment capacity between conventional pressure packed (CPP) and self cured (SC) sample groups ($p<0.05$). Self cured PMMA resin samples were shown to exhibit significantly less candidal attachment after 1 hour adhesion than conventionally processed samples, which is illustrated in Figure 3.7.

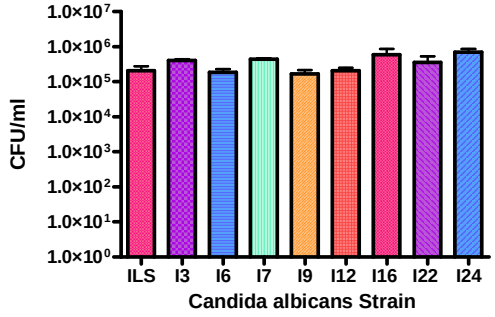
Figure 3.6 Attachment of individual *Candida albicans* strains to different denture acrylics

Colony forming unit attachment of nine strains of *C. albicans* on three denture materials: (A) SC, (B) CPP and (C) IM. No significant differences were observed between the attachment of these strains to each of the materials examined ($p>0.05$).

A



B



C

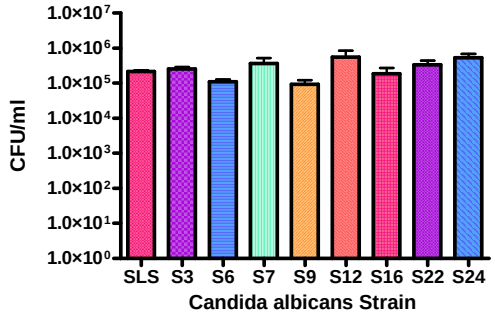
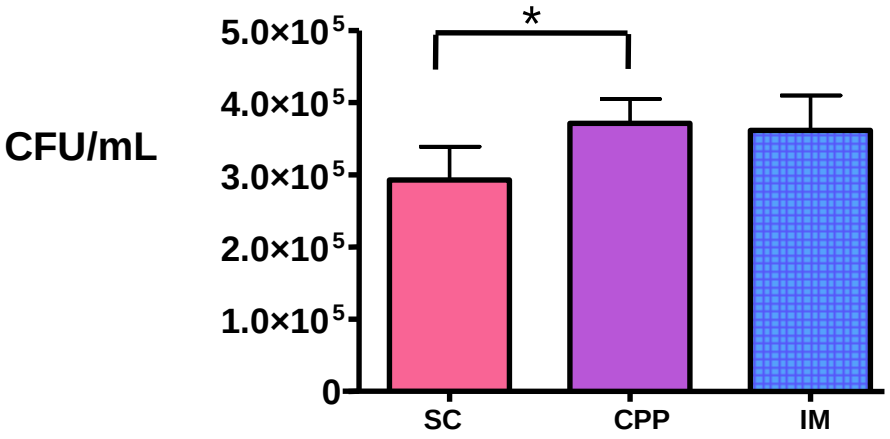


Figure 3.7 Mean attachment of all *Candida albicans* strains different denture acrylics

Mean colony forming unit attachment of nine strains of *C. albicans* on three denture materials: SC, CPP and IM. A significant reduction in attachment was observed between SC and CPP *p<0.05.



3.3.2 SEM examination of acrylic surfaces after Candida albicans inoculation

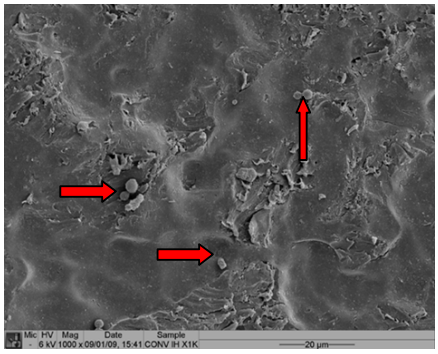
SEM examination of the acrylic resin samples, as described in Section 3.2.2, indicated the presence of varying surface morphology between the groups. Further to this initial examination, scanning electron microscopy was therefore performed to determine the influence of these differing surface features upon the sample groups, following inoculation with *C. albicans* cells for 1 h and 24 h (post biofilm formation). These SEM images are presented in Figures 3.8 and 3.9.

The images in Figure 3.8 illustrate the aggregation of *C*. *albicans* cells in surface irregularities for example, porosity in conventional pressure packed (A), cracks in injection moulded sample (B) and bubbles in self cured sample (C). Figure 3.9 demonstrates the minimal influence surface morphology appears to have over subsequent biofilm formation. Complex intertwining hyphae were evident on all the material samples, irrespective of initial candidal adherence patterns.

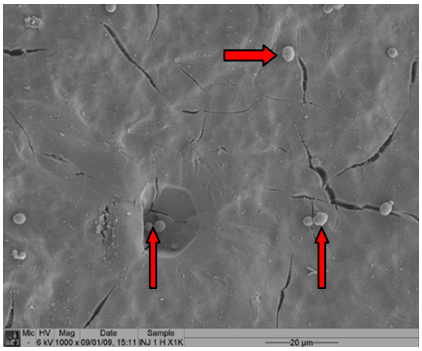
Figure 3.8 SEM of *Candida albicans* attachment to denture acrylics after 1 h

Images of (A) conventional pressure packed (B) injection moulded and (C) self cured acrylic resin samples. Note apparent porosity of conventionally processed sample. Cracks are evident in the surface of the injection moulded sample. Bubbling is evident on the surface of the self cured sample. Greater numbers of adherent candidal cells appear to be present on the self cured sample surface (x1000).

(A)



(B)



(C)

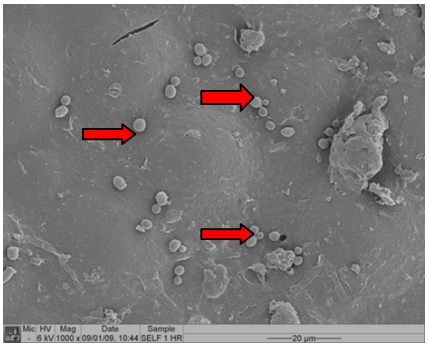
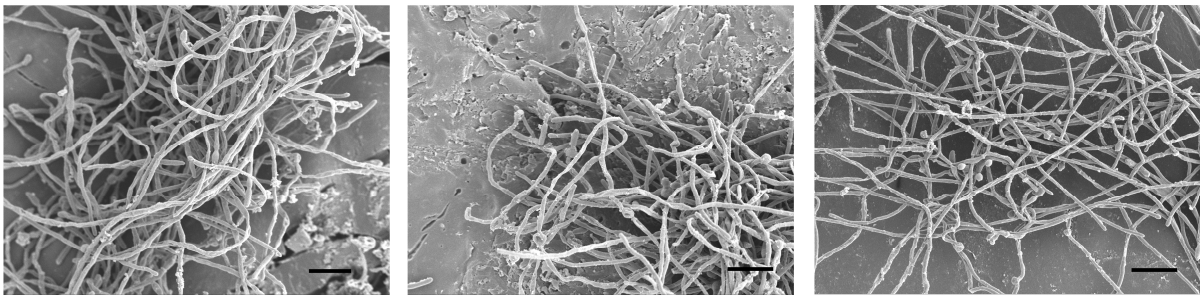


Figure 3.9 SEM of *Candida albicans* biofilms grown on three denture acrylics

C. albicans biofilms were formed on (A) self cured, (B) conventional pressure packed and (C) injection moulded acrylics. Note the expansive biofilms comprised of intertwined hyphae that are similar irrespective of the material (x1000).

A **B** **C**



3.3.3 Discussion

The ability of *Candida* spp. to form a biofilm quickly has important repercussions, in both the resulting biofilms ability to evade host defences and subsequent resistance to the effects of antifungal agents. The formation of a biofilm on the denture surface also serves as a reservoir for continuous and repeated infection (Douglas, 2002; Kojic, 2004). Ultimately the impression surface of the maxillary denture is in contact with the patient's palatal mucosa; therefore, it is this region of the denture which may clinically act as reservoir for *Candida* spp. (Budtz-Jorgensen, 1981; Samaranayake *et al.*, 1980).

SEM was undertaken to assess variation in the physical aspects of three material surfaces, and to investigate the attachment characteristics of *C. albicans* and its subsequent ability to form biofilms. Material surface flaws, either in the form of porosity or surface cracks, may increase candidal attachment to the denture base. This may result in varying degrees of denture induced stomatitis (Radford *et al.*, 1999). The formation of biofilms in cracks and imperfections has been shown to increase difficulty of biofilm removal by brushing (Baillie & Douglas, 1999; Ramage *et al.*, 2002).

In this study the difference in levels of *C*. *albicans* attachment between the materials tested may be owing to their surface characteristics and in particular their physical properties. Although not quantifiable in this study, SEM images of self cured PMMA resin samples did visually appear to show a higher density of *C. albicans* cells initially adhering to the resin surface. As has been

already discussed, the self cured PMMA samples were found to be rougher than either injection moulded or conventional sample groups when subjected to profilometry. The finding that, initially, more candidal cells are adherent to the self cured samples concurs with previous research indicating that increased surface roughness increases the adherence of *Candida albicans* to the acrylic resin surface (Nevzatoğlu *et al.*, 2007; Verran & Maryan, 1997).

The presence of *Candida* spp. within saliva, adhesion to the oral mucosa, and colonisation and development of biofilms on the denture surface are associated with mild to severe pathophysiological effects. According to Newton's classification, there is a range of fungal induced stomatitis (Newton, 1962). This pathogenesis is related to attachment of *C. albicans* cells to the impression surface of a denture, which is considered to be dependent on non-specific factors affecting the material surface, and specific factors relating to *Candida* spp. (Cannon *et al.*, 1995; Holmes, 1995; Millsap, 1999; Pereira-Cenci *et al.*, 2007). Candidal specific factors include the presence of adhesins and the ability to recognise ligands on the cell surface. *Candida* spp. are also able to coaggregate and/or bind to bacteria (Cannon, 1999; Holmes, 1995; Millsap, 1999).

The findings regarding adhesion to self cured PMMA samples are contrary to those which may have been expected based on the profilometry, and perhaps SEM images taken of the material surfaces after initial candidal adhesion. Previous studies have shown a direct correlation between increasing surface roughness and candidal adhesion (Radford *et al.*, 1999; Verran & Maryan,

1997). Although sonication is an effective way to remove micro-organisms (Kobayashi *et al.*, 2009; Ramage *et al.*, 2001), increasing surface roughness, and therefore retentive characteristics of the material, may, in a similar way to surface imperfections and tooth brushing, decrease the efficacy of such methodology. Thereby, although a higher number of candidal cells may adhere to the sample; these cells may not be efficiently removed for counting by sonication. Therefore, lower quantities of adherent cells would be observed.

The physical and chemical composition of denture acrylic is particularly important in terms of reducing the ability of pathogenic yeast cells to attach and form biofilms (Nevzatoğlu *et al*., 2007). This study has clearly shown that although there was no significant variation of strain attachment between the acrylic sample groups, a significant reduction in attachment to self cured denture acrylics was observed. These data indicate that material surface factors may play a greater role in promoting or preventing candidal adhesion, than the organism *per se*.

The chemistry of the various materials may also influence levels of candidal attachment determined. It has been demonstrated that storing PMMA resin denture bases in water decreases residual monomer levels, resulting in increased candidal adherence (Waltimo *et al.*, 2001). In order to minimise this as a source of error, all samples in this study were stored in water for one month prior to adhesion testing. However, within the restraints of this study, it was not possible to measure residual monomer levels within the 3 sample

groups. Both SC and IM materials are known to contain a higher level of barbituric acid based initiators than conventional materials (Ruyter, 1982). The effects of barbituric acid on *Candida spp.* are unknown, however, other materials containing this initiator have been shown to contain higher residual monomer levels (Frangou *et al.*, 1990). The content of this agent in the material samples may therefore also have influenced the data obtained. SC PMMA resins have been demonstrated to be more cytotoxic than conventionally processed PMMA resins (Sheridan *et al.*, 1997), this may be due to the initiators mentioned above, along with higher residual monomer levels, however this requires further examination.

C. albicans were found to adhere to all 3 types of PMMA resin studied. If left undisturbed, the *Candida* cells will proliferate to form a biofilm following initial adherence (Davenport *et al.*, 2001; Ramage *et al.*, 2004; Ramage *et al.*, 2005). This was demonstrated by the SEM images of the 24 hour biofilms. Candidal infection has been indicated by previous clinical studies to be directly related to a decrease in frequency of denture hygiene procedures (Kanli *et al.*, 2005). Therefore, in those patients most at risk of *Candida* infection, the differences exhibited between the material groups in this study are likely to be of little relevance. Only where the biofilm is removed by frequent brushing and disinfection, would the small differences seen between adhesion levels for the three sample groups perhaps become relevant.

Chapter 4:

DISCUSSION

There are currently some 15 million denture wearers within the United Kingdom. A minority of these experience problems, such as denture induced stomatitis. This condition represents a significant clinical and socioeconomic burden (Coulthwaite & Verran, 2007). Denture stomatitis is of multi-factorial origin influenced by factors such as salivary flow, denture cleanliness, age of prosthesis, denture base material, denture trauma, continuous denture wearing, smoking and nutritional intake (Oksala, 1990; Soysa *et al.*, 2004; Soysa & Ellepola, 2005; Soysa *et al.*, 2006). The data presented in this thesis demonstrates that the physical and mechanical properties of denture acrylics are influenced by processing methods, which has been discussed in detail within each section. These properties may then impact upon how microbial factors, including yeasts, adhere and are retained upon denture acrylic. Ultimately, as soon as dentures are placed into the oral cavity they become colonised by microbial species resident to this environment irrespective of the physical properties of dentures. Therefore, there are also more general factors that must be considered in relation to the production of dentures.

With such close similarities between injection moulded and conventional PMMA materials studied herein, it is necessary to examine other factors before being able to recommend one technique over the other. As stated in previous research, implementation of new therapies is usually governed by financial considerations, so efficacy must take into account cost comparisons (Heydecke *et al.*, 2005). Overall costs of conventional pressure packed complete dentures have been examined in multiple studies in comparison to mandibular implant retained overdentures (Heydecke *et al.*, 2005; Lewis,

1998; MacEntee & Walton, 1998). These studies have demonstrated that although implant retained over dentures were more expensive, they did result in significantly better oral health related quality of life scores than conventional complete dentures. However, no data exists on the cost effectiveness of conventionally processed compared to injection moulded complete denture bases, when related to quality of life scores.

In addition to the cost implication, the time required to process the materials may vary significantly between differing techniques. According to manufacturers' instructions, conventional heat cured acrylic processing takes 9 hours. In contrast, the processing time for injection moulding systems, is 35 min combined with 5 min to inject and 30 min polymerisation in the water bath (approximately 70 min in total). This represents only 13% of the time for conventional manufacture. Whilst the initial set up cost appears to be relatively high, in the long term, this expenditure may be re-couped. The injection moulding technique also has the added benefit that 2 denture bases can be produced simultaneously within the apparatus. Ganzarolli *et al.* (2007) stated that for injection moulding it was 'necessary to evaluate and balance the cost-effectiveness of techniques that are more expensive and time-consuming'. They also reported from their studies, more time consuming resin processing techniques may not be cost effective, considering the material's overall physical properties and clinical results.

There are some caveats to injection moulding. Specialised equipment is required, with increased initial outlay costs. The flasking technique is also more complex. Care must be taken during wax up of injection moulding sprues to ensure they are attached in the optimal region and that no undercuts are present in the wax up. Should an undercut be present where the sprue attaches to the denture base, fracture of the base in this region may occur when attempting to de-invest. Denture teeth must also be pre-treated after the boiling out stage to avoid de-bonding from the finished denture base. Additionally, care must be taken to ensure injection of the acrylic is performed at the appropriate moment. If injection is undertaken too early, then acrylic resin will be injected too rapidly through the flask set-up and voids or porosity may occur. These may result in enhanced microbial retention (Karaagaclioglu *et al.*, 2008; Nevzatoğlu *et al.*, 2007; Pereira-Cenci *et al.*, 2007). If injection is left until too late, then the material will become too viscous and again may result in voids or other inaccuracies. Interestingly, Ganzarolli *et al.* (2007) determined injection moulded denture base samples to possess significantly greater porosity than either conventional or microwave processed PMMA resins.

A reduction in the magnitude of occlusal inaccuracies has been reported with use of the injection moulding system (Keenan *et al.*, 2003; Nogueira *et al.*, 1999). This is likely to be favourable to the clinician both in terms of ease of denture delivery, but also in terms of clinical time costs. This was confirmed by Nogueira *et al.* (1999), who stated that although there was no appreciable difference in laboratory working time between injection moulding and

conventional processing techniques; injection moulding would save considerable time post-denture processing. They reported that with the improved dimensional accuracy of the injection moulding technique, less adjustment would be required at chairside (Nogueira *et al.,* 1999).

Prevalence of edentulousness is gradually decreasing. However, over 25% of the UK population wears complete or partial dentures and 13% are currently edentulous (Kelly *et al*., 2000). The UK has been ranked sixth highest WHO region/country with regards to prevalence of edentulous elderly (Peterson & Yamamoto, 2005). It is therefore likely that, for the considerable future at least, provision of complete dentures will continue to be of significant importance within dentistry. Since its introduction over 70 years ago, PMMA resin has been the most widely used denture base material (Johnson, 1994). It seems somewhat surprising that in all this time, little has changed in the way dentures are processed despite an ever present demand. This thesis has demonstrated that although injection moulding may possess some advantages over conventional pressure packed acrylic resins, the method is not without its flaws.

It is possible that in the future we may see a move towards other techniques aimed towards reducing denture inaccuracies, for example CAD/CAM techniques. This technique was described as far back as 1997 for duplication of complete dentures. However, difficulties were encountered with scanning acutely curved denture surfaces (Kawahata *et al.*, 1997). CAD/CAM technology has been reportedly successful in construction of removable

partial dentures (Williams *et al.*, 2006). In this report, the construction of a cobalt chrome framework by CAD/CAM technology was judged to be at least as accurate as conventional methodology. With high initial set up costs, reported in this paper to be in the region of $25,000, it seems unlikely that the use of CAD/CAM technology in denture construction will be permissible unless it is able to exhibit a greater magnitude of improvement compared to existing techniques.

In this study, injection moulded materials were not determined to exhibit any significant reductions in candidal adhesion, when compared to conventionally processed materials. Once candidal cells are adherent to material surfaces, they will rapidly proliferate to form a biofilm (Davenport *et al.*, 2001; Ramage *et al.*, 2004; Ramage *et al.*, 2005). Denture hygiene of dependent elderly people is known to be poor (MacEntee, 2000; Preston *et al.*, 2006; Sweeney *et al.*, 2007). In such cases as these, surface modification to prevent the adherence of *Candida* may show promise (Chandra *et al.*, 2005), Yoshinari *et al.* 2006). Acrylic resin surface treatments have included incorporation of methacrylic acid to alter surface charge (Park *et al.*, 2003) and incorporation of apatite-coated TiO_2 photocatalyst and ammonium compound (Pesci-Bardon *et al.*, 2006). All of these modifications have been shown to effectively reduce candidal adherence and may warrant further investigation.

4.1 Conclusion

From the results obtained, both the conventionally processed and injection moulded PMMA materials are acceptable for use as denture base materials. In terms of overall base-plate adaptation, mean weights of vinyl polysiloxane (VPS) present between denture impression surface and master cast for both the injection moulded and conventional pressure packed PMMA samples were small. Such magnitudes of dimensional inaccuracy, as demonstrated herein, are unlikely to impact upon the clinical success of the denture base. Polymerisation shrinkage of PMMA type denture base materials is, however, a well recognised problem (Craig, 2002). There is a desire to overcome this shrinkage via the development of changes in processing methods and modified or new materials.

The studies undertaken within this thesis have demonstrated injection moulded PMMA resin to be superior in terms of dimensional accuracy compared to conventional pressure packed PMMA resin. However, as the differences observed were very small, and the sample size also relatively small, it is debatable whether they would actually be of significance clinically.

Overall adaptation of the denture base is also of paramount importance when considering likelihood of candidal infection. A poorly fitting denture base is more likely to cause trauma to the denture bearing tissues. It is also more likely to allow the ingress of food and thereby plaque formation on the denture impression surface. Both these factors increase the likelihood of candidal infection of the denture base material, which may act as a reservoir, and of

the denture bearing tissues. The dimensional accuracy of the chosen denture base material and candidal adhesion to this are therefore closely associated.

The data did not indicate any statistically significant differences between injection moulded PMMA resin and conventional pressure packed PMMA resin materials in terms of candidal adhesion. As was previously mentioned, Candida will proliferate following initial cell adhesion. Therefore unless adhesion cell counts were found to be zero, or denture hygiene procedures were carried out often enough to prevent biofilm formation, the observed differences in attachment are likely to be of limited clinical importance in the prevention of candidal infection.

Having discussed the described techniques with experienced laboratory technicians and having used them throughout the laboratory stages of the research reported in this thesis myself, it is difficult to say if one technique is hugely advantageous over the other, either in terms of the dimensional or candidal adherence results described, or the ease of laboratory processing. However, it seems reasonable to suggest that a busy commercial laboratory, with a high volume of complete denture cases may wish or elect to choose the injection moulding system, which has a significant reduction in processing time, with similar or slightly more favourable material properties.

4.2 Suggestions for further work

The three *in vitro* studies reported in this thesis have been performed in order to compare the *in vitro* performance of two, or in the case of the microbiological study, three commercially available PMMA resin type denture base materials. As a result, a number of other areas for potential investigation have been identified. These may provide useful in supplementing the data obtained so far.

Suggestions for further research include:

1) An *in vivo* study of candidal adherence to injection moulded and conventional pressure packed acrylic resins to determine any significant differences.

2) An analysis of the patients involved in above the study to determine if statistical differences present in candidal attachment levels to the two materials correlate with clinical differences in levels of candidal infection and clinico-pathological effects.

3) In the experiments reported in this thesis, the un-finished surface of PMMA resin samples was examined. It would be useful to establish if significant differences exist in candidal adherence between the two materials after polishing.

4) As mentioned in Section 1.4.3.5, there are 2 possible heat regimes used for curing heat activated PMMA denture bases. The first of those involves heating at 72^{o}C for at least 16 hours. The second technique involves heating at 72^{o}C for 2 hours, then increasing the temperature to 100^{o}C and heating for a further 2 hours. The samples used in this study were processed by the latter method. A study comparing conventional pressure packed PMMA samples produced by the first method and injection moulded PMMA samples, in terms of both candidal adherence and dimensional accuracy may be useful.

5) Residual monomer is present in denture base acrylic resin to some degree (Lung & Darvell, 2005). Various post polymerisation treatments have been described in attempts to reduce this (Jorge *et al.*, 2007). An investigation may be performed to determine residual monomer levels present in conventional pressure packed PMMA samples versus injection moulded samples, and the impact of this on candidal adherence levels.

6) The data in this study indicated that material surface factors may play a greater role in promoting or preventing candidal adhesion, than the organism *per se*. Further consideration should therefore be given to studies investigating the modification and/or development of improved denture base materials that may provide a more biocompatible material to the oral environment.

7) The chemistry of the three materials used in this study varied. In order to explain the differences seen in adhesion levels between the groups, further investigation as to the impact of barbituric acid on candidal adhesion is required.

8) The results of this *in vitro* study demonstrated statistically significant, but very small differences in the dimensional accuracy of conventional pressure packed versus injection moulded PMMA samples. An investigation to determine whether or not these differences are actually significant in the clinical success of the two materials as denture base materials would be of benefit.

9) The use of potentially more accurate 3D imaging techniques to determine and compare overall base-plate adaptation for the two denture base material sample groups would be of interest.

10) Further investigation into the relationship between the injection moulding inlet location and magnitude of dimensional inaccuracies present in denture base samples in relation to this, would be beneficial.

Chapter 5:

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