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Characterisation of FDM 3D printed PMMA filament for manufacturing removable prostheses

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By

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إلى بسمة الحياة وسر الوجود الى من كان دعائها سر نجاحي

أمي الغالية

إلى من قال لي وهو يتحسر على فراقي لن أستطيع الوقوف عائقا امام مستقبلك وطموحك

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إخواني وأخواتي

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Abbreviations

PMMA	Poly Methyl Methacrylate
CAD/CAM	Computer Aided Design/ Computer Aided Manufacturing
Stl. file	Standard Tessellation Language format file
ISO	International Organisation for Standardisation
3D	3 dimensional
SLA	Stereolithography
FDM	Fused deposition modelling
mm	Millimeter
µ	Micron
µm	Micron Meter
µL	Micron Litre
µg	Microgram
mm^3	Cubic Millimeter

Abstract

Background: Polymethylmethacrylate (PMMA) has been used as a denture base material for over 70 years, but recently its formulation and manufacturing routes have undertaken significant developments with the development of PMMA based 3D printing filaments for Fused Deposition Modelling (FDM) and photocuring resins for Stereolithography (SLA). The use of these approaches has potential benefits to the patient in terms of cost, enhanced biocompatibility, improved mechanical and chemical properties. These advanced manufacturing routes also have potential to improve the working environment for dental technicians as well as to decrease environmental waste. Although key studies have been published in the area during the past 5-10 years, these tend to include limited comparisons between techniques, lacking to provide a comprehensive view that encompasses the whole available range of available manufacturing routes. Therefore, there is a need to develop more complex studies that incorporate conventional and new manufacturing techniques and refer their performance to specific ISO (International Organisation for Standardisation) standards. This study aims to characterise the properties of 3D-printed (FDM) PMMA denture base materials in comparison to conventional processed PMMA, milled PMMA, and photo-cured resins via stereolithography (SLA).

Methods: Five sample groups were compared: Heat cured acrylic resin, Cold cured acrylic resin, 3D printed PMMA FDM, 3D printed Methacrylate resin SLA, and CAD/ CAM milling PMMA. 3D printed samples divided into three subgroups depending on the printing orientation (X, Y &Z). All groups were subjected to mechanical tests (flexural strength, impact strength and hardness), water sorption and solubility tests, tooth bonding test, microbiological assessment and accuracy of fit. The data was analysed using one–way ANOVA.

Results: The FDM group performed within ISO specifications for mechanical testing and water sorption and solubility tests. However, the FDM group failed to achieve ISO requirements for the tooth bonding test. Also, the FDM group showed a rough surface finish which may lead to high candida adhesion. For accuracy of fit, FDM dentures showed discrepancies when compared to the original denture design (depending on printing orientation), which means FDM can present a lower degree of accuracy than other manufacturing routes.

Significance: FDM is a cost-effective and easy to use manufacturing route and our initial results regarding its use for denture base fabrication are promising, however, further research is required to ensure FDM 3D-printed denture bases can meet the requirements for clinical acceptance. In essence, the testing regime of denture base polymers is not complied with 3D printed polymers for denture manufacturing, so recommendations for future research have been made based on this study.

Introduction

Edentulism, either complete or partial, is a public oral health issue that affects millions of people worldwide. It has a high prevalence globally with 10% of adults aged ≥ 50 years getting affected and it represents a condition where patients' performance of key daily activities such as speaking and masticating is compromised (Tyrovolas et al., 2016). Edentulous patients can also experience poor oral and general health, low nutritional intake as well as a poor quality of life (Fenlon and Sherriff, 2008). Different causes result in edentulism; dental caries, periodontal diseases and trauma (Divaris et al., 2012; Cooper, 2009) being the most common. Although the prevalence of tooth loss has been reduced as middle-aged individuals have had better dental health in comparison to previous age groups, there are still a considerable number of edentulous patients seeking treatment around the world (Douglass et al., 2002; Han et al., 2017).

Treatment options for the edentulous include the use of a removable dentures (complete or partial), an implant supported or retained overdenture and, in some cases, implant supported fixed prostheses. A removable dental prosthesis is the most suitable and affordable treatment option for tooth loss, considering the main obstacle of dental implant therapy which is the high cost (Fenlon and Sherriff, 2008; Carlsson et al., 2010). Also, a removable prosthesis is preferred as an alternative treatment option over an implant with some edentulous patients who show anatomical restrictions such as poor bone quality or quantity (Saponaro et al., 2016).

Conventional techniques of fabrication of a removable prosthesis have been used for more than 80 years and it has shown functional and reliable results (Wimmer et al., 2016). However, fabrication is labour intensive, with several clinical and laboratory steps involved; at least 5 visits to the dental surgery are required to obtain a removable prosthesis. Accordingly, several disadvantages accompany these steps including: (a) a high treatment cost, (b) extensive laboratory time and expense, (c) material related issues such as improper fit due to polymerisation shrinkage and porosity that leads to aggregation of microorganisms (leading to infection) (Bidra et al., 2013).

3D printing technology has been utilised for fabrication of anatomical models for surgery since the 1990s and recently, 3D printing has demonstrated such progression that it shows potential for manufacturing of removable prostheses (Barazanchi et al., 2017; Almufleh et al., 2018). Computer Aided Design and Computer Aided Manufacturing (CAD/CAM) have been used for fabricating removable dentures (Bilgin et al., 2016). This technology can potentially provide a removable dental prosthesis with fewer clinical visits, shorter treatment time and lower cost. It has also shown to provide an enhanced fit, due to absence of polymerisation shrinkage (Schwindling and Stober, 2016). Fabrication of a removable denture by CAD/CAM technology is achieved by either additive manufacturing (3D printing) or by subtractive manufacturing (milling). One of the additive manufacturing techniques in which dentistry has been putting its attention to is Fused Deposition Modelling (FDM) and this is, among other factors, due to its cost-effectiveness and ease of use.

FDM additive manufacturing has presented advanced evolution in the field of prosthetic dentistry, but there is little evidence presented in the literature. Therefore, this study aims to evaluate the feasibility of using FDM 3D printed PMMA filament for manufacturing removable dental prostheses via the development of a comparative study using a range of conventional and CAD/CAM-based manufacturing techniques.

Chapter I. Literature Review

1. Background

Tooth loss is a major oral health problem that mostly affects elderly people, and it can be created by trauma or periodontal disease. A survey conducted by UK Adult Dental Health found that 5% of individuals aged 55–64 years and 15% of individuals aged 65–74 years were edentulous (Peltzer et al., 2014). In 2009, the NHS reported that one in five adults wear partial or complete removable dentures (Steele et al. 2012). Some consequences of tooth loss involve alteration in normal physiology such as alveolar bone resorption which adversely affects facial appearance. Furthermore, this problem impacts daily activities such as communication and, consequently, affected individuals may lose self-confidence. Mastication difficulty is another problem associated with tooth loss and edentulous people are more likely to consume less significant nutritious foods such as fruit and vegetables. Because of this diet shift, the risk of developing systemic diseases is high, and this has implications for the lessening of the quality of life of edentulous patients (Emami et al., 2013). The most common treatment for significant tooth loss is the use of a removable denture which is the affordable treatment of choice for edentulous patients since they tend to present low socioeconomic status. A 'better' treatment (retention, stability, tactility) is an implant retained prostheses, but the high cost of implants (Cunha et al., 2013) and the anatomical and health conditions of the edentulous patients make this option restricted in many cases (Saponaro et al., 2016).

2. The history of removable denture materials

Materials being used to overcome tooth loss have been described throughout history. Various materials have been used for removable denture base manufacturing involving ivory, wood and animal bones. Vulcanized rubber was commonly used as a dental polymer until 1937 when poly methyl methacrylate (PMMA) emerged as a denture base material. Acrylic resins, with a chemical formula $(C_5O_2H_8)_{n,}$ are formed by a reaction of PMMA powder particles with liquid monomer. The acrylic powder consists of a mixture of PMMA polymer, methyl methacrylate monomer and dibenzoyl peroxide while the liquid is a mix of methyl methacrylate monomer and ethylene glycol dimethacrylate monomer (Anusavice et al., 2012).

Polymerisation processes can be initiated by various methods; heating activation, chemical activation by adding substances such as dimethyl-p-toluidine, or light activation; all these methods are used in dentistry (Çelebi et al., 2008; Blagojevic et al., 1999). PMMA is not only used in the fabrication of removable prostheses, but it also has many uses: (1) fabrication of provisional crowns and orthodontic appliances; (2) bone cements; (3) ocular lenses; (4) filler of bone defects (Frazer et al., 2005).

An ideal denture base material should show specific mechanical, biological, physical and chemical properties. These properties involve natural appearance, dimensional stability, high strength, non-toxicity, non-absorption of oral fluids, ease of repair, ease of manipulation, resistance to microorganisms, good thermal conductivity, radiopacity and ease of cleaning (Noort & Barbour, 2013). PMMA acrylic resin is the most prominent material among all polymeric denture base materials although it does not fulfil all specifications of ideal denture base materials (Muhsin et al., 2019). It is the material of choice and most commonly used for denture base fabrication due to its favourable features such as surprising biocompatibility (Gautam et al., 2012), ease of processing and fixing, low water sorption and solubility, and adequate degree of strength (Urechescu et al., 2017).

However, there are several defects that make acrylic resin not ideal for denture base manufacturing, such as poor fitting due to polymerisation shrinkage and high coefficient of thermal expansion, and a possibility of an allergic response (Anusavice et al., 2012). These limitations also extend to a relatively low mechanical strength and high microbial colonisation (Akalin-Evren et al., 2012). Another common disadvantage of PMMA is the presence of residual monomers (due to uncured polymer) which affects the biocompatibility of acrylic denture base; a study by Austin and Basker (1980) reported that there is a strong relation between residual monomers released and oral mucosa irritation. Several alternatives have been explored to overcome these problems. Some studies have attempted different techniques to enhance PMMA performance via reformulation or the incorporation of other materials. These attempts included metal reinforcement, addition of fibres and alteration of chemical features of the PMMA (Muhsin et al., 2019).

Alternative polymeric materials for manufacturing removable prosthesis are also being researched. One of these materials is nylon which is a type of thermoplastic polymer known

as polyamide. Nylon was first introduced as denture base material in the 1950s and it was not used commonly in that time (Stafford et al., 1986; Yunus et al., 2005). It is suitable to be used as an alternative to acrylic prostheses in special situations such as repeated fracture of dentures and with patients who demonstrate an allergy to methyl methacrylate monomer (Stafford et al., 1986). In addition, nylon shows high flexibility which permits it to fill undercuts for superior retention. However, the early form of nylon exhibited some disadvantages including high water sorption, staining and developing a rough surface after a short time of use. These drawbacks were overcome by using other forms of nylon and reinforcement by glass fibres (Yunus et al., 2005).

Polyoxymethylene (POM) is another polymeric material that can be considered as a substitute of PMMA for fabrication removable prosthesis since 1986. POM is also called acetal resin which is composed by the polymerization process of formaldehyde. This material is more commonly used for the framework of removable partial denture since it shows high impact strength, esthetic appearance and high fatigue resistance (Meenakshi et al., 2016). POM is also used as implant material for hip replacement prosthesis and artificial heart valves (Fitton et al., 1994).

Another alternative material is Polyetheretherketone (PEEK), which is a thermoplastic polymer of the Polyaryletherketone (PAEK) family. PEEK is considered as a promising dental material since it has favourable physical, chemical and mechanical properties (Najeeb et al., 2016). In dentistry, PEEK has been used in many applications since 1992, for such applications as provisional crowns for anterior teeth, an implant material and orthodontic wire (Liu et al., 2017). PEEK has an aesthetic appearance and superior mechanical properties, so it is a good candidate material for fabrication fixed and removable prostheses (Najeeb et al., 2016). A clinical study published by Costa-Palau et al (2014) revealed that PEEK was successfully used as a part of maxillary obturator prosthesis. Another study has revealed that PEEK offered excellent mechanical properties compared to PMMA, and this finding indicates potential of using PEEK as an alternative removable prosthesis material (Muhsin et al., 2019). Despite all PEEK advantages', there is a lack of studies evaluating long term clinical performance and complications of this material (Zoidis et al., 2016). With all the efforts made to introduce new

techniques and develop new materials, PMMA is currently the best material for the fabrication of removable denture bases (Aguayo et al., 2017).

3. Denture Base Manufacturing

Denture bases are most commonly processed using a compression moulding technique (Nogueira et al.,1999). This technique composed of investment a wax up of removable prosthesis in flask of two-part gypsum mould and after setting of gypsums the wax is eliminated then the denture base material is mixed and packed in the flask which is placed in curing machine for polymerization (Oliva and Lowe, 1982). Regardless of the popularity of this technique, it shows some limitations. This technique is time consuming in terms of the dental laboratory procedure since processing of a denture base might take up to 48 hours. In addition to time, many processing errors might result from this technique, such as dimensional change and resulting increase in the vertical dimension of occlusion (Gharechahi et al., 2014; Nogueira et al.,1999). Efforts to overcome the drawbacks of compression moulding have led to the development of a new system of manufacturing. In 1942, the injection moulding technique was introduced by Pryor and shows less polymerisation shrinkage due to a closed mould, and the resin being under a higher constant pressure during polymerisation (Pryor, 1942) resulting in less dimensional change (Anderson et al., 1988). Despite these advantages, injection moulding requires almost the same laboratory time as compression moulding (Nogueira et al.,1999). Another manufacturing technique that has been explored is computer aided design and computer aided manufacturing (CAD/CAM) milling. Denture bases fabricated by the milling technique show proper fit, good strength, low residual monomer release and low bacterial adherence (Miyazaki et al., 2009; Srinivasan et al., 2018). Milling process of a denture base is wasteful as a significant amount of material is removed from a pre-polymerised block (Van Noort, 2012). Furthermore, the cost of a milling system is high and usually requires additional expenses such as training for staff (Srinivasan et al., 2019).

Another approach of CAD/CAM technology is additive manufacturing or 3D printing. Additive manufacturing can produce an object with complex structures at affordable cost with low or no waste materials (Van Noort, 2012; Liu et al., 2006; Attaran, 2017). The digital workflow of a denture base either by subtractive or additive manufacturing might be challenging since

several sets of data need to be recorded, transferred and re-evaluated during the process of denture base manufacturing. These data include occlusal vertical dimension (OVD), the occlusal plane and maxillomandibular relationship (MMR). Another challenge with digital manufacturing is the lack of opportunity to try a trial denture intraorally (Bidra et al., 2013; Kattadiyil et al., 2013). With the advent in technology and software, most of the challenging issues of digital manufacturing have been resolved to the point where non-3D design specialists can produce a viable outcome with minimal training. A clinical report presented that digital records of maxillomandibular relationship and occlusal plane can be registered by using a scannable recording material (Schwindling and Stober, 2016). Another demonstrated the possibility of a functional try-in, but with additional cost (Bidra et al., 2013). Additive manufacturing has been considered as competitive to conventional manufacturing methods in terms of cost, reliability and speed (van Noort, 2012).

4. Additive manufacturing

Manufacturing methods can be divided into 'subtractive' or 'additive'. The subtractive or milling technique can be illustrated as the procedure of cutting down block materials such as resin, metal, or ceramic to produce a physical object (Liu et al., 2006). This technique was first used in dentistry in 1980's in the form of the CEREC machine (Moörmann et al., 2006). Subtractive (milling) technique can be used for dental crown fabrication and removable denture construction (Uzun, 2008). Additive manufacturing or 3D printing relies on build up technique until the desired object is completed. 3D printing is known as a technique of joining layers of materials to produce a 3D object (Bourell et al., 2017). This technique consists of three essential elements: (i) modelling software, (ii) a manufacturing machine (3D printer), and (iii) materials that are used for printing. 3D printing materials include polymers, ceramics, and metals (Asprone et al., 2018).

Many advantages associated with 3D printing technology make this technique attractive and favourable. Such advantages include production of complex objects, efficiency in terms of materials (lack of) consumption, producing low manufacturing waste, and reinforcing sustainability (Attaran, 2017). The significant evolution of 3D printing markets has opened the door for various industrial fields to set up this technique in their manufacturing. In addition

to industries, 3D printers now are common in public libraries, schools, universities and even in homes (Stansbury and Idacavage, 2016).

The process of integration of 3D printing techniques in dental clinics and laboratories was actively promoted by Maeda et al (1994) and Inokoshi et al (2012). Current 3D printed dental applications include surgical guides, orthodontic patterns, complete removable dentures (Stansbury and Idacavage, 2016) and dental prostheses such as bridges, implants, and crowns (Bogue, 2013). Further innovation and developments in the processing techniques and materials control the potentiality of using 3D printing in various aspects of dentistry (Stansbury and Idacavage, 2016).

3D printing techniques include stereolithography (SLA), selective laser sintering (SLS), and fused deposition modelling (FDM) (Gaal et al., 2017; Stansbury and Idacavage, 2016) among others. The SLA technique is based on using a source of power such as a laser to cure photo-curable resins, which makes it relatively expensive in terms of materials availability and the equipment itself (Carneiro et al., 2015). FDM technique is based on using heat to extrude a filament of thermoplastic material to produce a 3D structure (Ngo et al., 2018). SLS technique is based on utilizing CO_2 laser to build parts by sintering a powder form of materials such as: polymers, ceramics and metals (Raghunath and Pandey, 2007). Each of these techniques shows advantages and disadvantages across the manufacturing process.

4.1 Stereolithography (SLA)

This technique is considered the earliest type of additive manufacturing, which was commercially developed in 1986 (Ngo et al., 2018). The SLA technique relies on a photo-polymerisation process, where UV light is used to cure layer by layer a photo-curable resin to produce a solid 3D object (Dizon et al., 2018). Photo-curable resins consists of monomers, such as urethane dimethacrylate (UDMA), photo-initiators and additives such as light blockers which can be either colorants or fluorescent agents (Lin et al., 2020; Bayarsaikhan et al., 2021). Additives have the role of achieving the desired details of the printing by finely managing the curing thickness of the printed layers (Liu and He, 2017). When the 3D object is fully formed, the support structures and unreacted resin are removed. A post processing treatment such as photo curing might be applied to the object to achieve good mechanical

performance (Ngo et al., 2018). In this process, many factors determine the quality of the final object such as the polymerisation depth (Heller et al., 2009), speed of scanning, laser power and exposure time (Cho et al., 2005). The SLA technique can print objects with high quality and high resolution and this feature makes SLA advantageous among the existing additive manufacturing techniques (Dizon et al., 2018). However, it shows some disadvantages such as limited range of printing materials, complexity of the curing process (Ngo et al., 2018) and high cost of setting up the system (Dizon et al., 2018). Dentistry has taken advantage of the rapidly developing technology to print structures with high resolution and smooth surfaces, printing clear structures and also where fast printing is necessary (Stansbury and Idacavage, 2016). In 1994, a Japanese research group used the SLA technique to fabricate removable dentures revealing that it was a time-consuming method. They concluded that SLA technique is a promising concept that requires further development to fabricate clinically acceptable denture (Maeda et al., 1994).

4.2 Fused Deposition Modelling (FDM)

Fused deposition modelling (FDM) or fused filament fabrication (FFF) is another additive manufacturing approach which was introduced in the early 1990s (Stansbury and Idacavage, 2016). This technique can be defined as the process of joining layer by layer a thermoplastic material to create a 3D object (Carneiro et al., 2015). FDM follows an extrusion principle where a molten thermoplastic material is extruded from the head of a printer on the platform (van Wijk and van Wijk, 2015).

The FDM technique can provide many advantages, for example, it is a simple production process and presents low cost and a high speed of manufacture. However, it does show some drawbacks that include poor surface appearance, mechanical weakness due to inter layer distortion and low quality of products (Chohan et al., 2017). Products by FDM show roughness on their surface due to the supporting overhangs which are inherent in the layer-by-later extrusion principle. To allow for printing of overhangs and across gaps, there are two types of support; the first is built from the same material with low strength while the second requires a double head machine for other materials (Bourell et al., 2017). The important factor for successful FDM printing is the gap between the nozzle head and the building plate. A space

that is too small may lead to obstruction of the extrusion while too large space may prevent the adhesion of semi liquid filament to the print bed (Gaal et al., 2017).

Many thermoplastic materials can be used in the FDM technique including polycarbonate (PC), polylactic acid (PLA), nylon, acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET) (Lee et al., 2017) and PMMA (most recently). Also, recycled polymer stocks can be used as a printing filament in FDM (Hunt et al., 2015). The FDM technique is widely known to represent the lowest cost of manufacturing among other 3D printing technologies (Dizon et al., 2018). Therefore, a removable denture that could be fabricated using FDM could involve a more cost-effective manufacturing approach (Fafenrot et al., 2017). Deng and coworkers have used FDM technique to fabricate a PLA complete denture pattern which was used for indirect production of a complete denture. Their main concern was the accuracy of the FDM product in comparison to a wax printed pattern (Deng et al., 2018).

5. Other possible dental applications of 3D printing

5.1 3D printed custom dental impression trays

Taking an accurate impression of edentulous jaws is a crucial step for a successful removable prosthesis. A good impression should show a replica of morphological structures of edentulous area and surrounding tissue (Zarb et al., 2013). The process of taking a primary impression is usually performed by using a 'stock' prefabricated impression tray which rarely matches the form of a patient's dental arch (Petrie et al., 2005). After pouring the primary impression, the gypsum cast is made. The undercuts are filled, and a uniform thickness of wax is applied onto the gypsum cast to create a uniform space for impression materials. After that, the wax surface is covered by photo curable or chemical curing resin and then this resin is cured to form a customized impression tray – the special tray (Chen et al., 2016). From the steps discussed above, the process of manufacturing handmade custom trays takes more time and materials, and it is easy to get an irregular space for the impression material because of the modification of wax when it covers gypsum cast. With advanced technology, the handmade custom tray can be replaced with a digital one which can provide a more efficient and accurate product (Cohen et al., 2014; Fasbinder, 2013; Andreiotelli et al., 2013). Chen et al (2016) describes the successful design and production of a custom tray by using 3D printing

technology (FDM) and polylactic acid (PLA) filament as printing material. They measured the time required for digitally manufacturing one mandibular custom tray which is about 50 minutes and they revealed that this time can be reduced by developing special CAD software for custom trays (Chen et al., 2016). Therefore, more advancement in the field of digital custom tray manufacturing is required to possibly reduce the treatment time and enhance the accuracy of prosthetic products.

5.2 3D printed surgical guides

Dental implants have become a common treatment option that is broadly used in many edentulous cases. A successful dental implant depends on many factors and one of them is good surgical planning (Widmann et al., 2006). Placement of dental implant should be in the optimal position of planned implant to minimise surgical complications and place dental implant in the most appropriate position for prosthetic treatment (Vlahović et al., 2017). A surgical guide is a device used for this purpose and it can be defined as a guide that facilitates the suitable surgical insertion and angulation of a dental implant (Ramasamy et al., 2013). There are many ways to fabricate surgical guides including conventional radiographic methods. A digital method of fabrication surgical guide is more accurate than conventional one due to accurate transferring of simulating plan to the surgical site (Ramasamy et al., 2013). Fabrication of digital surgical guides requires collecting data from patients; scanning intraoral tissues and Cone Beam Computed Tomography (CBC) images (Ma et al., 2018; Ramasamy et al., 2013). Stereolithography is one of 3D printing techniques that was used to fabricate surgical guides (Vlahović et al., 2017; Ramasamy et al., 2013).

5.3 3D printed educational and anatomical models

Emergence of Cone Beam Computed Tomography (CBCT) technology opened the door to 3D print anatomical models which were used in surgical planning to reduce surgical complications and lowering treatment time. These models can be useful in cases of traumatic reconstruction, resection of pathological tissue, obturator appliance fabrication and orthodontic diagnostic models (Anderson et al., 2018). Furthermore, anatomical models which are used for demonstration purposes can be fabricated by 3D printing. Dental students can use these models for training in their preclinical courses (Rengier et al., 2010). The education process in dental schools depends on extracted teeth or commercial teeth for

preclinical training. Extracted teeth are a good choice for clinical simulation but the availability, disinfection and storage limit their use. However, commercially teeth can offer a good alternative to the natural teeth but can be costly. Employing 3D printing in artificial tooth manufacturing can help dental students in preclinical training. For example, in preclinical endodontic training, transparent artificial teeth are required for students to differentiate between the internal structures of the tooth such as root canals and pulp champers (Nassri et al., 2008). Using multi material 3D printing (FDM) can provide this kind of structure where different materials with different textures can be used (Cresswell-Boyes et al., 2018). Furthermore, 3D printing technology can be utilized in the preclinical prosthodontic courses where the tooth preparations are performed on artificial teeth. 3D printed tooth preparations can help in lowering the time of preparation by staff course, reduce the cost of production compared to conventional stone models and assist dental students to compare their tooth preparations to the ideal tooth preparations (Boonsiriphant et al., 2019).

6. Criteria for denture success

Denture base materials should show adequate mechanical, morphological and microbiological properties prior to use intraorally. Acrylic resin has been the most commonly used option for denture base fabrication for more than seventy years (Craig, 2002), as it has proven to meet to an acceptable standard many of these criteria, Table 1 summarising these criteria. These will be described in the following chapters.

Evaluation success of removable dentures is extended to include other factors. Such factors include patient and clinician factors or laboratory related factors (Young, 2010). Not all edentulous patients demonstrate ideal anatomical landmarks such as enough alveolar ridge which are essential for denture success. Some of them have mucosal structure abnormalities such as flat palates, shallow ridges or flabby ridges. These abnormalities lead to low functional efficiency of denture which may be generated by instability, lack of retention or fracture. Clinicians need to pay special attention to patients presenting such abnormalities during the treatment procedure. Competent clinicians can understand the consequences of these abnormalities on denture success and use their clinical judgment to provide a functional denture (Patel et al., 2018). Also, clinicians are responsible to get accurate impressions before

sending them to the dental laboratory. In addition, dental technicians should be skilled in terms of understanding clinicians' instructions and use a proper material to fabricate dentures (Al-AlSheikh, 2012).

Table 1: Summary of the acceptable values of required criteria for denture base materials.

Criteria / Requirement		**Pass/fail determination**	**Source/ Reference**
Mechanical Properties	Flexural Strength	> 65 MPa	ISO 20795-1
	Impact Strength	> 1.9 kJ/m^2	ISO 20795-1
	Hardness	N/A	N/A
Water Sorption and Solubility		**Wsp**: not exceed 32 µg/mm^3 **Wsl**: not exceed 1.6 µg/mm^3	ISO 20795-1
Tooth Bonding		showing cohesive or mixed fracture	ISO/TS 19736
Microbiological Properties		N/A	N/A
Accuracy of Fit		N/A	N/A

7. Measuring the success of a denture/ Testing methods

Mechanical properties of denture base materials can be evaluated by various tests, and most of published studies have followed standards of ISO 20795-1, Denture base polymers (Abhay & Karishma, 2013; Mumcu et al., 2011; Ajaj-ALKordy & Alsaadi, 2014; Choksi & Mody, 2016; Lee et al., 2018). ISO 20795-1 specifies that a flexural strength test is conducted by a three-point bending test where a rectangular sample is subjected to vertical force with a speed of 5 mm/min until fracture. In addition, ISO 20795-1 determines the impact strength test specifications', which consist of a notched strip sample hit by the testing device plunger until

break. Impact strength value is demonstrated by ultimate load before break (ISO, 2013). For hardness evaluation, Vickers hardness test was used widely in literature as a testing method and it is based on using a force to apply indenter on a sample surface then recording diagonal indent dimensions optically (Alhareb et al., 2017; Farina et al., 2012; Duymus et al., 2016; Ali et al., 2008).

Literature showed wide variation in the methods used for tooth bonding tests. Some published studies used shear bond strength (Cunningham, 2000; Nishigawa et al., 2006) or tensile bond strength (Schneider et al., 2002; Chaves et al., 2009), others performed additional procedure such as mechanical modification or chemical treatment on artificial teeth or different angulated load direction (Chai et al., 2000; Cunningham, 2000; Beuer et al., 2006; Chung et al., 2008; Moffit et al., 2008). Tooth bonding can be evaluated by ISO 22112: Artificial teeth for dental prostheses, which is based on tensile test and evaluates bond strength in relation to the strength of artificial teeth material (ISO, 2017). Tooth bonding test can also be carried out by another ISO/TS 19736: Bonding test between polymer teeth and denture base materials, which is based on shear bond strength (ISO, 2017). Requirement of passing tooth bond test on both ISO standards based on the fracture mode that is presented by the tested sample. Mode of fracture divided to cohesive fracture which means tooth remnants stick to denture base or remnants of denture base stick to artificial tooth, adhesive fracture where the fracture path run clearly along the adhesive layer between artificial tooth and denture base, and mixed fracture which combine both cohesive and adhesive. Tested samples need to display cohesive or mixed fracture mode in terms of achieving ISO requirements.

For water sorption and solubility tests, most of published studies (Rahal et al., 2004; Tuna et al., 2008; Jang et al., 2015; Hemmati et al., 2015) followed the ISO 20795-1 specifications where samples need to be dried and weighed until reach constant mass (m1). Then they are immersed in water at 37 C for 7 days and after that they are weighed (m2). Then Samples need to be reconditioned (m3) to the constant mass by repeating the cycle of drying and weighing.

For microbiological characterisation, many studies have evaluated the adherence of microbial species such as Candida albicans which can cause denture stomatitis (Ferreira et al., 2009;

Murat et al., 2019; Wady et al., 2012). The process of growing colonies of candida and adhering to the surface is known as the biofilm formation which is significant in the development of denture stomatitis (Zamperini et al., 2013; Blankenship and Mitchell, 2006). The process of adherence is promoted by some factors; the roughness of the denture surface, the wettability / hydrophobicity of the denture resins (Ali et al. 2013) and factors related to the patient such as, dry mouth and poor oral hygiene (Kawasaki et al. 2016). Surface roughness can be measured by using a profilometer device which can determine the degree of roughness on the surface of denture materials. Also, scanning electron microscope (SEM) is used for qualitative assessment of surface topography of denture materials. Another feature of the denture surface is the wettability which is determined by measuring the contact angle between the solution and the surface. Contact angle is measured with the help of an automated device supplied with a camera and a software for image analysis (Ferreira et al., 2009; Murat et al., 2019; Zamperini et al., 2013). Once the candida biofilm is formed on the denture surface, it is evaluated by viability assay such as XTT (2,3-bis2-Methoxy-4-nitro-5-sulfophenyl- 2H-tetrazolium-5-carboxyanilide) which relies on the metabolic activity of candida cells (Ramage et al., 2001; Kuhn et al., 2003; Wady et al., 2012).

The assessment of the accuracy of fit of the denture relies on the amount of space between the fitting surface of the denture and the edentulous cast which represents the underneath mucosa (Darvell and Clark, 2000; Oğuz et al., 2021). This space should be as narrow as possible to attain good retention and consequently better adaptation of the denture base to the mucosa (Oğuz et al., 2021). Literature has concluded that the evaluation of accuracy of fit of the denture can be carried out by either physical or digital techniques (Goodacre et al., 2016; Hsu et al., 2020; Lee et al., 2010). One of the methods of physical technique is to measure the microscopic gap distance of the trimmed denture on the cast (Lee et al., 2010; Sayed et al., 2019). Furthermore, another method of physical technique depends on physical weighing (McLaughlin et al., 2019; Lee et al., 2010) or thickness measurement of a silicone impression material film that duplicates the space between the denture and the cast (Goodacre et al., 2016; Yoon et al., 2018). By using advanced technology, accuracy of fit can be evaluated by digital matching/superimposition analysis where the digital surfaces of the denture and its corresponding cast are aligned then the distance between these surfaces are measured. Also, this surface matching method offers a visualization analysis by colour surface maps (Hsu et

al., 2020; Oğuz et al., 2021). Micro-computed tomography is another digital method that is described in the literature for evaluating the accuracy of fit. This method allows a 3D visualization of objects images' and an accurate volumetric analysis of the gap between objects (Swain et al., 2009; Scotti et al., 2020).

All the previous criteria are needed to be shown in the denture in terms to achieve acceptable clinical requirement so the assessment of the denture performance should consider these criteria. Thus, we need a comprehensive study that evaluates/ measures these key points and compares different manufacturing techniques of the denture base.

8. Aim and Objectives

Aim

To evaluate the feasibility of using 3D Printing filament and FDM manufacturing for removable dental prostheses via the development of a comparative study using a range of conventional and CAD/CAM-based manufacturing techniques.

Objectives

- To identify and compare the mechanical properties (flexural strength, impact strength and hardness) of 3D printed acrylic based denture materials against ISO standards of denture base polymers (ISO 20795-1:2013).

- To identify and compare water sorption and solubility of 3D printed acrylic based denture materials against ISO standards of denture base polymers (ISO 20795-1:2013).

- To identify and compare tooth bonding of 3D printed acrylic based denture materials against ISO standards bonding test between polymer teeth and denture base materials (ISO/TS 19736:2017).

- To evaluate the surface properties (roughness and wettability) and bio-acceptability of 3D printed acrylic based denture materials by using candida albicans.

- To evaluate the accuracy of fit of 3D printed acrylic based denture materials and other manufacturing techniques by using a matching software.

Chapter II. General methods and materials

1. Introduction

The purpose of this chapter is to present a summary of the manufacturing methods and materials that will be used throughout the thesis. All the samples for all the following chapters were manufactured using the same principles and hence these methods are described in this "General Methods Chapter". Throughout the thesis referrals will be made back to this chapter, however, other more specific methods will be described in each of the chapters.

Acrylic based samples were distributed in 5 groups according to the manufacturing methods; conventional compression moulding (heat and cold cured PMMA), PMMA samples milled from a block (subtractive manufacturing), PMMA formed by using additive manufacturing (FDM and SLA 3D printing) outlined in Table 2. Also, this study includes the comparison of two SLA resins: grey resin and denture base resin. The process of fabrication of the denture via conventional and CAD/CAM techniques is shown in Figure 1.

The 3D printing orientation is known as in which position a 3D object is aligned within the build platform. As Ezair et al (2015) have shown that orientation can impact the printing outcome in many aspects including mechanical strength, surface finish and the printing time among others. In this work, 3D printing groups were subdivided to 3 subgroups: X, Y & Z based on printing orientation, as shown in Figure 2.

Table 2: Sample group distribution.

Group number	**Description**
Group 1/ Heat cured	Conventional heat cured acrylic resin
Group 2/ Cold cured	Conventional cold cured acrylic resin
Group 3/ Milling	Subtractive (milled) from PMMA block
Group 4/ FDM	Additive (FDM), PMMA filament; X, Y & Z
Group 5/ SLA	Additive (SLA), Grey and Denture base resins; X, Y & Z

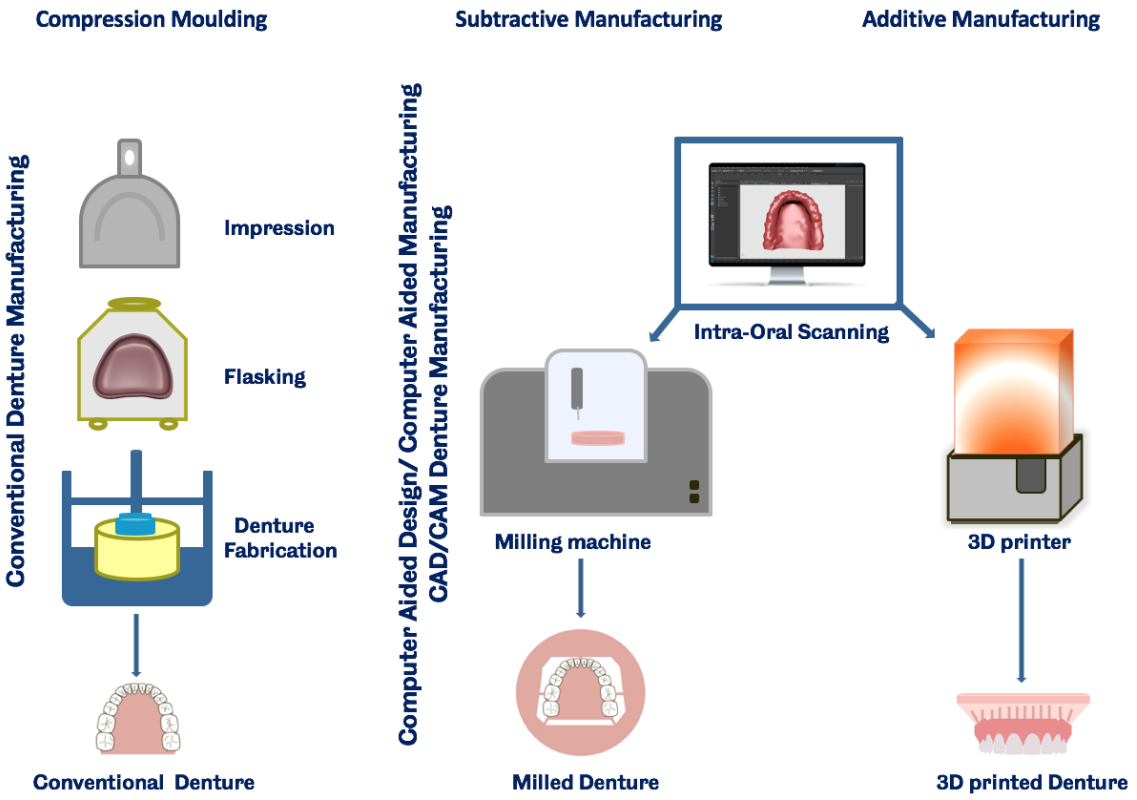


Figure 1. Schematic chart of denture fabrication via conventional and digital manufacturing approaches. Conventional denture manufacturing by alginate impression and flasking (compression moulding). Digital denture manufacturing using intra-oral scanner (digital impression); fabrication of denture either by subtractive technique (milling) or additive technique (3D printing).

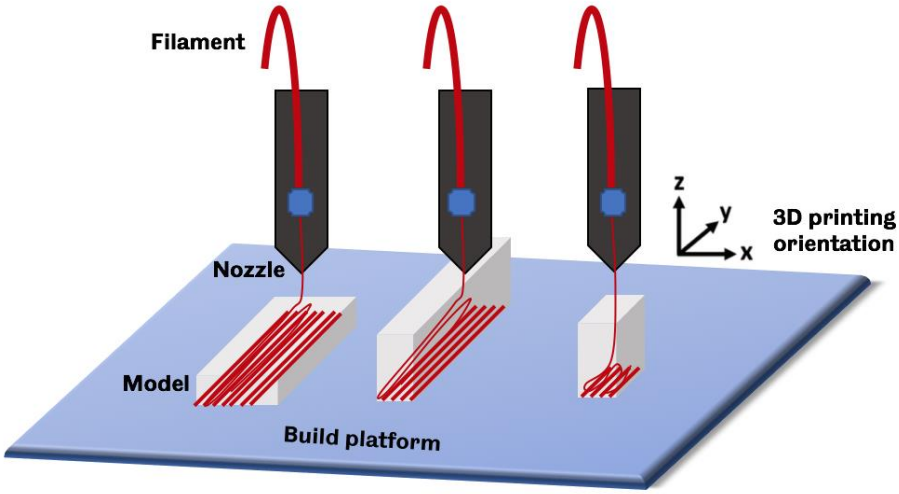


Figure 2. Schematic chart showing how the same object can be aligned and 3D printed by different orientations.

2. Methods of manufacturing

Conventional heat-cured samples (Group 1): samples were prepared by investing desired shaped wax patterns in a dental white plaster mould flask. After plaster setting, these patterns were boiled out and removed by a boiling out machine (Labormat TH, Dreve). After bench cooling, separating medium Iso K (Candulor, Zurich, Switzerland) was used on both parts of the flask to prevent the flask halves adhering to each other. The acrylic resin: 22.5 g of powder and 10 ml of liquid (ProBase® Hot, Ivoclar Vivadent AG) was mixed and packed into the stone mould, according to the manufacturer's instructions then the flask was placed in a heat polymerization unit (Eclipse Paco) for 6 hours. Then after bench cooling, the acrylic samples were removed from the flask.

Conventional cold-cured samples (Group 2): samples were prepared by mixing 15g powder and 10ml liquid of cold cure acrylic resin ProBase® Cold (Ivoclar Vivadent AG), according to the manufacturer's instructions. The resin was either poured into the custom silicone mould or packed in a plaster mould flask as discussed above. The flask was placed in a pressure polymerisation unit (Polymax 5, Dreve) and after bench cooling, the acrylic samples were removed from the flask.

Subtractive (milled) from PMMA block (Group 3): the desired geometry of the sample was designed using Fusion 360™ software, (Autodesk Inc.). An stl. file of the design was created and imported to Sum3D (CIMsystem), which was used to configure the milling settings. Samples were milled with a 5-axis milling machine (Roland DWX-50) from a PMMA disc with dimensions 98.5mm x 30mm (IvoBase CAD). 5-axis milling machines can provide efficient and accurate cutting, so they are widely used with more complex surfaces (Jung et al., 2002). The number of axes of a machine indicates the amount of separate manageable movements on the machine slides. Within a 3-axis machine, the tool axis angle remains immovable during the machining thus the flexibility of the tool angle is restricted. However, 5 axis machines show more freedom of tool angle movement by affording rotational slides (Bohez ,2002).

Additive (FDM), PMMA filament (Group 4): the stl. file of the design was imported into Cura LulzBot Edition version 2.6.52, which was used to set up (slice) the printing parameters: layer height, line width, infill density, infill pattern, printing temperature and support

placement. Samples for each printing position were printed using a desktop 3D printer (LulzBot TAZ 6, Aleph Objects, Inc., USA) and a 3D printer filament of Poly Methyl Methacrylate (PMMA) (Material4print, Germany) was used for this printing, with manufacturer's instruction to build the plate temperature 100°C and 240°C for the printing temperature. The diameter of the filament was 2.85mm, and the infill density of printing was 100% (to produce a solid sample, and to eliminate variables due to infill design and density).

Additive (SLA), Grey and Denture base resins (Group 5): the design file was imported into PreForm Software version 2.18.0, which was used to set up the printing parameters. Samples for each printing position were printed using a desktop 3D printer (Form 2, Formlabs Inc.) and two different 3D printer resins (Grey resin & Denture resin, Formlabs Inc.) were used for this printing. The grey resin was printed at 100-micron resolution while the denture base resin was printed with 50-micron resolution.

Chapter III. Mechanical characterisation

1. Background

Denture base resins should present adequate mechanical properties to provide successful clinical treatment of removable prostheses (Mumcu et al., 2011). Failures of denture base material are mostly associated with fractures, which have a high rate of occurrence and 68% of dentures have been shown to fracture during a few years of service/insertion (Al-Dwairi et al., 2020). In addition, edentulous patients are mostly elderly people with likely less muscle control, resulting in accidental denture fracture (Ucar et al., 2012). Acrylic denture base is more susceptible to fracture and this results from two different reasons: flexural fatigue (stresses) and impact. Flexural fatigue is raised from frequent masticatory load which may induce the prominence of microscopic cracks and joining of these cracks over a period lead to denture fracture. Impact, which is another reason for denture fracture, occurs mostly due to the denture falling onto a hard surface during cleaning, sneezing or coughing (Jagger et al., 1999; Johnston et al., 1981). In addition, stress concentration areas such as around the labial frenulum area may contribute to denture fracture and examples of this fracture can be seen across the midline of a denture (Ajaj-AlKordy and Alsaadi, 2014; Takahashi et al., 2012). Sasaki and co-workers reported that impact force is the reason for the fracture of 80% of mandibular dentures while maxillary denture fracture is caused commonly by the mixed forces of flexural fatigue and impact (Sasaki et al., 2016). Other factors involved in causing denture fracture include the forming around anatomical structures such as tori and bony prominences (Lee et al., 2012) and poor fitting of the denture to the underlying mucosa (Faot et al., 2006).

Therefore, resistance to flexural fatigue and impact is a desirable property of denture base resins when considering the long term of functional and clinical performance of a denture (Gurbuz et al., 2012). Various approaches have been investigated to optimise fatigue and impact resistance of the denture base, so as to overcome denture fracture. One study has used metal wires in the denture base, but this technique showed poor adhesion between acrylic resin and metal and unsatisfactory appearance (Vojdani and Khaledi, 2006). Another study investigated fibre reinforcement, and this technique has found an aesthetically satisfactory result but with the drawback of being time consuming, complicated and technique sensitive (Uzun et al., 1999). The technique of using glass fibres to reinforce PMMA

is widely described in the literature (Uzun et al., 1999; Aydin et al., 2002; Cokeliler et al., 2007). In addition to improving the mechanical properties of PMMA, glass reinforcement techniques have shown high polishability, good appearance and ease of manipulation (Jagger et al., 1999; Cokeliler et al., 2007).

Recently, Computer Aided Design/ Computer Aided Manufacturing (CAD/CAM) technology has been applied in the denture manufacturing field. Manufacturers of this technology have declared that dentures fabricated from CAD/CAM PMMA blocks have superior mechanical properties in comparison to conventional techniques (Steinmassl et al., 2017). Computerised Numerical Control (CNC) milling subtractive fabrication is a type of CAD/CAM technology that uses PMMA discs in the denture manufacturing process; these discs are pre-polymerized under high heat and pressure. Studies have concluded that the pre-polymerisation process of the PMMA discs is behind the improvement of mechanical performance of CAD/CAM dentures. Another CAD/CAM technology is additive manufacturing (3D printing) where dentures can be fabricated from PMMA photo-polymerised resin (Al-Dwairi et al., 2020). Many studies have evaluated the mechanical properties of conventional and milling PMMA denture resins (Al-Dwairi et al., 2020; Iwaki et al., 2020; Aguirre et al., 2020; Bedrossian et al., 2019).

While there is growth in the development, use and discussion of 3D printed PMMA materials for denture manufacturing, there are insufficient published studies evaluating the mechanical properties of 3D printed PMMA denture materials. This chapter aims to evaluate the mechanical properties of 3D printed denture base resins.

2. Materials and methods

Acrylic based samples were distributed in 5 groups according to the manufacturing methods; conventional compression moulding (heat and cold cured PMMA), PMMA samples milled from a block (subtractive manufacturing), PMMA formed by using additive manufacturing (FDM and SLA 3D printing), see Table 3. Flexural and impact samples were prepared and tested in accordance with ISO specifications (ISO, 2013). In addition, SLA and FDM groups were subdivided to 3 subgroups; X, Y & Z based on 3D printing orientation to test variabilities in properties caused by the layer-by-layer manufacturing process as shown in Figure 3 (A). In

addition to the ISO tests, a hardness test was conducted by following common methods reported in the literature.

Table 3: Sample group distribution.

Group number	**Description**
Group 1/ Heat cured	Conventional heat cured acrylic resin
Group 2/ Cold cured	Conventional cold cured acrylic resin
Group 3/ Milling	Subtractive (milled) from PMMA block
Group 4/ FDM	Additive (FDM), PMMA filament; X, Y & Z
Group 5/ SLA	Additive (SLA), Grey and Denture base resins; X, Y & Z

2.1 Manufacturing of Samples

2.1.1 Flexural samples

Conventional heat-cured samples (Group 1): samples (n=5) were prepared using a plaster mould made by investing rectangular shaped wax patterns (64 x 10 x 3.3 mm) then these patterns were removed after plaster setting as shown in Figure 3 (B) and (C). The acrylic dough ProBase® Hot (Ivoclar Vivadent AG) was mixed and packed into the plaster mould, according to the manufacturer's instructions. The rectangular resin samples were then removed from the flask, Figure 3 (D).

Conventional cold-cured samples (Group 2): (n=5) samples with dimensions (64 x 10 x 3.3mm) were prepared by mixing powder and liquid of cold cure acrylic resin ProBase® Cold (Ivoclar Vivadent AG), according to the manufacturer's instructions. The resin was poured into the custom mould as shown in Figure 3 (E) and after bench cooling, the acrylic samples were removed from the mould.

Samples for groups 3, 4 & 5 were designed with the dimensions (64 x 10 x 3.3 mm) using Fusion 360™ software, (Autodesk Inc.) as shown in Figure 3 (F). **Group 3,** the design was imported to another computer software (Sum3D), which was used to set up the milling

settings. 5 samples were milled with a 5- axis milling machine (Roland DWX-50) from a PMMA disc (IvoBase CAD) as shown in Figure 3 (G). **Group 4,** the design file was imported into Cura LulzBot Edition (version 2.6.52), which was used to set up the printing parameters. 5 samples for each printing position were printed using a desktop 3D printer (LulzBot TAZ 6, Aleph Objects, Inc., USA) and a 3D printer filament of Poly Methyl Methacrylate (PMMA) (Material4print, Germany) was used for this printing, following the manufacturer's instructions to set the build plate temperature to 100°C and the printing temperature to 240°C. The diameter of this filament was 2.85mm, and the infill density of printing was 100%. **Group 5,** the design was imported to into PreForm Software (2.18.0), which was used to set up the printing parameters. 5 samples for each printing position were printed using a desktop 3D printer (Form 2, Formlabs Inc.) and two different 3D printer resins (Grey resin and Denture resin, Formlabs Inc.) were used for this printing.

Flexural samples' shape and dimension were determined according to the ISO 20795-1:2013 specification for denture base polymers. Flexural samples were finished by using wet grinding with P500, 1000, and 1200 grit paper (SiC grinding paper, Buehler, Germany) and stored in water at 37°C for 50 ± 2h prior to the flexural test (ISO, 2013).

2.1.2 Impact samples

All samples (n=10) for each group were produced using the same method as previously described in section 2.1.1 but with the dimensions of 39 x 8 x 4mm. Following production, all samples were subjected to motorised notch cutting, Figure 3 (H), using a (Ray-Ran Test Equipment Ltd, UK) to create a ''v''-shaped notch at the centre of each impact sample according to the ISO 20795-1:2013 specification for denture base polymers. The impact samples were again finished using the method described previously and stored in water at 37°C for 7 days ± 2 h prior to impact test (ISO, 2013).

2.1.3 Hardness samples

The remaining samples of all groups that were prepared for the impact test were used as hardness samples (n=2) from each group sample. Additionally, these samples were subjected to mechanical polishing with polishing paste using a Dental Lathe (wet pumice on the rag

wheel) then polishing with an ultra-shine rag wheel to mimic the conventional method of polishing an acrylic denture base. All these procedures were performed by one investigator.

2.2 Mechanical characterisation (testing)

2.2.1 Flexural strength test

Flexural samples were subjected to the three-point flexural strength test as shown in Figure 3 (I), according to the ISO 20795-1:2013 using a universal testing machine (Lloyd LRX, AMETEK, Inc.). Samples were centrally located and a load of 2.5kg at a crosshead speed of 5 mm/min was applied until fracture occurred. The span length was 50mm. Computer software (NEXYGEN 4.1, Lloyd Instruments) was used to obtain the flexural strength value for each group.

2.2.2 Impact strength test

Impact samples were subjected to a Charpy impact test by using an impact tester (H503 Impact test, Tinius Olsen Ltd). The sample was positioned centrally with the V notch facing the opposite side of the pendulum of the testing machine as shown in Figure 3 (J). With the pendulum released from its rest position, the sample can be fractured and the maximum load before fracture is the impact strength in kJ/m2.

2.2.3 Vickers hardness test

Hardness was measured using a Vickers hardness tester (Foundrax, UK) with an applied load of 1kg at a 10 seconds' dwell time. The diagonal lengths (D1 and D2) of a square shape trace were measured by a scaled microscope as shown in Figure 3 (K). The mean value of five points of indentation for each hardness sample was investigated to obtain the Vickers hardness.

2.3 Statistical analysis

All data were statistically analysed with one–way ANOVA, using SPSS software (version 22). p-values less than 0.05 were considered statistically significant.

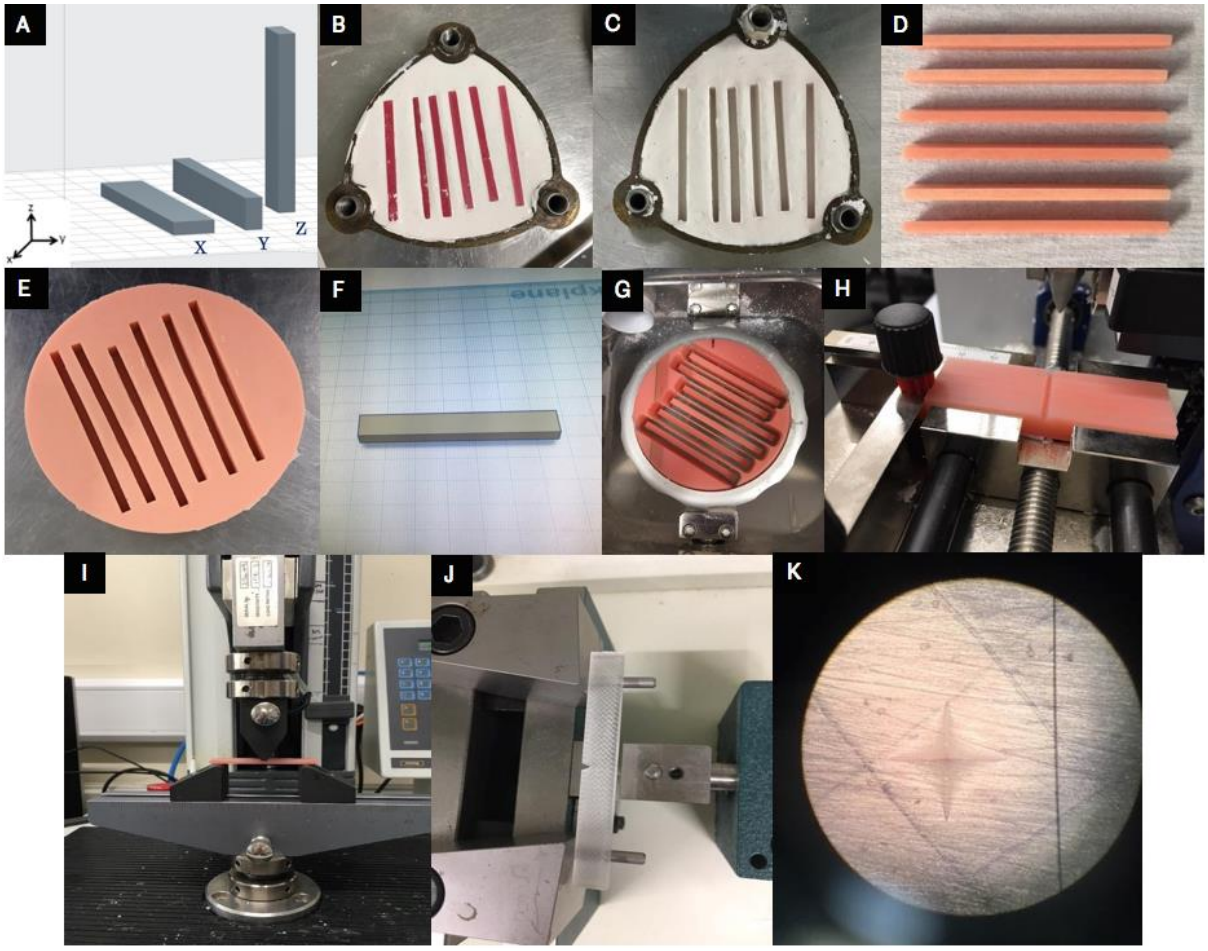


Figure 3. The process of preparing and testing mechanical samples; (A): different orientation of 3D printed samples, (B): wax patterns of flexural samples on stone mould before setting, (C): wax removed after stone setting, (D): conventional heat cured flexural samples, (E): custom silicone mould for flexural samples, (F): flexural sample design (64 x 10 x 3.3 mm) using Fusion 360™ software, (Autodesk Inc.), (G): flexural samples after milling from PMMA disc, (H): impact samples after ''v'' shaped notch creation, (I): three-point flexural test using the universal testing machine (Lloyd LRX, AMETEK, Inc.), (J): position of impact sample in impact tester (H503 Impact test, Tinius Olsen Ltd), (K): hardness sample under microscope.

3. Results

Table 4 shows the results of flexural strength, impact strength, and Vickers hardness for each group. As shown in Figure 4, cold cured samples showed the highest mean value of flexural strength, while the lowest mean value was in the Z FDM group. For impact strength, the X SLA denture resin group showed the highest mean value while the lowest mean value was in the Z FDM group, as shown in Figure 5. The Y SLA denture resin group showed the highest Vickers hardness values while SLA grey resin groups exhibited the lowest Vickers hardness values, as

shown in Figure 6. One way ANOVA test shows significant differences for flexural strength, impact strength and Vickers hardness, as shown in Table 1 in the appendix.

Table 4: Mean and SD of flexural strength, impact strength and Vickers hardness.

Samples Groups		**Flexural strength (MPa)** **Mean ± SD (n = 5)**	**Impact strength (kJ/m^2)** **Mean ± SD (n = 10)**	**Vickers Hardness (kg/ mm^2)** **Mean ± SD (n = 10)**
Heat cured		91.8 ± 17.18	1.38 ± 0.21	17.38 ± 0.65
Cold cured		117.22 ± 3.8	1.47 ± 0.34	17.03 ± 0.71
Milling		103.64 ± 2.15	1.76 ± 0.11	13.82 ± 0.41
FDM	X	64.87 ± 23.31	2.08 ± 0.71	10.51 ± 0.50
	Y	34.57 ± 19.05	2.36 ± 0.56	10.23 ± 0.31
	Z	23.28 ± 1.23	0.93 ± 0.08	9.23 ± 0.51
SLA Grey resin	X	53.15 ± 1.91	1.65 ± 0.15	5.71 ± 0.69
	Y	61.29 ± 2.33	1.59 ± 0.13	5.54 ± 0.32
	Z	61.2 ± 4.98	1.68 ± 0.14	5.13 ± 0.80
SLA Denture resin	X	89.16 ± 18.98	2.45 ± 0.2	13.25 ± 0.89
	Y	69.73 ± 12.43	2.27 ± 0.13	19.24 ± 3.17
	Z	91.26 ± 16.43	2.13 ± 0.09	13.65 ± 1.95

n: number of samples

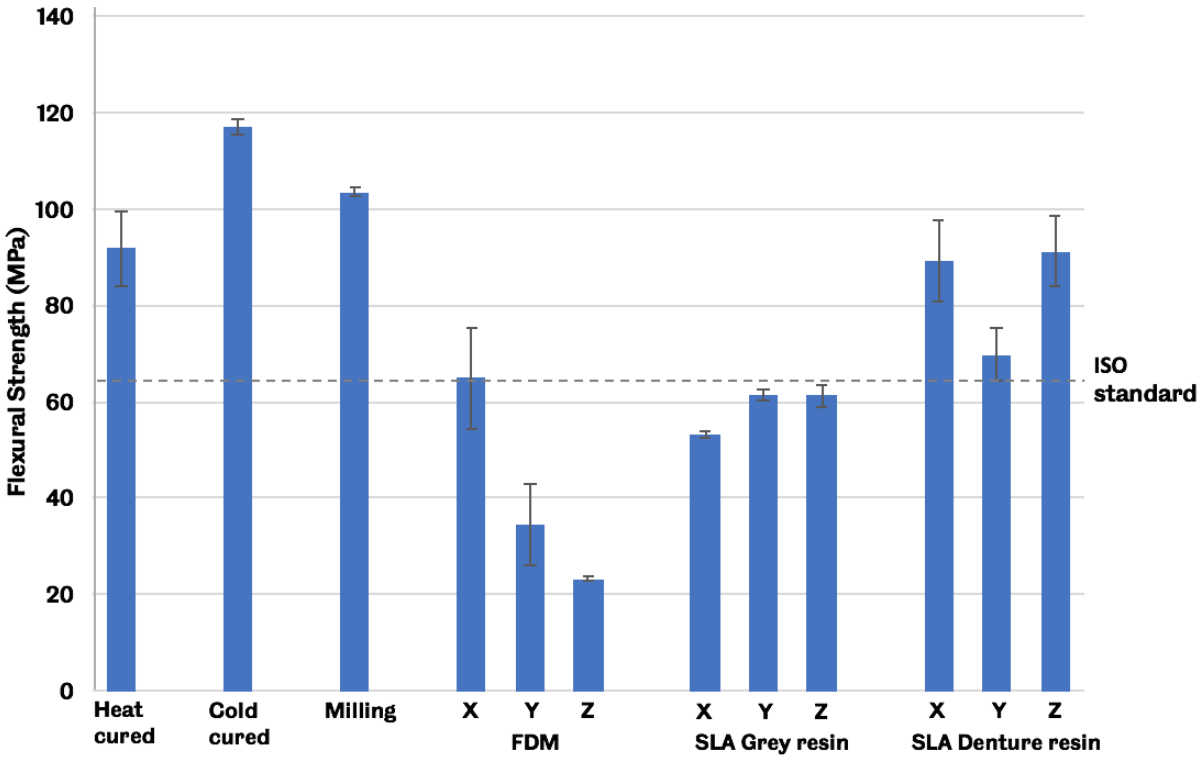


Figure 4. Mean values of flexural strength for all groups. Error bars represent standard errors.

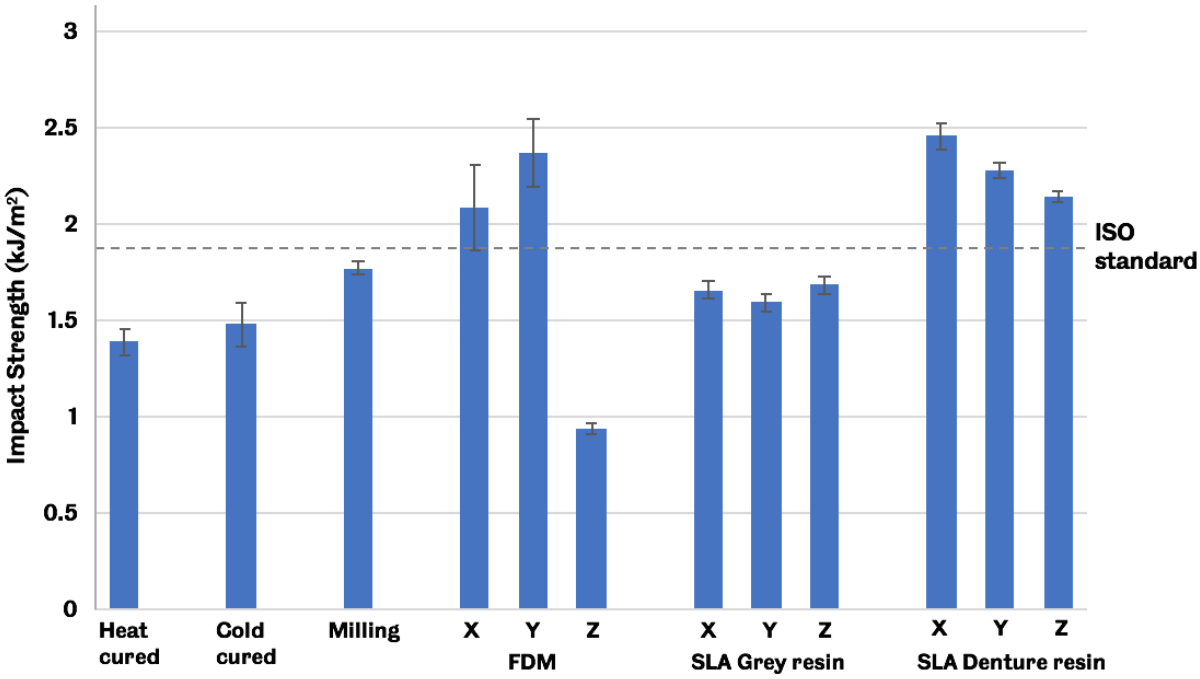


Figure 5. Mean values of impact strength for all groups. Error bars represent standard errors.

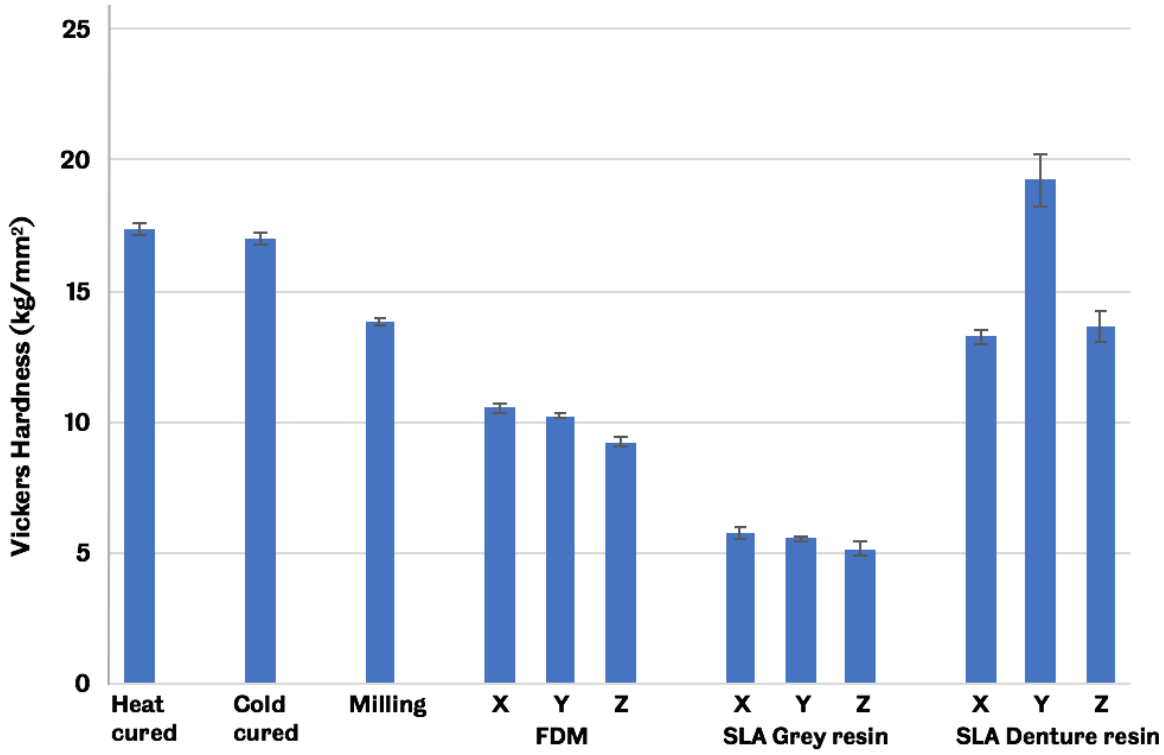


Figure 6. Mean values of Vickers hardness for all groups. Error bars represent standard errors.

4. Discussion

This study aimed to compare mechanical properties (flexural strength, impact strength and hardness) of acrylic denture base resins using different manufacturing methods and to compare these results against ISO standards of denture base polymers (ISO 20795-1:2013). Mechanical properties are one of the key factors that are used to evaluate the functional performance of removable prostheses materials. These properties include (i) flexural strength, which is defined as the maximum force applied to deform the material to fracture or permanent yield (Jaikumar et al., 2015; Aguirre et al., 2020) (ii) impact strength, which is the amount of energy that required to cause the fracture (Alhotan et al., 2021), and (iii) hardness, which is a significant feature to resist scratch or deformation on a material's surface (Batisse and Nicolas, 2021). High values of these properties are important to avoid fracture induced from high occlusion force or accidently dropping of removable prosthesis and to resist surface scratching that may induce from food or negligence (Ali et al., 2008).

Flexural strength results showed variation among the sample groups, the cold cured group performed higher than heat cured, with the milling group being comparable. The SLA grey

resin samples did not show high differences between subgroups (X, Y &Z), but all were below the ISO requirements. The FDM group showed large variation, with the 'X' printed samples passing the ISO standard, and the 'Y & Z' samples below the standard. The SLA denture resin group presented differences between subgroups (X, Y &Z), and all achieved the ISO requirements. The findings for this study agree with a number of research investigations which have revealed that printing orientation is a significant factor that impacts the mechanical properties of 3D printed objects (Dizon et al., 2018; Chantarapanich et al., 2013; Mohamed et al., 2015).

Impact strength results showed that only two of the FDM subgroups (X & Y) and the SLA denture resin group passed the ISO specifications. Heat and cold cured groups showed similar values (mean: 1.38, 1.47), while the milling group (mean: 1.76) presented better performance. 3D printed groups showed differences based on the design and manufacture approach of removable prostheses. Impact strength of SLA grey resin group was not significantly affected by the printing orientation of the samples which means more flexibility in design and manufacturing. The FDM group demonstrated a significant influence with printing orientation, with the Z samples (where the printing layers were in alignment with the direction of impact force) showed significantly low values. This means Knowledge of the likely applied force needs to be considered in a design and printing orientation.

The hardness test showed that the Y SLA denture resin group produced the hardest samples followed by heat and cold cure groups, and all samples offered a shiny appearance after the polishing procedure. The degree of surface smoothing is crucial in decreasing the accumulation of microorganisms and retaining undesirable particles, thus enhancing the cleaning procedure of removable dental prostheses (Al-Rifaiy, 2010).

This chapter has explored new fabrication methods of acrylic based removable dental prostheses and has revealed the achievement of superior mechanical properties by considering the design and production route. In addition, the finding of this study would be helpful in the future design and manufacture of low-cost items and FDM technique can provide dental applications such as custom trays and surgical guides with a cost-effective manufacturing approach.

This part of study has limitations; 3D PMMA filament and SLA grey resin were not optimised for manufacturing removable dental prostheses. Further enhancement for dental use would potentially improve mechanical properties. In addition, the investigation was restricted to a specific PMMA filament diameter of 2.85mm, as the LulzBot TAZ 6 (Aleph Objects, Inc., USA) used in this study only accepts 2.85 mm diameter. Moreover, several parameters that control the printing process, such as line width, print speed and layer height were not evaluated in this investigation. The inserted values of these parameters might impact the mechanical properties of 3D printed samples. Previously published work has advocated that mechanical feature of 3D printed products get affected by processing parameters such as layer height and width (Mohamed et al., 2015).

5. Conclusions

- Flexural behaviour of conventional and milling groups performed within the ISO specifications, while the impact strength of these groups failed to achieve the ISO specifications.

- Only two groups (X&Y FDM and SLA denture resin) met the ISO requirements for impact test.

- Printing orientation was significant to the mechanical properties measured, where superior properties were achieved when the printed layer oriented vertically to the direction of load.

- Y SLA denture resin group produced the hardest samples, and all samples could be polished to a glossy finish.

Chapter IV. Water sorption and solubility

1. Background

Many intraoral environmental factors might impact the durability of denture base resins and in consequence affect the life service of these resins (Agarwal et al., 2015); water sorption and solubility are two of these factors. ISO standards have defined the water sorption of denture base polymers as an increase in mass per volume, where solubility is the loss in mass per unit volume (ISO Standard 20795-1:2013). PMMA can absorb water gradually through time and this absorption is promoted by polarity features of the resin molecules due to unsaturated bonds of the molecules (Miettinen and Vallittu, 1997). The range and amount of water intake into polymer networks are mostly dominated by resin polarity, guided by a) the number of polar sites applicable to create hydrogen bonds with water, and b) network format (Tuna et al., 2008). Dogan and co-workers studied the water sorption and found that water dispersed in the polymer matrix as voids which formed as a result of gas bubbles or involved residual monomers (Doğan et al., 1995).

High water sorption can lead to adverse impact on the physical features of the denture base material, such Young's modulus and flexural strength, since water performs as a plasticizer of PMMA (Anusavice et al., 2012; Miettinen & Vallittu, 1997). Studies have shown that low mechanical properties of acrylic resin are mainly due to absorption of water and losing residual monomer which leaches out into saliva or water (Arima et al., 1996). Another study concluded that immersing a dental prosthesis in water would reduce the mechanical properties of resin and followed by enlargement of the polymer due to capacity of the water molecules to move the polymer chain apart (Dhir et al., 2007). In essence, dimensional performance of denture base resins is affected by water sorption and solubility (Cucci et al., 1998; Pfeiffer & Rosenbauer, 2004) as internal stresses within the resin can be formed which can be followed by crack development and finally denture fracture (Tuna et al., 2008). Water sorption has an advantage of compensating polymerization shrinkage while extended use could build a leaning for water, leading to long term plasticizing impact on the denture base resin (Dhir et al., 2007). This decreases its hardness, transverse strength and fatigue limit (Cucci et al., 1998; Tuna et al., 2008;).

In addition to dimensional instability of denture base resins, solubility may promote allergic reactions and irritation of the oral soft tissues (Pfeiffer & Rosenbauer, 2004). The major cause behind these adverse tissue reactions is residual monomer which exists more in auto polymerized denture base than heat polymerized one. Two obstacles are related to the presence of residual monomer: 1) the unreacted monomer has the ability to irritate soft tissues, affecting the biocompatibility of the denture base resin and 2) mechanical performance of the polymer is being affected by high concentration of methyl methacrylate monomer (Lung and Darvell, 2005). Therefore, it is desirable that denture base resins show low solubility degree (Figuerôa et al., 2018).

With advance progress in using additive techniques for denture base manufacturing, there is a lack of published studies evaluating the water sorption and solubility of 3D printed denture base resins. Therefore, this study is aimed to evaluate the water sorption and solubility properties for conventional, milling and 3D printing techniques.

2. Materials and Methods

Water sorption and solubility samples were distributed in 5 groups according to the previously described manufacturing methods as shown in Table 5. 3D printed groups were subdivided to two subgroups; X & Y based on the orientation of the 3D print see Figure 7 (A). Samples were designed and tested in accordance with ISO 20795-1:2013 (Denture Base Polymers).

Table 5: Sample group distribution.

Group number	**Description**
Group 1/ Heat cured	Conventional heat cured acrylic resin
Group 2/ Cold cured	Conventional cold cured acrylic resin
Group 3/ Milling	Subtractive (milled) from PMMA block
Group 4/ FDM	Additive (FDM), PMMA filament, X & Y
Group 5/ SLA	Additive (SLA), Grey and Denture base resin, X &Y

2.1 Manufacturing of Samples

Conventional heat-cured samples (Group 1): samples (n=5) were prepared using a plaster mould made by investing disc shaped wax patterns (50 x 1 mm) then these patterns were removed after plaster setting. The acrylic resin ProBase® Hot (Ivoclar Vivadent AG) was mixed and packed in the flasks according to the manufacturer's instructions. Then the flasks were placed in a wet curing unit (Eclipse Paco) for the polymerisation process.

Conventional cold-cured samples (Group 2): (n=5) samples with dimensions (50 x 1 mm) were prepared by mixing powder and liquid of cold cure acrylic resin ProBase® Cold (Ivoclar Vivadent AG), according to the manufacturer's instructions. The resin was poured into the custom mould as shown in Figure 7 (B), then placed in a pressure polymerisation unit (Dreve Polymax 5) at 23 C for 30 minutes.

Subtractive (milled) samples (Group 3): cylindrical shaped specimen with dimensions (50 x 25 mm) was designed using Fusion 360 ™ software, (Autodesk Inc.). The design was imported to another computer software (Sum3D), which was used to set up the milling settings followed by milling the cylinder with a 5- axis milling machine (Roland DWX-50) from a PMMA disc (IvoBase CAD) as shown in Figure 7 (C). To save the material, one milled cylinder was used to get 5 samples with dimensions (50 x 1 mm) using a precision cutter (Precision Saw-Isomet-1000, Buehler).

Additive (FDM) and (SLA): disc shaped specimen with dimensions (50 x 1 mm) was designed using Fusion 360™ software, (Autodesk Inc.). **For FDM group,** the design file was imported into (Cura LulzBot Edition version 2.6.52), which was used to set up the printing parameters. 5 samples for each printing orientation were printed using a desktop 3D printer (LulzBot TAZ 6, Aleph Objects, Inc., USA) and a 3D printer filament of Poly Methyl Methacrylate (PMMA) (Material4print, Germany) was used for this printing, with manufacturer's instruction to build the plate temperature 100°c and 240°c for the printing temperature. The diameter of this filament was 2.85mm, and the infill density of printing was 100%. **For SLA group,** the design was imported into (PreForm Software 2.18.0), which was used to set up the printing parameters. 5 samples for each printing orientation were printed using a desktop 3D printer (Form 2, Formlabs Inc.) and the 3D printer resins (Grey and Denture base, Formlabs Inc.) for

this printing. The grey resin was printed with the 100-micron resolution while the denture base resin was printed with 50-micron resolution.

All group samples were finished by using wet grinding with P500, 1000, and 1200 grit paper (SiC grinding paper, Buehler, Germany) to reach the desired thickness of (0,5 ± 0,1) mm according to the ISO 20795-1:2013.

2.2 Testing procedure of water sorption and solubility

The process of the test consists of three steps, as follow:

2.2.1 Conditioned samples

Each group samples were placed in the rack inside a desiccator containing the dried silica gel as shown in Figure 7 (D). The desiccator was kept in the oven at 37 °C for 24 h followed by transfer of samples to the second desiccator supplied with freshly dried silica gel. The second desiccator was kept at 24°C for 1 h then the samples weighed using an analytical weighing balance (Mettler, AJ-100). The desiccator remained closed in all the process except for the removing and replacing samples. After weighing all samples, the silica gel in the first desiccator was replaced with freshly dried gel. The cycle described above was repeated until obtaining a constant mass, called conditioned mass m1, where the loss in mass of each sample is not more than 0,2 mg between two consecutive weightings. After that, the volume (mm^3) of each sample was measured by calculating the mean of three diameter measurements and the mean of five thickness measurements at four equally spaced locations at the circumference of the sample, together with a centre measurement.

2.2.2 Wet samples

The samples were immersed in water bath (VWR Collection) at 37°C for 7 days. After that, the samples were removed and wiped with a clean, dry towel and waved in the air for 15 s. Then they were weighed after 60 s of removal from the water and the samples' weight recorded as wet mass, m2.

2.2.3 Reconditioned samples

The samples were reconditioned to constant mass with the same conditions applied to the first drying process as described early in the first step. The samples' weight is recorded as reconditioned mass, m3.

Calculation of water sorption and solubility:

Water sorption, Wsp, value can be calculated by applying the following equation:

Wsp = m2- m3 / v

Water solubility, Wsl, value can be calculated by applying the following equation:

Wsl = m1- m3 / v

Where

m1 is the conditioned mass of the sample in μg.

m2 is the wet mass of the sample in μg.

m3 is the reconditioned mass of the sample in μg.

V is the volume of the sample in mm^3.

2.3 Statistical analysis

All data were statistically analysed with one–way analysis of ANOVA, using SPSS software (version 22). P-values less than 0.05 were considered statistically significant.

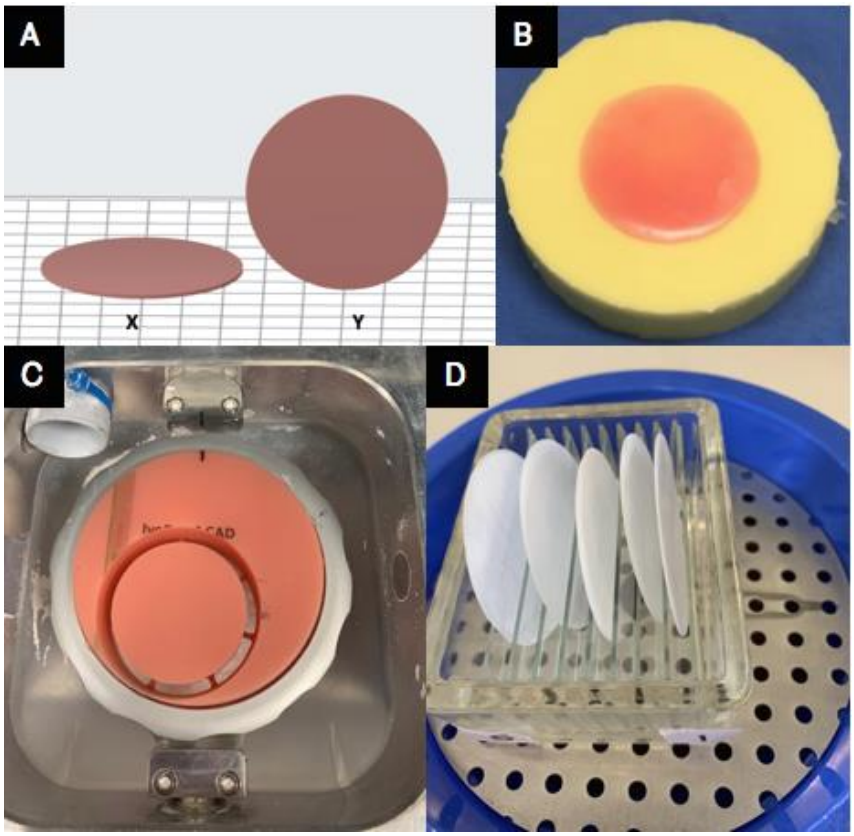


Figure 7. The process of manufacturing and testing water sorption and solubility sample; (A): the orientation of the 3D printed sample, (B): cold cure resin poured in the custom mould, (C): milled cylinder from PMMA block, (D): samples inside the desiccator containing the silica gel.

3. Results

All group samples were prepared and measured according to ISO 20795-1:2013 specifications. Table 6 shows the mean and the standard deviation of water sorption and solubility for each group. As shown in Figure 8, The highest mean value of water sorption can be seen in SLA (Grey resin) groups while the lowest mean value among the groups is shown in both the milling group and SLA (X Denture resin). For solubility, the SLA (X Grey resin) group showed the highest mean value, however the SLA (Y Denture resin) group showed the lowest mean value among groups, as shown in Figure 9. One way ANOVA test shows significant differences for water sorption and solubility, as shown in Table 2 in the appendix. The raw data of the conditioned mass (m1), the wet mass (m2), the reconditioned mass (m3) and the volume (v) of each sample are presented in the appendix (Table 3 to 11).

Table 6: Mean and SD of water sorption and solubility.

Samples Groups			Water sorption (µg / mm³) Mean ± SD (n = 5)	Water solubility (µg / mm³) Mean ± SD (n = 5)
Heat cured			23.127 ± 2.248	0.119 ± 0.410
Cold cured			20.951 ± 3.025	0.676 ± 0.163
Milling			20.132 ± 1.249	1.544 ± 2.213
FDM	X		28.248 ± 2.290	- 0.117 ± 0.037
	Y		25.642 ± 1.482	- 0.165 ± 0.120
SLA	Grey resin	X	54.728 ± 2.412	5.124 ± 0.355
		Y	55.647 ± 1.381	2.623 ± 0.500
	Denture base resin	X	20.204 ± 1.145	0.113 ± 1.134
		Y	22.317 ± 1.253	- 0.372 ± 0.234

n: number of samples

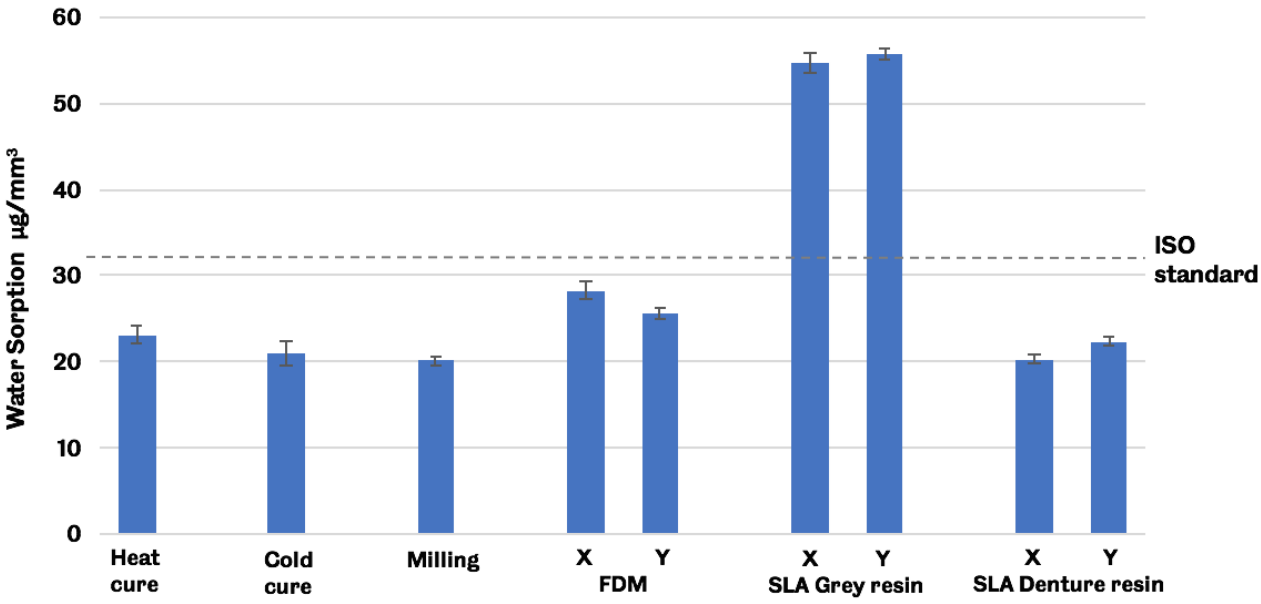


Figure 8. Mean values of water sorption for all groups. Error bars represents standard errors.

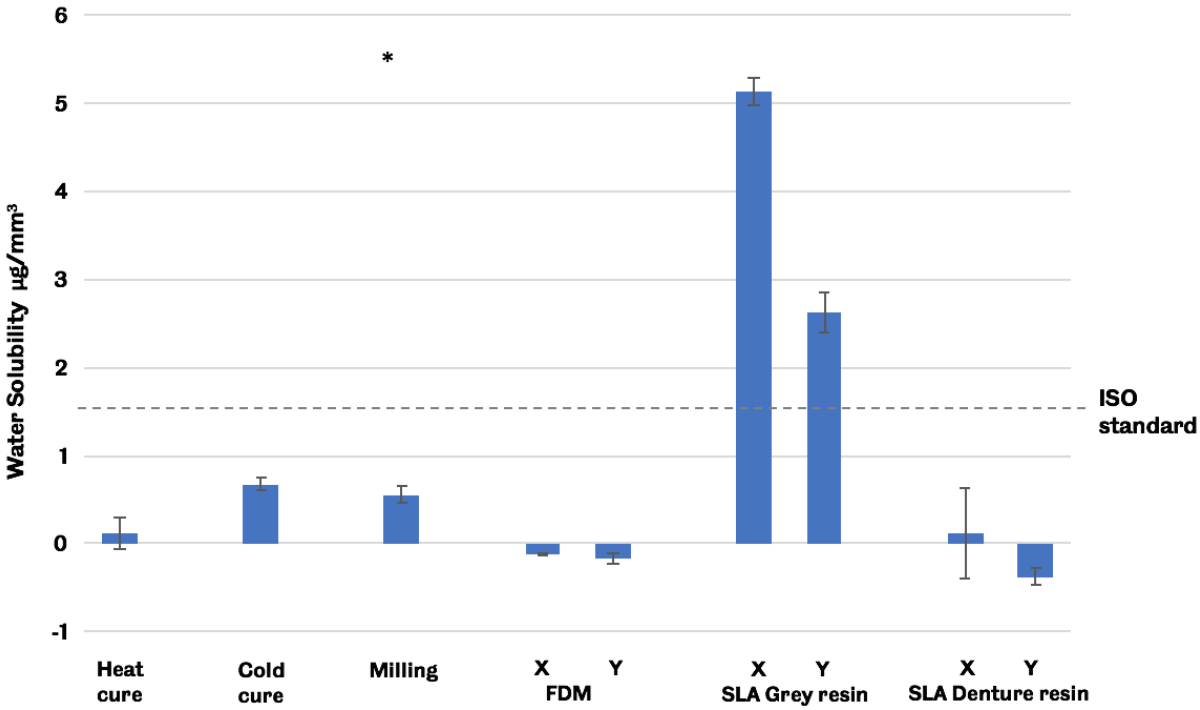


Figure 9. Mean values of water solubility for all groups. * (5.49) is a value of one sample of milling group. Error bars represents standard errors.

4. Discussion

This study was aimed to investigate water sorption and solubility of acrylic denture base resin manufactured through different methods including 3D printing techniques. The American Dental Association recommends that water sorption is measured by deducting the sample's conditioned mass (m1) from sample's saturation mass (m2) (Figuerôa et al., 2018). Another method of evaluating water sorption, which is recommended by ISO standard and used in this study, is measured by deducting the sample's final dry (reconditioned mass, m3) from the sample's saturation mass (m2) and should not exceed 32 µg/mm^3. While solubility displays the mass of the soluble materials from denture resin and should not exceed 1.6 µg/mm^3 (ISO, 2013). These properties determine how the denture base material acts in a wet environment.

The study showed several limitations; samples were fabricated with the dimension of 1mm thickness and then polished to achieve the desired thickness (0.5 ± 0.1 mm) which is required by the ISO standard. This way of manufacturing was followed since it was difficult to fabricate samples with the 0.5 mm thickness especially with the 3D printing techniques. Polishing

procedure does not affect the chemical composition of the sample, but it would change the surface properties of the sample through eliminating the irregularities of the sample's surface and theoretically the contact area between the sample' surface and the solution will decrease. There is a positive correlation between the water sorption degree and the surface roughness (Rahal et al., 2004). Another limitation is related to the solution, distilled water, was used in this study at a temperature of 37 ºC and it is known to be effective for leaching before delivering dental prostheses, but nothing is identified about the solubility of intraoral fluids that are absorbed by edentulous patients. Also, samples were kept in the water bath for 7 days with a stagnant status without freshness of the solution while in the mouth, saliva is produced and absorbed regularly. From that, we can conclude that the test conditions did not mimic the oral environment of edentulous patients.

Water sorption results presented that all groups achieved ISO specifications except both subgroups (X&Y) of SLA grey resin group. Cold cure and milling groups showed similar values (mean: 20.95, 20.13), while the heat cure group (mean: 23.12) presented slightly higher sorption performance. For additive techniques, FDM group and SLA denture resin group showed no significant difference between the two subgroups (printing orientations groups, X & Y). This means there are no restrictions about printing the denture base with specific position.

Same as water sorption results, all groups passed the ISO specifications for solubility test except the SLA Grey group in both orientations. Cold cure group showed higher solubility values than heat cure, while the milling group performed within the border of the ISO requirements. For 3D printing groups, both subgroups (X&Y) of FDM group and Y SLA denture resin group showed negative mean values. The X SLA denture resin group showed similar values with the heat cure group (mean: 0.113, 0.119).

The finding of this study about low solubility rate in heat cure resin than cold cure one is coincidence with literature which explained that heat cure resin is processed under high temperature for long duration and thus resulting in low residual monomer concentration and therefore low solubility rate (Figuerôa et al., 2018; Miettinen and Vallittu, 1997; Tuna et al., 2008). In this study, different techniques of manufacturing with different commercial forms of PMMA materials (resin, block and filament) were used to fabricate the samples. These

materials are different in their chemical composition and this composition plays a significant role in determining the sorption and solubility of the material (Perea-Lowery et al., 2021).

The SLA grey group showed a different pattern behaviour after 7 days on the water bath and this behaviour was not noticed in other groups where samples kept their initial pattern, flat disc pattern. As seen in Figure 10, samples of this material are getting curved, and the outer form changed. This resin is classified by the manufacturer as a standard resin and not characterised as a resin for dental appliance manufacturing. So, it is not surprising that this resin didn't pass the ISO specifications for water sorption and solubility of denture base polymers.

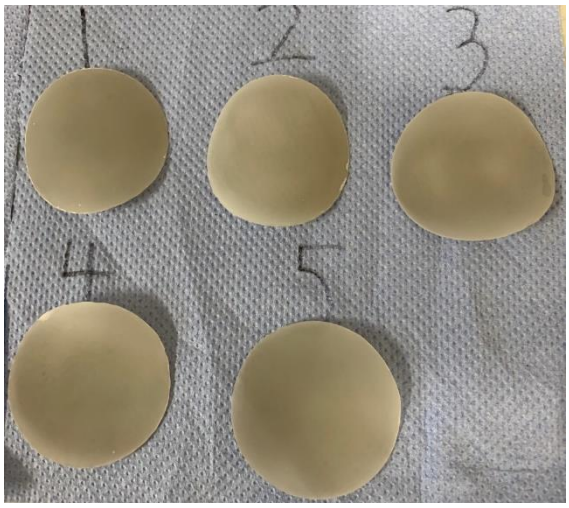


Figure 10. Samples of SLA grey resin after 7 days of soaking in water.

The findings of this study add worthwhile knowledge about fused deposition modelling (FDM) which can be used as an alternative method for manufacturing denture prostheses. Although the PMMA filament that is used within FDM is not custom to prepare dental prostheses, this filament presents promising data in terms of water sorption and solubility properties. Further development on this filament would make this material a cost-effective choice in prosthodontic dentistry.

5. Conclusions

- All tested groups except SLA grey resin group are working within ISO limit for water sorption and solubility.
- The printing orientations (X &Y) of FDM and SLA groups have no significant effect on the water sorption and solubility properties, which means no restriction for denture printing with specific orientation.

Chapter V. Tooth bonding

1. Background

Bonding between the denture base material and prefabricated artificial denture teeth is a key factor for achieving a successful and functional removable denture. PMMA resin is a common material for producing denture teeth and acrylic teeth are favoured over porcelain teeth since they can chemically bond to the denture base resin. However, these teeth can show problems in use such as fracture, tooth wear, and debonding from the denture base (Clements et al., 2018). Between all failures of removable dentures, the frequency of tooth debonding represents an estimated percentage between 25 and 33% (Nakhaei, 2018; CHUNG et al., 2008). Detachment of acrylic teeth from the denture base can occur inside or outside the patient's mouth and many causes can drive to this detachment. Such causes include processing errors during the fabrication procedure, problems related to the material itself or excessive forces applied during function (Palitsch et al., 2012; CHUNG et al., 2008). The masticatory forces created in the denture teeth during function differ among various conditions of opposing teeth; whether they are natural teeth, or denture teeth, or implant supported prosthesis. Occlusal forces are high with implant supported prosthesis and this means high risk of tooth fracture or debonding (Moffitt et al., 2008; Clements et al., 2018).

The bond between acrylic teeth and the denture base resin is affected by two mechanisms: the physical contact of denture teeth with the denture base resin during polymerization process and the formation of an interwoven polymer network (IPN) during the polymerization process (Patil et al., 2006). Sufficient bonding of acrylic teeth to the denture base is crucial for increasing the strength and durability of removable prosthesis and consequently low failure rate. Attempts have been made to enhance the bond strength including chemical treatments as for instance application of methyl methacrylate monomer and mechanical adjustment of the base surface of acrylic teeth such as surface grooving (Lang et al., 2012; Krishna et al., 2014).

Since the fabrication method of removable denture is one of significant factors that affect the bonding strength between the artificial teeth and the denture base (CHUNG et al., 2008), other methods have been explored and applied for denture manufacturing. These methods

include subtractive manufacturing (milling) and additive manufacturing (3D printing). Milling technique is based on using a PMMA block while 3D printing technique is based on using photopolymer resins to fabricate both the denture base and the denture teeth. It is important to highlight that currently available literature is poor regarding studies that evaluated the bonding strength of acrylic teeth to denture base resin fabricated either by milling or 3D printing. Thus, this study is aimed to compare the bonding strength of acrylic teeth to PMMA denture base resin fabricated by conventional methods, milling and 3D printing.

2. Materials and methods

Tooth bonding samples were distributed in 5 groups as shown in Table 7 and the SLA and FDM groups were subdivided to two subgroups; X & Y based on the orientation of the 3D print, see Figure 11 (A). Master waxed sample was prepared according to the ISO specifications of the tooth bonding test (ISO/TS, 2017). These specifications include preparing a cylinder with dimensions (20 mm height and 25 mm diameter) and then placing a central incisor artificial tooth on the vertical axis of the cylinder with 1 mm of wax covering the neck of the tooth. Then the master waxed sample (Figure 11 (B)) was duplicated using silicone-based duplication material (FINOSIL 15 Duplicating Silicone, A and B, FINO, Germany) according to manufacturer's instructions to produce a master silicone mould, as shown in Figure 11 (C). After that, the tooth was removed from the master sample and the scanner machine (Identica Blue Scanner, Medit) was used to scan the master sample and convert this scanning to stl. file. The central incisor artificial tooth was scanned by intraoral scanner (Primescan, Dentsply Sirona) and a stl. file was created from this scanning to print the tooth. To standardize the process of manufacturing, the silicon mould was used in heat & cold cure groups and the stl. files were used in all digital manufacturing.

Table 7: Sample group distribution.

Group number	**Description**
Group 1/ Heat cured	Conventional heat cured acrylic resin
Group 2/ Cold cured	Conventional cold cured acrylic resin
Group 3/ Milling	Subtractive (milled) from PMMA block
Group 4/ FDM	Additive (FDM), PMMA filament, X &Y
Group 5/ SLA	Additive (SLA), Grey and Denture base resin, X &Y

2.1 Manufacturing of Samples

Conventional heat-cured samples (Group 1): samples (n=6) were prepared by using the silicon mould to create wax investment patterns. These wax patterns were invested in plaster mould by flasking then the wax was removed after plaster setting. The acrylic resin ProBase® Hot (Ivoclar Vivadent AG) was mixed and poured according to the manufacturer's instructions. Then samples were removed from the flask.

Conventional cold-cured samples (Group 2): samples (n=6) were prepared by the silicon mould where the artificial tooth was placed and then cold-cured acrylic resin ProBase® Cold (Ivoclar Vivadent AG) was mixed and poured according to the manufacturer's instructions. Then samples were removed from the mould.
Subtractive (milled) Samples (groups 3): the stl. file of master sample design was imported to computer software (Sum3D), which was used to set up the milling settings. Then 6 samples were milled by a DWX-50 milling machine (5-axis, USA) from a PMMA disc (IvoBase® CAD, Ivoclar Vivadent AG). Artificial teeth were bonded to milled samples by self-curing two component bonding system (IvoBase® CAD Bond, Ivoclar Vivadent AG).

Additive (FDM) PMMA samples (group 4): the stl. file of master sample design was imported into (Cura LulzBot Edition version 2.6.52), which was used to set up the printing parameters. 6 samples were printed in each different orientation (X&Y) using a desktop 3D printer (LulzBot TAZ 6, Aleph Objects, Inc., USA) and a 3D printer filament of Poly Methyl Methacrylate (PMMA) (Material4print, Germany) was used for this printing, with manufacturer's instruction to build the plate temperature 100°c and 240°c for the printing temperature. The diameter of this filament was 2.85mm, and the infill density of printing was 100%. Artificial teeth were bonded to printed samples by self-curing acrylic (Orthoresin, Dentsply Sirona).

Additive (SLA) PMMA samples (group 5): the stl. file of master sample design was imported into (PreForm Software 2.18.0), which was used to set up the printing parameters. 6 samples were printed in each different orientation (X&Y) using a desktop 3D printer (Form 2, Formlabs Inc.) and the 3D printer resins (Grey and Denture base, Formlabs Inc.). The grey resin was printed with the 100-micron resolution while the denture base resin was printed with 50-micron resolution. Artificial teeth were bonded to grey and denture base printed samples by

self-curing acrylic (Orthoresin, Dentsply Sirona). In addition, teeth were printed by using 3D printer resin (Denture teeth resin, Formlabs Inc.), Figure 11 (E), and bonded to the denture base cylinder, Figure 11 (D), by uncured denture base resin following manufacturer instructions to get 3D printed tooth bonding sample, as seen in Figure 11 (F).

Additional part (practice based) / not ISO specifications based:

The previous methods of fabrication for heat cure, cold cure, milling and the X SLA denture resin groups were repeated but, in addition to this, the fitting surface of prefabricated teeth was roughened by hand piece (in order to remove the glaze layer) and then painted using methyl methacrylate monomer. Also, X SLA denture resin groups were prepared using uncured denture base resin (A) and self-cure (ortho resin) (B) as bonding agents.

2.2 Tooth bonding test

Tooth bonding samples were subjected to shear strength test, according to ISO/TS 19736:2017 using a Lloyd LRX Universal Testing Machine (AMETEK, Inc.). A vertical load of 2.5kg with a speed of 1 mm/min was applied by shear pin on the incisal edge of palatal surface of artificial tooth, Figure 11 (G), until fracture occurred. Then shear strength values were obtained from computer software (NEXYGEN 4.1, Lloyd Instruments) connected to the testing machine. Also, the mode of fracture (adhesive, cohesive or mixed) was visually examined.

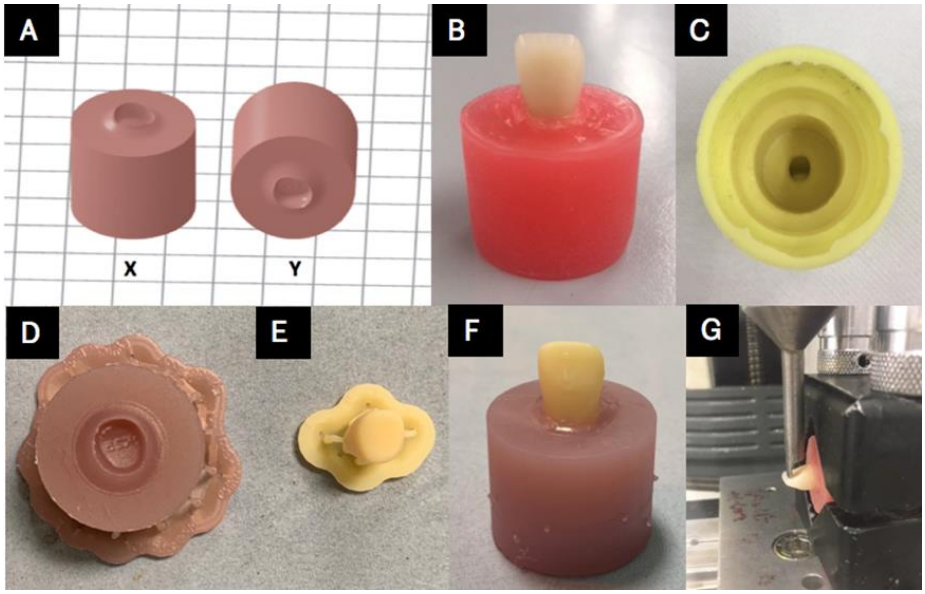


Figure 11. the process of manufacturing and testing tooth bonding sample; (A): the orientation of the 3D printed sample, (B): master waxed of tooth bonding sample, (C): silicon mold for tooth bonding sample, (D): 3D printed cylinder base, (E): 3D printed tooth, (F): 3D printed tooth bonding sample (after assembling D & E), (G): shear strength test using the universal testing machine (Lloyd LRX, AMETEK, Inc.).

3. Results

Results of the tooth bonding test will be presented in 2 cycles; the first cycle is ISO based only (with no adjustment on the prefabricated teeth) while the second cycle is ISO based and practice based (with mechanical and chemical adjustment on the prefabricated teeth). Each cycle will show shear bonding strength (SBS) and mode of fracture. Regarding SBS, Table 8 and Figure 12 show mean and standard deviation of all sample groups for the first cycle, where the Y SLA denture resin group displayed the highest SBS among tested groups. Table 9 and Figure 14 show mean and standard deviation of all sample groups for the second cycle, where the cold cure group showed the highest SBS among groups. One way ANOVA test shows significant differences between and within groups for shear bonding strength of the first cycle and no significant differences for shear bonding strength of the second cycle. P-values less than 0.05 were considered statistically significant. The second way of presenting tooth bonding results is counting and mode of fracture which is used to determine the pass or fail of the samples according to ISO specifications, as shown in Figure 13 for the first cycle and Figure 15 for the second cycle.

Table 8: Mean and SD of shear bonding strength of the first cycle.

Samples Groups			**Shear bonding strength (N) Mean ± SD (n = 6)**
Heat cured			108.43 ± 61.05
Cold cured			211.54 ± 28.25
Milling			97.53 ± 20.32
FDM	X		112.56 ± 23.17
	Y		132.26 ± 40.82
SLA	Grey resin	X	90.63 ± 25.99
		Y	115.95 ± 35.52
	Denture base resin (A)	X	76.86 ± 39.55
		Y	140. 82 ± 50.15
	Denture base resin (B)	X	297.36 ± 51.11
		Y	326.25 ± 51.92

(n) number of samples, (A) with prefabricated teeth, (B) with printed teeth.

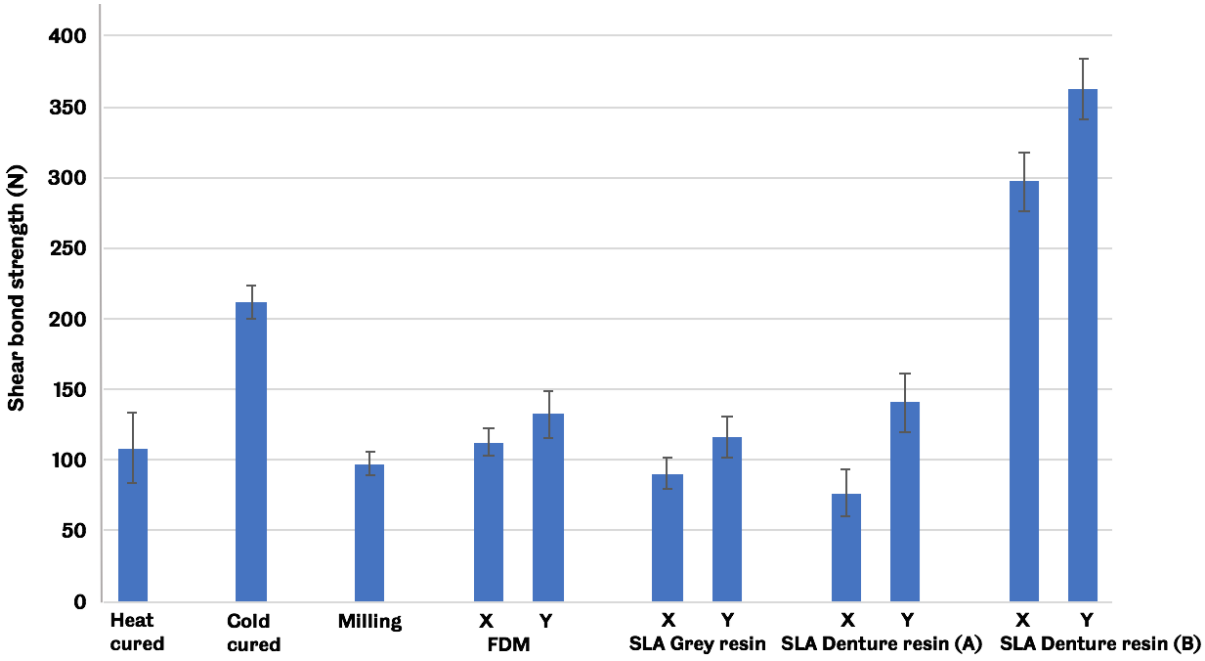


Figure 12. Mean values of shear bonding strength for all groups of the first cycle. A: with plastic teeth, B: with printed teeth. Error bars represents standard errors

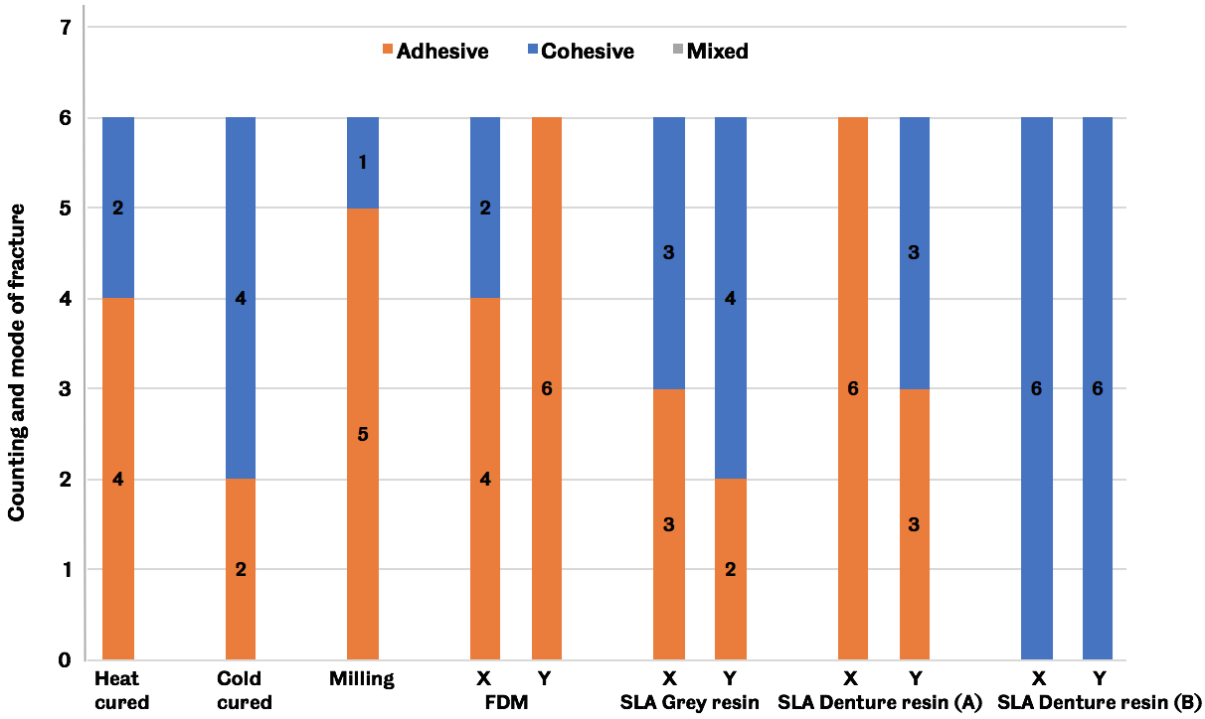


Figure 13. Counting and mode of fracture (adhesive, cohesive or mixed) of tooth bonding samples for the first cycle. A: with plastic teeth, B: with printed teeth.

Table 9: Mean and SD of shear bonding strength of the second cycle.

Samples Groups	Shear bonding strength (N) Mean ± SD (n = 6)
Heat cured	213.78 ± 66.40
Cold cured	252.43 ± 86.06
Milling	298.48 ± 72.39
X SLA Denture base resin (A)	237.32 ± 128.64
X SLA Denture base resin (B)	228.46 ± 59.46

(n) number of samples, (A) with uncured denture base resin, (B) with self-cure resin.

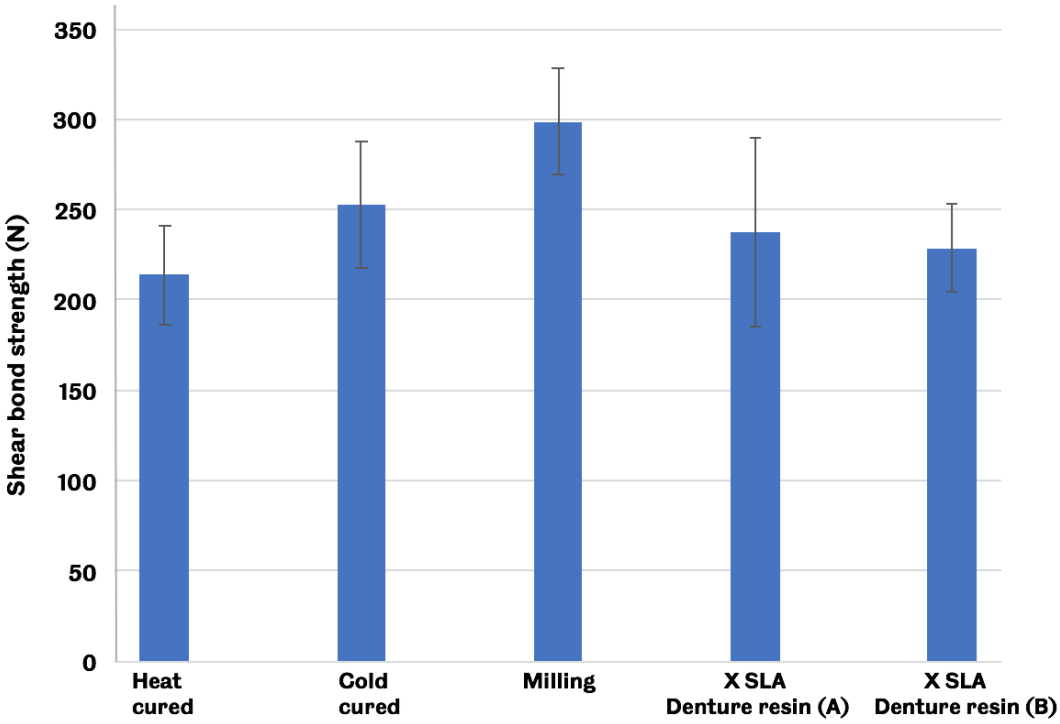


Figure 14. Mean values of shear bonding strength for all groups of the second cycle. A: with uncured denture base resin, B: with self-cure resin. Error bars represent standard errors.

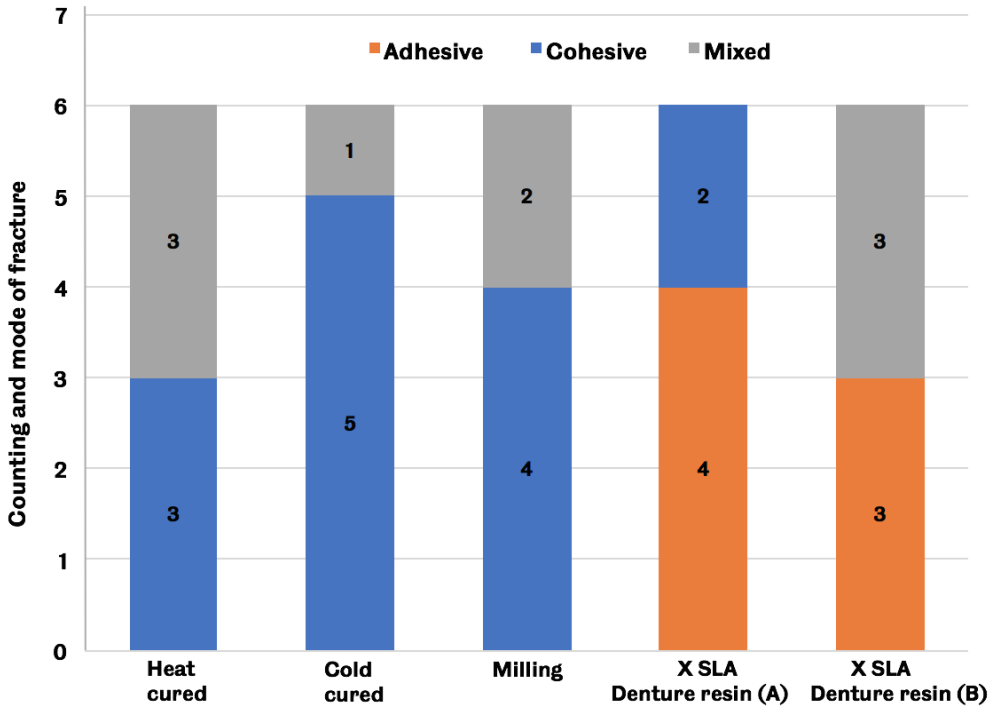


Figure 15. Counting and mode of fracture (adhesive, cohesive or mixed) of tooth bonding samples for the second cycle. A: with uncured denture base resin, B: with self-cure resin.

4. Discussion

Tooth bonding test aimed to evaluate the bonding strength between artificial teeth and acrylic denture bases manufactured by different methods and compare the results against ISO specifications of tooth bonding test (ISO/TS, 2017). This ISO classified fracture mode to adhesive where fracture occurs along the interface between teeth and base material, cohesive where fracture occurs within base material or the tooth, or mixed where the fracture mode is partially adhesive and cohesive. At least 4 out of 6 samples of each group should display cohesive or mixed fracture to achieve ISO standards (ISO/TS, 2017).

This study presented some limitations; the first one is related to the nature of applied load. In the oral environment, the masticatory load is dynamic during the denture function while in the performed test the load was static. Also, the denture is retained against resilient oral mucosa which may absorb some stresses that generate from masticatory force while this condition was not simulated in this study. Another limitation is related to unsuitable surface aspect at the junction of the prefabricated tooth and the denture base resin. One study has

revealed that different structures of the prefabricated tooth and the denture base resin since they processed via different routes cause the debonding (Patil et al., 2006).

In this study, the test was carried in two cycles; the first cycle is without any modification on the prefabricated teeth (as recommended by ISO specifications) while the second cycle is with modifications (the fit surface of the prefabricated teeth was roughened and painted by methyl methacrylate monomer). The count and mode of fracture of the first cycle was investigated and it was found that adhesive failure occurred in most of the groups. Only cold cure, Y SLA grey resin and SLA denture base resin (B) groups passed the ISO requirements for tooth bonding test. Orientation of print layers does not show significant difference in fracture behaviour of 3D printed samples. Also, shear bonding strength was evaluated, and Y SLA denture base resin (B) group displayed high shear bonding strength among tested groups.

The present method of the first cycle is problematic since the variation in monomeric contacts between the groups possibly influences the results. And to avoid this problem and to standardize on all tested groups, the second cycle was created where the fitting surface of the prefabricated teeth was roughened and painted with methyl methacrylate monomer. All groups of the second cycle except (A)&(B) X SLA denture base groups pass the ISO specifications in terms of counting and mode of fracture of tooth bonding test. For shear bonding strength, milling groups showed the highest value among tested groups. Although X SLA denture resin groups showed similar SBS values to heat and cold cure, they failed to achieve the ISO standards. This was expected since the method that was used in fabrication was not recommended by the manufacturer instructions. However, when the manufacturer instructions were followed for SLA denture base resin group in the first cycle, this group showed excellent SBS results and passed the ISO requirements.

Tooth bonding failure is a common scenario in clinical practice (Bhochhibhoya et al., 2016) and in order to minimise this failure, many attempts such as mechanical modification and chemical treatment have been investigated. Many studies have revealed that mechanical modification (retention grooves) on denture teeth can result in superior bond strength between denture teeth and denture base (Fletcher et al., 1985; Spratley, 1987; Cardash et al., 1990). Another attempt is chemical treatment where the ridge lap surface of an artificial tooth is painted using a chemical agent such as methyl methacrylate monomer. Published literature

supports the use of chemical treatment to develop bond strength (Geerts and Jooste, 1993; Saavedra et al., 2007). One study has reported that breaking the glaze of the fitting surface of prefabricated teeth significantly lowers the failure of bonding (CHUNG et al., 2008). The finding of the second cycle concurred with the literature, where the method of roughening the fitting surface of the prefabricated teeth to remove the glazed layer and applying methyl methacrylate monomer enhanced the results of fracture mode to be within the ISO standards specifications and increased the shear bonding strength. According to Bhochhibhoya et al 2016, maximum masticatory force created by complete denture individuals is about 90 N and based on the present results, shear bond strength of most groups is higher than the required force for masticatory function. Therefore, bond strength of the tested groups would be clinically acceptable. Scientific literature has no published studies evaluating the tooth bonding strength of milling and 3D printing techniques in accordance with ISO standards. Thus, the finding of this study has added effective /valuable knowledge in terms of evaluating the bonding behaviour of the tooth to the denture base fabricated by milling and 3D printing techniques.

5. Conclusions

- Only two groups (cold cure and Y SLA) performed within ISO specifications for tooth bonding test in the first cycle.

- All groups in the second cycle except (A) & (B) X SLA denture base resin achieve ISO standards for tooth bonding test.

- Modifications on the prefabricated teeth such as mechanical and chemical, are required to pass ISO standards and enhance the shear bond strength between the prefabricated teeth and the denture base.

- Following manufacturer instructions of 3D printing samples is mandatory to achieve acceptable results of tooth bonding strength.

Chapter VI. Microbiological characterisation

1. Background

Denture bases can act as a reservoir for many microorganisms that are present intraorally and these microorganisms may induce a variety of oral diseases (Budtz-Jorgensen, 1981; Samaranayake et al., 1980). One of these diseases is denture stomatitis which is developed mainly by the presence of candida albicans (Budtz-Jorgensen, 1981; Karaagaclioglu, 2008). Clinical studies have shown that denture stomatitis affects between 27 to 65% of denture wearers (Kulak et al, 1997; Morimoto et al., 1987; Salerno et al., 2011). Denture stomatitis is a pathogenic condition affecting the denture bearing the mucosa surface and the fitting surface of the maxillary denture is the popular place for aggregation of candida albicans colonies (Murat et al., 2019).

Adhesion of microorganisms such as candida albicans can be influenced by denture material surface properties such as surface roughness, surface free energy and hydrophobicity. Dentures with roughest surfaces tend to show high microorganism colonisation and dentures with high energy surfaces are more susceptible to plaque accumulation than low energy ones (Akalin-Evren et al. 2014). So, to minimise the adhesion of Candida albicans, the surface of denture base resins needs to be smooth, less porous and hydrophilic (Fouda et al., 2019). A study conducted by (Quirynen et al, 1990) concluded that a value of 0.2 µm is assumed as a roughness threshold where below this value no influence on microorganism adhesion can be observed.

In essence, smooth surfaces of dentures are required for prolonged use/ service of the denture, patient satisfaction, low plaque accumulation and good aesthetic appearance (Akalin-Evren et al. 2014). Studies have found that dependent elderly patients with dentures are neglected in term of denture hygiene (MacEntee, 2000; Preston et al., 2006) and therefore efforts have been applied to investigate surface treatments for acrylic dentures, one of them including alteration of surface charge by incorporation of methacrylic acid to minimise candida adhesion (Park et al., 2003). Another attempt of inhibition of candida adhesion is to apply antifungal coatings on denture resins. These attempts have shown good results in decreasing the candida adhesion to the acrylic denture surfaces; however, physical

properties and biocompatibility of these modified resins might get affected (Zamperini et al., 2013). Despite the advantages of PMMA highlighted in previous chapters, this material shows poor antimicrobial properties which can trigger oral infection and generate bad odour (Lee et al., 2016) as well as denture stomatitis which is a crucial problem with PMMA (Aguayo et al. 2017).

Technological improvements in digital dentistry have been implemented to introduce new materials that show sufficient mechanical and microbiological performance for clinical use. In order to fabricate digital dentures, CAD/CAM PMMA resins are used, which are different from conventional PMMA resins in chemical structure, mechanical properties and polymerisation techniques (Murat et al., 2019). CAD/CAM PMMA resins are polymerised under high temperature and pressure, and this enhances biocompatible outcome with less porosity and low residual monomer (Kawaguchi et al., 2014; Steinmassl et al., 2017). With the high increase in the use of 3D printing techniques for denture manufacturing, it is important to evaluate microorganism attachment on 3D printed denture resins. Thus, this study is aimed to evaluate the effect of surface characteristics such as surface roughness and wettability on candida adhesion of 3D printed acrylic based denture materials.

2. Materials and methods

2.1 Manufacturing of Samples

Table 10 shows the sample groups distribution and the SLA and FDM groups were subdivided to two groups; X & Y based on the orientation of the 3D print, as seen in Figure 16. The circle sample shape (with dimensions of 10 mm of diameter and 2mm thickness) was chosen to make sure the sample would fit inside the plastic well plate where candida was grown, and the biofilm were therefore developed. Also, the circle shape of the samples was suitable for scanning electron microscopy analysis.

Table 10: Sample group distribution.

Group number	Description
Group 1/ Heat cured	Conventional heat cured acrylic resin.
Group 2/ Cold cured	Conventional cold cured acrylic resin.
Group 3/ Milling	Subtractive (milled) from PMMA block.
Group 4/ FDM	Additive (FDM), PMMA filament, X & Y.
Group 5/ SLA	Additive (SLA) PMMA, Grey and Denture base resins, X & Y.

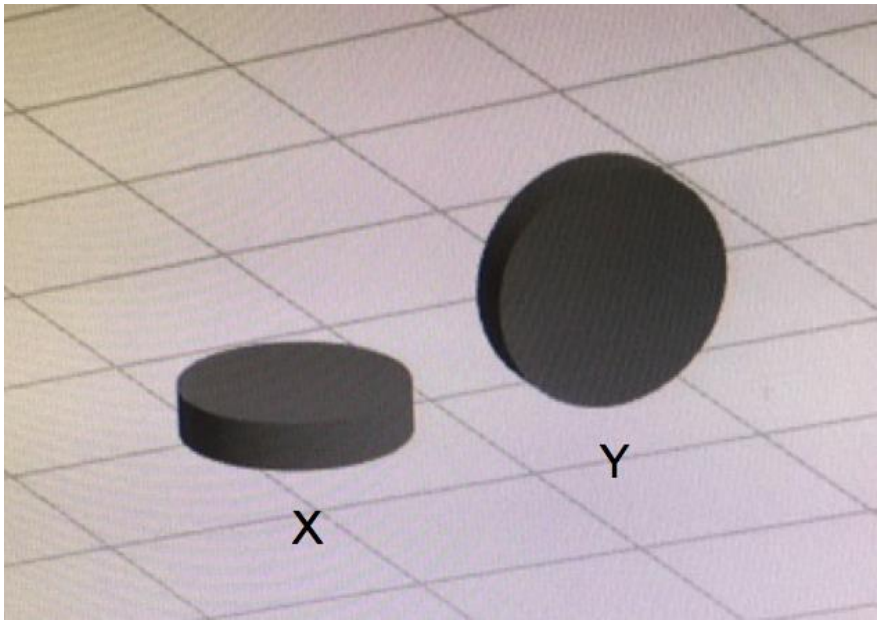


Figure 16. Orientation of 3D printed samples.

Conventional heat-cured samples (Group 1): samples were prepared using a plaster mould made by investing disc shaped wax patterns (diameter 10 mm and thickness 2mm) then the wax was removed after plaster setting. The acrylic resin ProBase® Hot (Ivoclar Vivadent AG) was mixed and poured according to the manufacturer's instructions. Then samples were removed from the flask.

Conventional cold-cured samples (Group 2): samples with dimensions (diameter 10 mm and thickness 2mm) were prepared by mixing and pouring of cold cure acrylic resin ProBase® Cold (Ivoclar Vivadent AG) according to the manufacturer's instructions into the silicon mould. After the polymerisation, the samples were removed from the mould.

Subtractive (milled) samples (groups 3): a cylinder shape with dimensions (diameter 10 mm and thickness 20 mm) was designed by using Fusion 360™ software, (Autodesk Inc.). Then the design was imported to another computer software (Sum3D), which was used to set up the milling settings. Cylinders with dimensions (diameter 10 mm and thickness 20 mm) were milled by a DWX-50 milling machine (5-axis, USA) from a PMMA disc (IvoBase CAD). PMMA cylinders were cut into discs with 2 mm thickness using a precision saw (IsoMet 1000, Buehler, USA).

Additive (FDM) PMMA samples (group 4): a disc shape was designed with the dimensions (diameter 10 mm and thickness 2mm) using Fusion 360™ software, (Autodesk Inc.). The design file was imported into (Cura LulzBot Edition version 2.6.52), which was used to set up the printing parameters. Samples were printed in two different orientations (X&Y) using a desktop 3D printer (LulzBot TAZ 6, Aleph Objects, Inc., USA) and a 3D printer filament of Poly Methyl Methacrylate (PMMA) (Material4print, Germany) was used for this printing, with manufacturer's instruction.

Additive (SLA) PMMA samples (group 5): the disc design that was used in group 4, was imported into (PreForm Software 2.18.0), which was used to set up the printing parameters. Samples were printed in two different orientations (X&Y) using a desktop 3D printer (Form 2, Formlabs Inc.) and the 3D printer resins (Grey and Denture base, Formlabs Inc.) for this printing.

Following manufacturing of samples, each sample group were divided to; A) as processed and B) standardised finishing surface by using wet grinding with P 600 Grit paper (SiC grinding paper, Buehler, Germany) on each surface; 5 times then rotate the sample around 90 degrees and do another 5 times. In addition to this, the FDM group was subjected to C) acetone vapour finishing which was performed in a glass desiccator containing acetone and the samples kept inside the desiccator for 2 hours.

2.2 Surface properties

2.2.1 Surface roughness

Surface roughness was measured using a profilometric device (TR200, Time Group Inc, UK) in conjunction with a 0.2 µm diamond tip. This profilometer was set to move the diamond sensor across the sample surface with a distance of 1.25 mm. Three samples of each sample group for each surface condition were subjected to three readings in three different directions (oblique, transverse, linear) to measure any expected surface irregularities. Then surface roughness (Ra) values were calculated in microns and all measurements were performed by one operator.

2.2.2 Surface wettability

Surface wettability is determined by measuring the contact angles between the distilled water drop and the sample's surface, as seen in Figure 17. Contact angles were measured by sessile drop method using a Drop Shape Analyzer device (DSA100- KRÜSS- Germany). Three samples from each group with different surface conditions were subjected to two distilled water drops (each 5 µl) in different areas of each sample. Then the mean of the right and left contact angle of each drop was calculated.

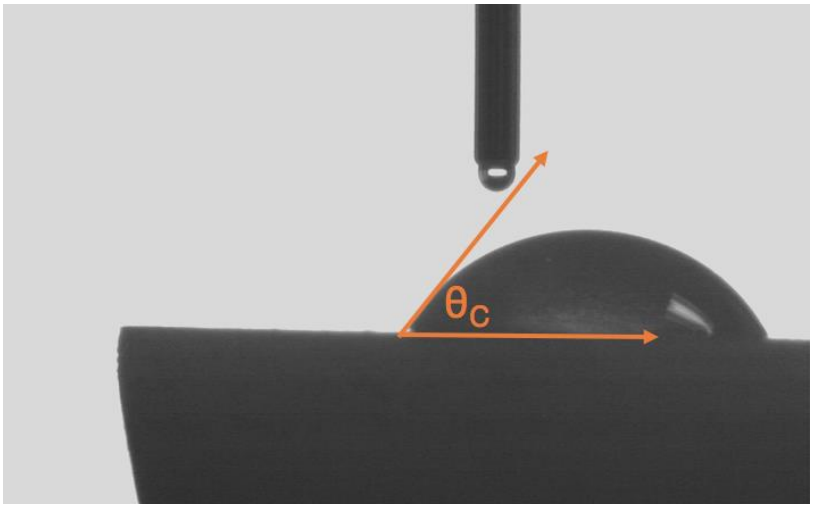


Figure 17. Sessile drop technique with a distilled water drops partially wetting the sample surface, θ_C is the contact angle.

2.3 Candida biofilm formation

Candida biofilm formation was grown on the surface of three samples of A, B, C from each group. Candida biofilms were grown on a glass disc inside the well plate (TCP) as a positive control. Samples not inoculated with candida were used as a negative control. The experiment was repeated three times. The following steps were followed in each time:

2.3.1 Candida growth

Candida albicans strains BWP17 was used in this experiment. Candida was grown in (yeast, peptone and dextrose - YPD) agar. A colony of Candida was transferred to 15 ml YPD broth and kept growing overnight in an incubator. Three samples of each group were sterilised by immersion in industrial methylated spirits (IMS) for 15 minutes. The samples were then transferred to a new 24 well plate and washed with 1 ml of phosphate-buffered saline (PBS). Candida was used at a concentration of 7.5×10^5 per ml, this was first established using a Miles and Misra counting method alongside an optical absorbance reading (0.75 absorbance). In each repeated experiment the yeast broth was altered to attain the same optical density. 1 ml of YPD broth was added to each sample including the positive and negative controls. 10 µl of yeast broth (10 µl ≈ 7.5×10^3 yeast cells) were added to each well with the exception of the negative controls. Then the plates were incubated at 37°C for 24 hours and after that, plates were ready for metabolic activity analysis.

2.3.2 Metabolic activity analysis of Candida biofilm

All samples were transferred to a new 24 well plate and were washed with (PBS). PrestoBlue (2.5 ml) was diluted in PBS (22.5 ml) to form a 10% PrestoBlue solution. Then 500 µl of this solution was added to each sample and controls and plates were incubated at 37°C for 1 hour. After incubation, 200 µl of the solution from each well was transferred to 96- well plate in duplicate (for a total of 400 µl) and absorbance was measured using a spectrophotometer Infinite 200 PRO Microplate Reader with an excitation wavelength of 550 nm and a reading wavelength of 590 nm (TECAN, Switzerland).

2.4 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used also to analyse candida biofilm formation on sample groups. Samples were washed twice with PBS then fixed with 2.5 % glutaraldehyde and 0.1 M Sodium Cacodylate buffer overnight, then washed three times with PBS and one time with distilled water. Following dehydration in a graded series of ethanol solutions, samples were dried by 1:1 mixture of hexamethyldisilazane (HMDS) and 100% ethanol and then dried finally in 100% HMDS. Following that, HMDS was removed, and samples were left to dry in a fume hood. Samples were fixed onto the pin specimen holder and then gold coated by a sputter coater unit (Edwards S150B). Samples were examined by Scanning Electron Microscope (Tescan Vega3 LMU) with operating voltage of 15kv. Scanning electron microscopy was performed at the microscopy department at the University of Sheffield.

3. Results

The surface roughness results of different surface conditions of tested groups are shown in Table 11 and Figure 18. There is a statically significant difference (P = 0.0) between different surface conditions (A & B) of all groups except FDM samples which showed no significant difference (P= 0.496) between A & C of X FDM group and (P= 0.031) between B & C of Y FDM group.

Table 11: Mean and SD of surface roughness.

Sample Group				Surface roughness (Ra, µm) Mean ± SD (n = 3)
Heat cured			A	0.992 ± 0.36
			B	0.401 ± 0.103
Cold cured			A	1.525 ± 0.224
			B	0.297 ± 0.067
Milling			A	1.162 ± 0.492
			B	0.379 ± 0.044
FDM		X	A	1.129 ± 0.348
			B	0.419 ± 0.059
			C	1.029 ± 0.155
		Y	A	3.193 ± 0.944
			B	0.42 ± 0.103
			C	0.739 ± 0.25
SLA	Grey resin	X	A	0.87 ± 0.118
			B	0.284 ± 0.049
		Y	A	0.945 ± 0.193
			B	0.258 ± 0.076
	Denture base resin	X	A	1.123 ± 0.297
			B	0.305 ± 0.116
		Y	A	1.203 ± 0.459
			B	0.311 ± 0.062

n: number of samples, A: as processed, B: standardised finishing, C: acetone vapour finishing.

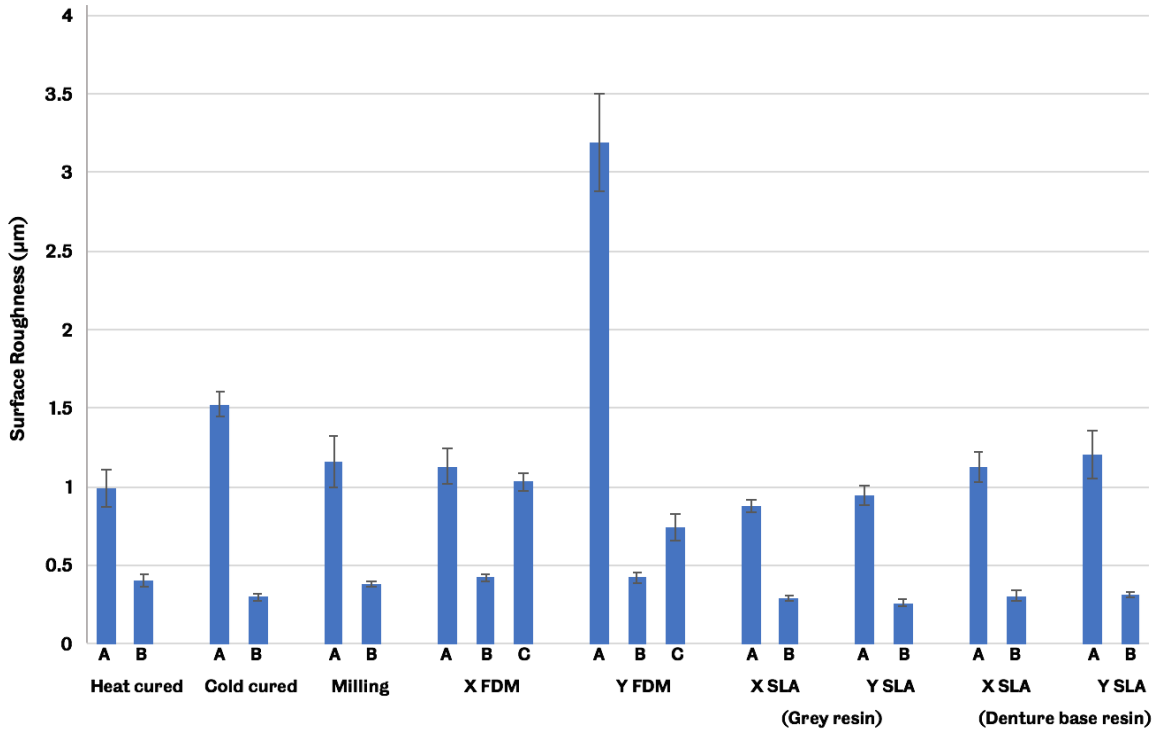


Figure 18. Mean of surface roughness measurement, A: as processed, B: standardised finishing, C: acetone vapour finishing. Error bars represent standard errors.

The mean values of contact angle measurements of each group against distilled water are shown in Table 12 and Figure 19. All groups except (heat cured and X FDM) show no statically significant difference between different surface conditions (A&B&C). Heat cured group shows a significant difference (P = 0.0) between different surface conditions (A & B) while the significant difference is found in X FDM when comparing A to B &C while there is no significant difference between B &C.

Metabolic activity of candida on all groups; except FDM, cell viability agent (Presto Blue) shows that standardised finishing samples (B) of all groups display lower metabolic activity of candida than as processed samples (A), as shown in Figure 20. While FDM samples (both orientations, X&Y) show that the standardised finishing samples (B) have higher metabolic activity than as processed samples (A) followed by the acetone vapour finishing samples (C). However, a one-way ANOVA test shows no statistically significant differences in the viability between all groups (P>0.05). SEM images are shown in Figure 21.

Table 12: Mean and SD of contact angle.

Sample Group				Contact angle (º) Mean ± SD (n = 3)
Heat cured			A	102.73 ± 2.53
			B	76.4 ± 12.2
Cold cured			A	80.73 ± 9.38
			B	72.15 ± 5.6
Milling			A	82.86 ± 13.97
			B	80.8 ± 12.48
FDM		X	A	59.05 ± 10.13
			B	81.06 ± 4.28
			C	79.85 ± 5.71
		Y	A	81.08 ± 9.77
			B	83.63 ± 11.73
			C	81.53 ± 6.3
SLA	Grey resin	X	A	85.78 ± 8.04
			B	97.96 ± 1.27
		Y	A	83.98 ± 5.86
			B	84.23 ± 10.53
	Denture base resin	X	A	84.36 ± 7.12
			B	68.68 ± 4.97
		Y	A	73.36 ± 7.62
			B	91.55 ± 15.09

n: number of samples, A: as processed, B: standardised finishing, C: acetone vapour finishing.

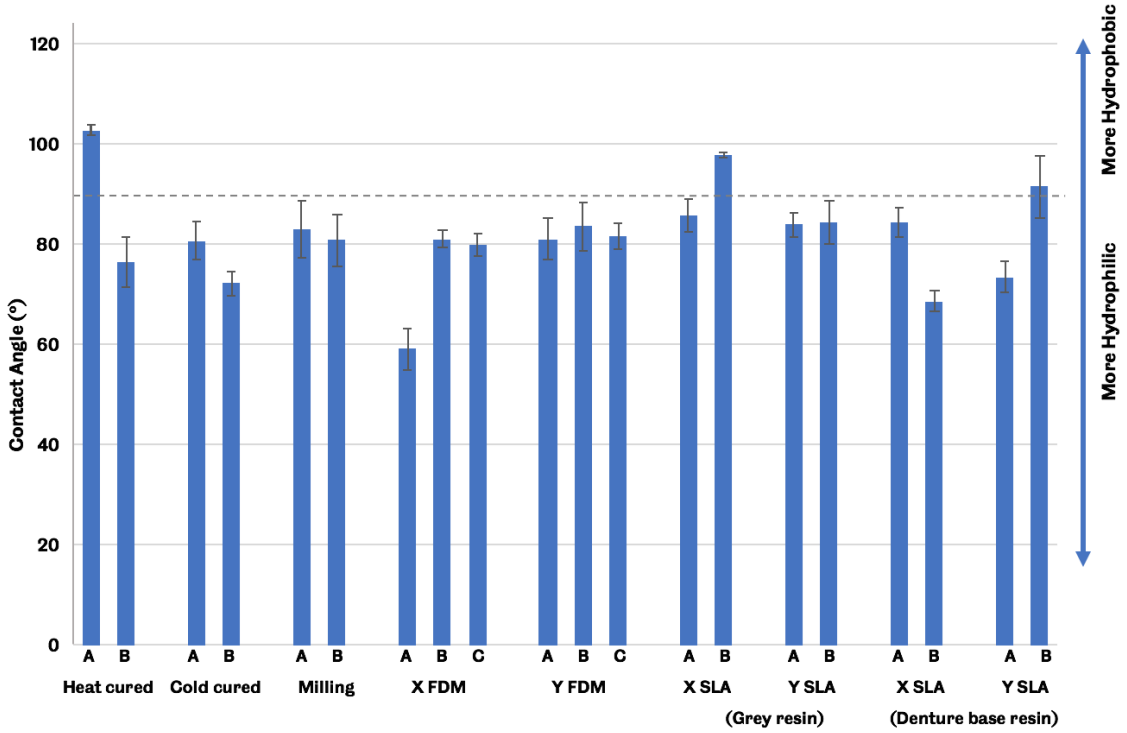


Figure 19. Mean of contact angle measurement, A: as processed, B: standardised finishing, C: acetone vapour finishing. Error bars represent standard errors.

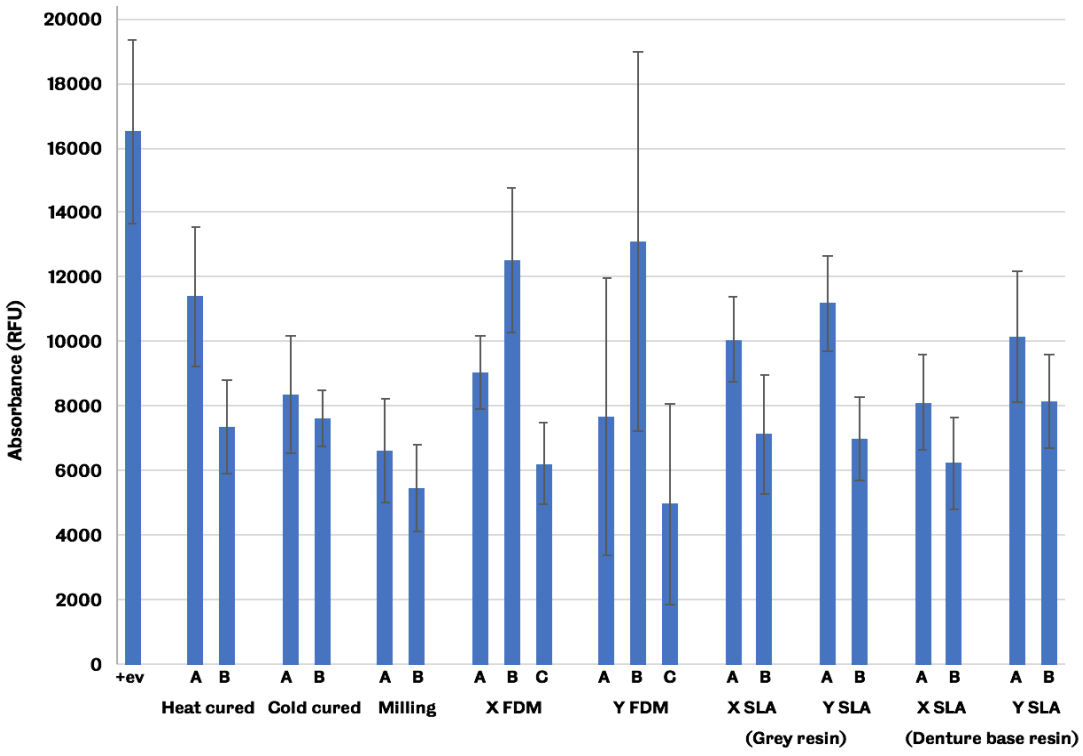


Figure 20. Mean of Candida viability according to Presto Blue. A: as processed, B: standardised finishing, C: acetone vapour finishing. Error bars represent standard errors.

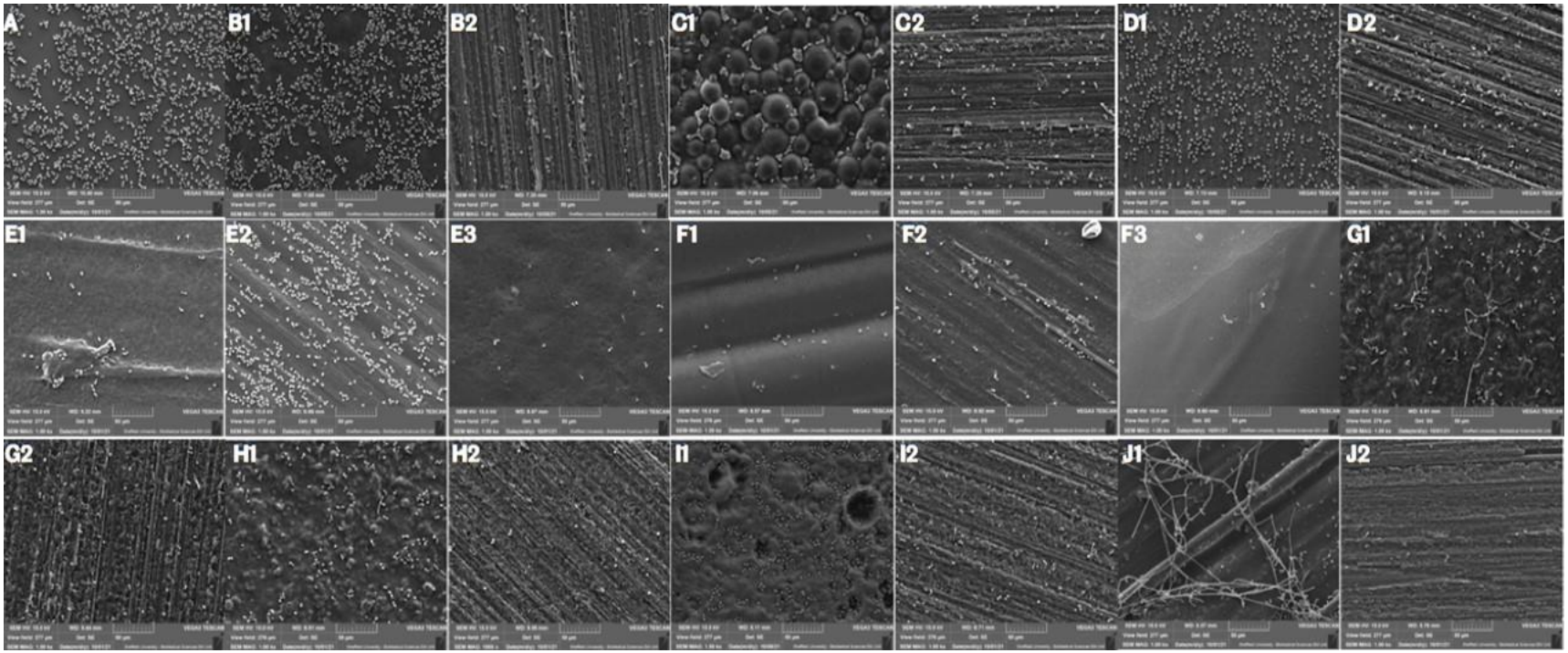


Figure 6: Scanning electron microscope (SEM) images (at 1000x magnification) of C. albicans colonisation on the surface of; **A:** glass disc (as a positive control), **B1:** as processed heat cured sample, **B2:** standardised finished heat cure sample, **C1:** as processed cold cured sample, **C2:** standardised finished cold cured sample, **D1:** as processed milled sample, **D2:** standardised finished milled sample, **E1:** as processed X FDM sample, **E2:** standardised finished X FDM sample, **E3:** acetone vapour finishing X FDM sample, **F1:** as processed Y FDM sample, **F2:** standardised finished Y FDM sample**, F3:** acetone vapour finishing Y FDM sample, **G1:** as processed X SLA (Grey resin) sample, **G2:** standardised finished X SLA (Grey resin) sample, **H1:** as processed Y SLA (Grey resin) sample, **H2:** standardised finished Y SLA (Grey resin) sample, **I1:** as processed X SLA (Denture base resin) sample, **I2:** standardised finished X SLA (Denture base resin) sample, **J1:** as processed Y SLA (Denture base resin) sample, **J2:** standardised finished Y SLA (Denture base resin) sample.

4. Discussion

Microbiological investigation was aimed to evaluate the adherence of C. albicans on different commercially acrylic based materials. Oral microorganisms such as C. albicans are responsible for denture stomatitis disease and this species can survive in the mouth by adhering to oral surfaces and beginning forming colonies (Blankenship and Mitchell, 2006). One of the important factors that affect biofilm formation is the surface topography of denture base materials. Surface roughness and hydrophobicity of denture base materials play a significant role in the process of candida biofilm aggregation (Ivković et al., 2013; Fouda et al., 2019). So, this investigation tried to evaluate the candida adhesion on different surface conditions of different manufacturing routes of PMMA denture materials.

In this study, the samples of each group were distributed according to their surface morphology to; as processed to mimic the fitting surface of the denture, standardised finishing, which was performed to make the samples surface smooth, then assess the effectiveness of the surface roughness on the candida adhesion, and acetone vapour finishing (only for FDM group) which was performed by using acetone vapour for finishing the samples' surfaces. This technique is known as vapour smoothing and has been introduced by Stratasys, Inc. where the chemical vapours react with outer layers of FDM parts (Chohan et al., 2017).

From results presented above, the surface roughness values are higher in the "as processed samples" group (A) than the "standardised finishing samples" group (B) of all groups except for the FDM group. The surface roughness values, Figure 3, of all denture base materials are higher than the threshold (0.2 µm) defined by Bollen and his team (Bollen et al.,1997). This finding has a significant relation to the amount of candida adherence where the viability analysis of candida shows that more candida cells adhere to rough surfaces than smooth surfaces and this finding is coincidence with literature (Murat et al., 2019; Verran et al., 1997; Radford et al., 1998). In addition, another investigation in this study is related to the contact angle measurement which is used to determine the hydrophobicity of the sample surface and its effectiveness on candida adherence. If the measured angle is high, that means the surface is hydrophobic while in the hydrophilic surface, the water droplet spreads quickly, and the measured angle is low. When evaluating the effectiveness of the surface hydrophobicity on

the candida biofilm formation, the results show varied findings. Some of the samples with as processed surface condition of some groups including heat cured, cold cured, milling and X SLA denture base resin, have a positive correlation between candida adhesion and the surface hydrophobicity and this finding agrees with published research (Kim et al., 2019; Lazarin et al., 2013; Zamperini et al., 2010). They reported that the higher adherence of candida occurs as a result of closer surface energy of the surface and the candida cell. However, the rest of groups display a negative correlation, and this finding is supported by literature too (Teughels et al., 2006; Pereira et al., 2007; Da Silva et al., 2015). They explained that a hydrophilic surface can generate a closely tied water layer which produces an active barrier that inhibits microbial adhesion.

The highest roughness result among the tested groups is found in as processed samples of Y orientation of the FDM group. This finding agrees with literature which concluded that objects processed by FDM show low surface finish which is generated due to curve approximation or chordal error and stair-stepping appearance; these terminologies explain the outer outline of depositioned layers (Weeren et al., 1995; Agarwala et al., 1996). Both orientations of the FDM group show a strange/ unexpected behaviour where the standardised finishing samples (B) have more candida than as processed samples (A) and acetone vapour samples (C). By comparing the results of candida biofilm on FDM samples to other groups, we found that FDM samples did not respond to mechanical polishing procedures in the same way the other groups did. Even though the standardised finishing surface (B) of FDM group displays the less surface roughness results among other samples surface conditions (A&C), it triggers higher candida colonisation than (A&C). Also, there is no clear relationship between hydrophobicity and candida adherence on different surface morphology of the FDM group. Furthermore, the SEM images show more candida adhere to as processed samples of all groups which have more porous surface and many surface irregularities. However, the SEM images of standardised finishing samples of all groups show less candida cell and the scratch lines that originated from the finishing procedure were detected on the surface of all sample groups.

This study shows some limitations including the testing conditions where the candida grows under static incubation conditions inside a thermal incubator device, while in an oral environment, candida species grow under dynamic incubation conditions. Another issue is

related to the presence of other factors in the oral cavity that might affect the oral biofilm formation; such factors are the saliva, change of temperature, and other microorganisms. These factors were not considered and simulated in this study. Furthermore, the adherence process of candida albicans is complicated and many factors may contribute to this process, such as mechanical attachment and electrostatic interaction (Minagi et al., 1985). Therefore, it is difficult to mimic these factors in in-vitro conditions.

Another limitation is related to the manufacturing process of the samples itself where each sample gets a different surface roughness degree. Surface properties of the samples should be standardised by attaining similar/close surface roughness degree before contaminating samples by candida to get consistent and reliable results. In addition, the acetone vapour finishing that is used in this study is causing dimensional discrepancies which means loss of dimensional accuracy of the denture. Chohan et al 2017 have concluded that acetone vapour smoothing leads to shrinkage in radical and linear dimensions since the outer layers of the 3D printed object reformed to establish a smooth surface (Chohan et al., 2017).

Future microbiological investigations should be established within in vivo conditions to enhance the clinical relevance of this research. Also, the candida biofilm formation on FDM samples is not fully understood and needs to be studied further. The vapour smoothing is a promising technique for achieving less Candida adhesion on FDM parts and future research should investigate other chemical agents with less side effects on the 3D printed objects.

5. Conclusions

- Surface roughness of denture base materials is crucial in the process of candida biofilm formation.
- Surface hydrophobicity of denture base materials has varied effectiveness on the candida adhesion among tested groups.
- Standardised finishing procedure seems to be ineffective on the sample surface of X&Y FDM group in terms of candida growing while acetone vapour smoothing leads to less candida growing.

- Acetone vapour smoothing technique shows promising results with FDM finishing but it leads to loss dimensional accuracy of objects so should be investigated to figure out this shortcoming/ drawback.

Chapter VII. Accuracy of fit

1. Background

Accuracy can be described by two terms: trueness and precision. Trueness interprets the proximity of agreement between the measured object and the reference object. High trueness means the measured object is in close or equal relation to the dimensions of the reference object. While precision refers to how the repeated measurement of object dimensions are close together (Ender and Mehl, 2013). Adaptation of the denture base is one of the crucial aspects determining the quality of removable prostheses (Jacobson and Krol, 1983). Inadequate fit between the denture base and underlying mucosa can lead to an uncomfortable feeling inside the patient's mouth, traumatic ulcers, and low retention and stabilisation. As a consequence of this, masticatory capability, patient satisfaction and quality of life are affected (Darvell and Clark, 2000).

Accuracy of fit of denture base is controlled by dimensional stability which is affected by three elements: polymerisation process, palate morphology and influence of cast material (Ono et al., 2004). Conventional methods such as compression moulding have been widely used for denture base fabrication even though this method shows around 7% volumetric shrinkage and 0.45%- 0.9%linear shrinkage (Parvizi et al., 2004; Kawara et al., 1998). To overcome the problem of polymerisation shrinkage, Pryor introduced injection moulding method where the polymerisation process is controlled directionally via the sprues and the using of persistent pressure to offset polymerisation shrinkage (Hsu et al., 2020). Since then, injection moulding has been widely used as an alternative to compression moulding because it improves dimensional accuracy within the denture base. Nevertheless, injection moulding shows limitations in terms of the ideal time for resin injection and the location of the polymerisation shrinkage which is likely to centralize on the polished surface of the denture base (Ono et al., 2004). Both techniques perform within the framework of conventional methods while the CAD/CAM technology is based on different approaches for manufacturing methods using either subtractive (milling) or additive (3D printing). These approaches can provide efficient alternatives to promote fast and accurate manufacturing of denture bases. Milled denture bases are fabricated from a block of acrylic resin that had been manufactured under excessive pressure and temperature, consequently avoiding polymerisation shrinkage

through manufacturing. This prefabricated block has a very compressed nature which results in less porosity, low residual monomer and less retention of Candida albicans (Bidra et al., 2013). Kattadiyil, (2013) reported that both CAD/CAM approaches minimised treatment time and improved denture base fitting. Many studies compared the fitting of denture bases made by conventional methods and CAD/CAM milling and concluded that milled denture bases show accurate and reproducible results (Tasaka et al., 2019). As previously highlighted, additive manufacturing (3D printing) is now the focus of attention in the medical care sector such as the prosthetic dentistry field. 3D printing can construct a structure with different materials based on the desired design (CAD data) and this enhances the outcome quality, the mechanical features of printed objects and the time and the cost of manufacturing (Alharbi et al., 2017). However, there is insufficient research evaluating the accuracy of fit of the denture base fabricated by additive techniques such as fused deposition modelling (FDM) and stereolithography (SLA). This study is aimed to compare the accuracy of fit of denture bases fabricated by conventional, milling and 3D printing techniques (FDM and SLA with two resins: 'grey resin' and 'denture base resin').

2. Materials and methods

A cast of an edentulous maxillary arch with three spherical reference points was defined as a master cast, Figure 22 (A). The three spheres were used to standardise the digital process of alignment later between the cast and the denture base through samples and groups. The master cast was duplicated using silicone-based duplication material (FINOSIL 15 Duplicating Silicone, A and B, FINO, Germany) according to the manufacturer's instructions to produce a master silicone mould, as seen in Figure 22 (B). This mould was used to fabricate the experimental model, Figure 22 (C); 6 experimental models were assigned for each heat and cold cure group and 1 experimental model was assigned for CAD/CAM groups (milling, fused deposition moulding (FDM) and stereolithography (SLA)). SLA and FDM denture bases were printed in two orientations, as shown in Figure 22 (D).

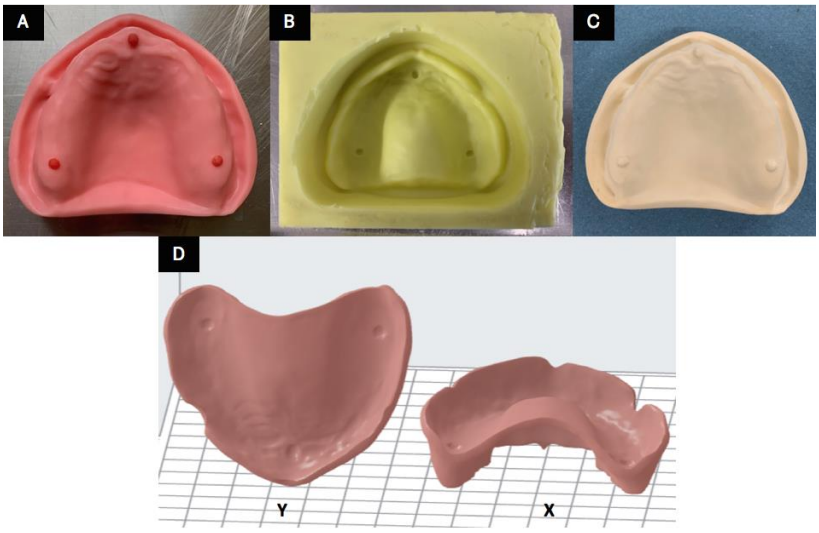


Figure 22: The process of manufacturing accuracy of fit sample; (A): the master cast with three spheres, (B): the silicon mould, (C): the experimental model, (D): printing orientations of 3D denture base.

2.1 Fabrication of experimental denture bases

Each experimental model was scanned by using a laboratory Identica Blue scanner (Identica Blue; Medit, Korea). This scanner was connected to the 3D software Identica Blue version 1.2 (Medit), which converts the geometry of the 3D object to a standard tessellation language (STL) format file which will be used later for measuring the accuracy of fit. **For conventional groups** as shown in Figure 23, a denture base wax pattern of each experimental model was prepared by using a pink wax sheet (Doric Pink Toughened Wax, Schottlander, England). The wax pattern and corresponding experimental model was invested in a white plaster mould by flasking, and after the plaster had set the wax was removed by a boiling out machine (Labormat TH, Dreve). The separating medium Iso K (Candulor, Zurich, Switzerland) was used on both parts of the flask to prevent stickiness. The acrylic resins ProBase® Hot (Ivoclar Vivadent AG) and ProBase® Cold (Ivoclar Vivadent AG) were mixed and packed according to the manufacturer's instructions and the experimental denture bases were polymerised. **For CAD/CAM groups** as shown in Figure 24, the scan of the experimental model was used to create a denture base design by using the Exocad software package (Dental CAD 3.0 Galway 7754). The CAD data was converted to a STL format file and was imported to **(i)** Sum3D which was used to set up the milling settings. The two denture bases were then milled by a DWX-50 milling machine (5-axis, USA) from PMMA discs (IvoBase® CAD, Ivoclar Vivadent AG). **(ii)**

computer software (Cura LulzBot Edition version 2.6.52), which was used to set up the printing parameters of the desktop 3D printer (LulzBot TAZ 6, Aleph Objects, Inc., USA) and a 3D printer filament of Poly Methyl Methacrylate (PMMA) (Material4print, Germany) was used to print two denture bases for each orientation with manufacturer's instruction. **(iii)** computer software (PreForm Software 2.18.0), which was used to set up the printing parameters of the desktop 3D printer (Form 2, Formlabs Inc.) and two different 3D printer resins (Grey resin & Denture base resin, Formlabs Inc.) were used to print two denture bases for each orientation with manufacturer's instruction. Each of the fabricated experimental denture bases was coated with a scanning spray (Scanspray, Renfert) and scanned by laboratory Identica Blue scanner then the (STL) format file for each denture base was created.

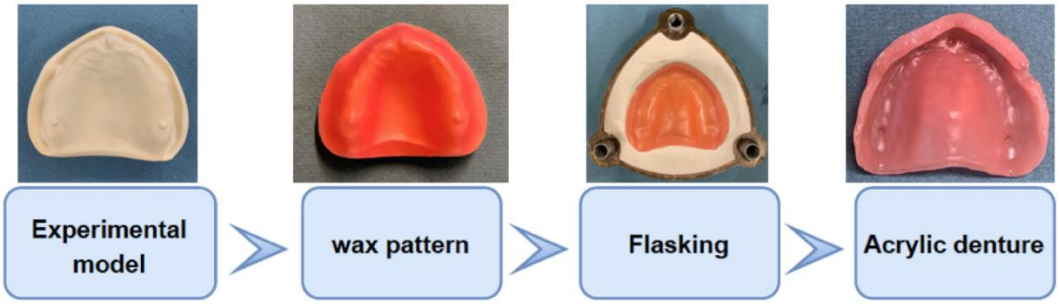


Figure 23. Schematic chart of denture base fabrication via conventional methods.

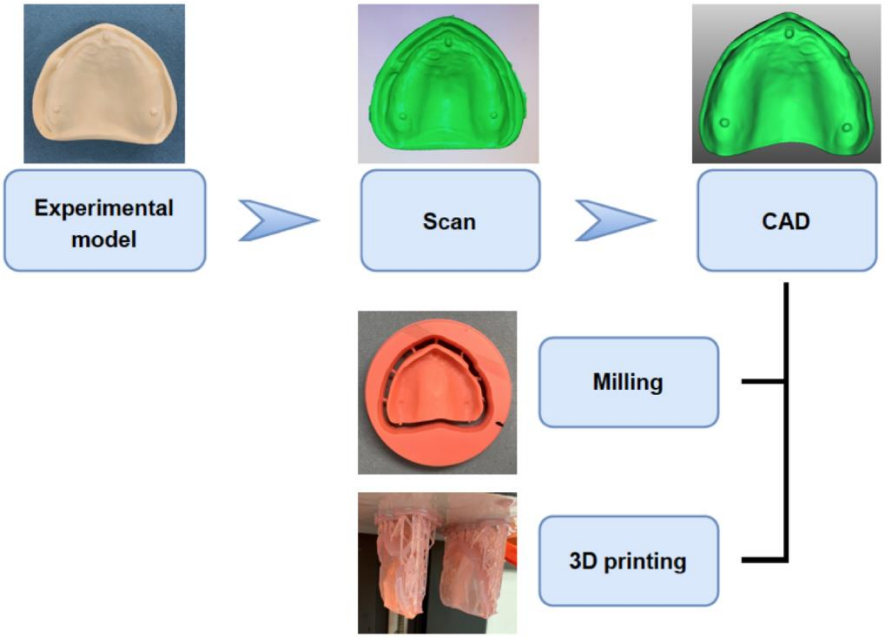


Figure 24. Schematic chart of denture base fabrication via CAD/CAM.

2.2 Evaluation of accuracy of fit

For conventional groups, each scan of the denture base was registered / aligned and compared with their corresponding scan of the experimental model by using matching software (CloudCompare v2.12 alpha). For CAD/CAM groups, the scan of the denture base was compared to the digital design and to the experimental model by using the same matching software. The alignment process was carried out with the help of the point pairs picking tool on the three spheres which was created for optimization of the registration by minimizing error distance existing between the 2 registered objects' surfaces as shown in Figure 25. Then the mean and standard deviation were calculated by measuring the distances existing between the various points on each denture base's surface and its corresponding experimental model. Also, a color-coded visualization map was created to express the result of comparison.

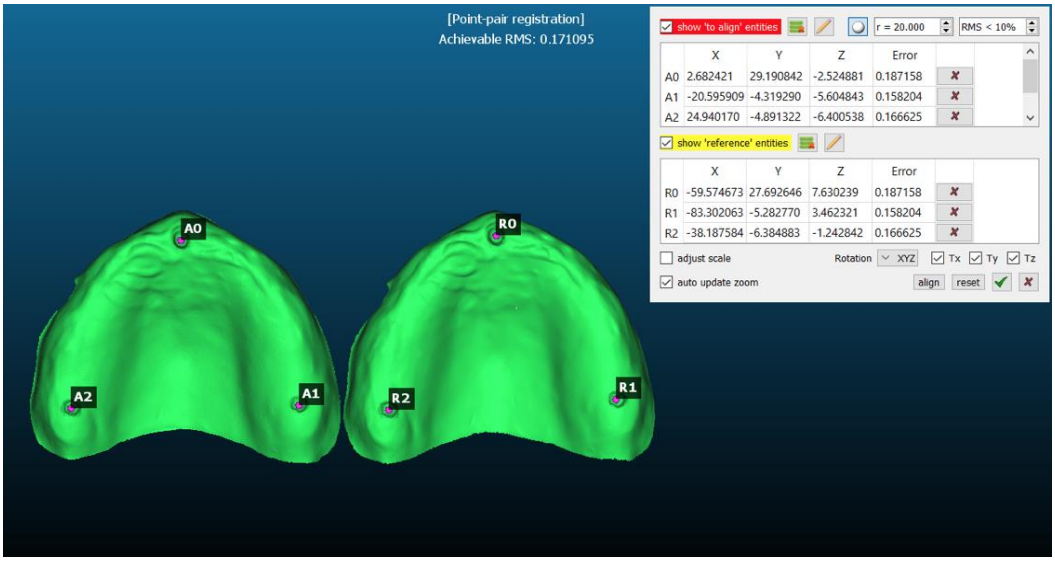


Figure 25. three-point paired registration /alignment process.

3. Results

Each conventional denture was compared to its corresponding model. Figure 26 shows a colour map of heat and cold cure samples, while Table 13 shows the mean and standard deviation for each sample. Cold cure samples showed less discrepancies of fit than heat cure samples. The scale of 0.5 to -0.5 in the colour map was set for conventional samples while the scale of 1 to -0.7 was set for CAD/CAM samples; the scale value is determined based on the worst result (the maximum distance) in the matching analysis. The process of evaluating the accuracy of fit for CAD/CAM groups passes through three steps as seen in Figure 27. Table 14 represents the results of the first and the second steps which are shared between all groups (milling, FDM and SLA) while the results of the third step for each group are demonstrated in Table 15 and Figure 28.

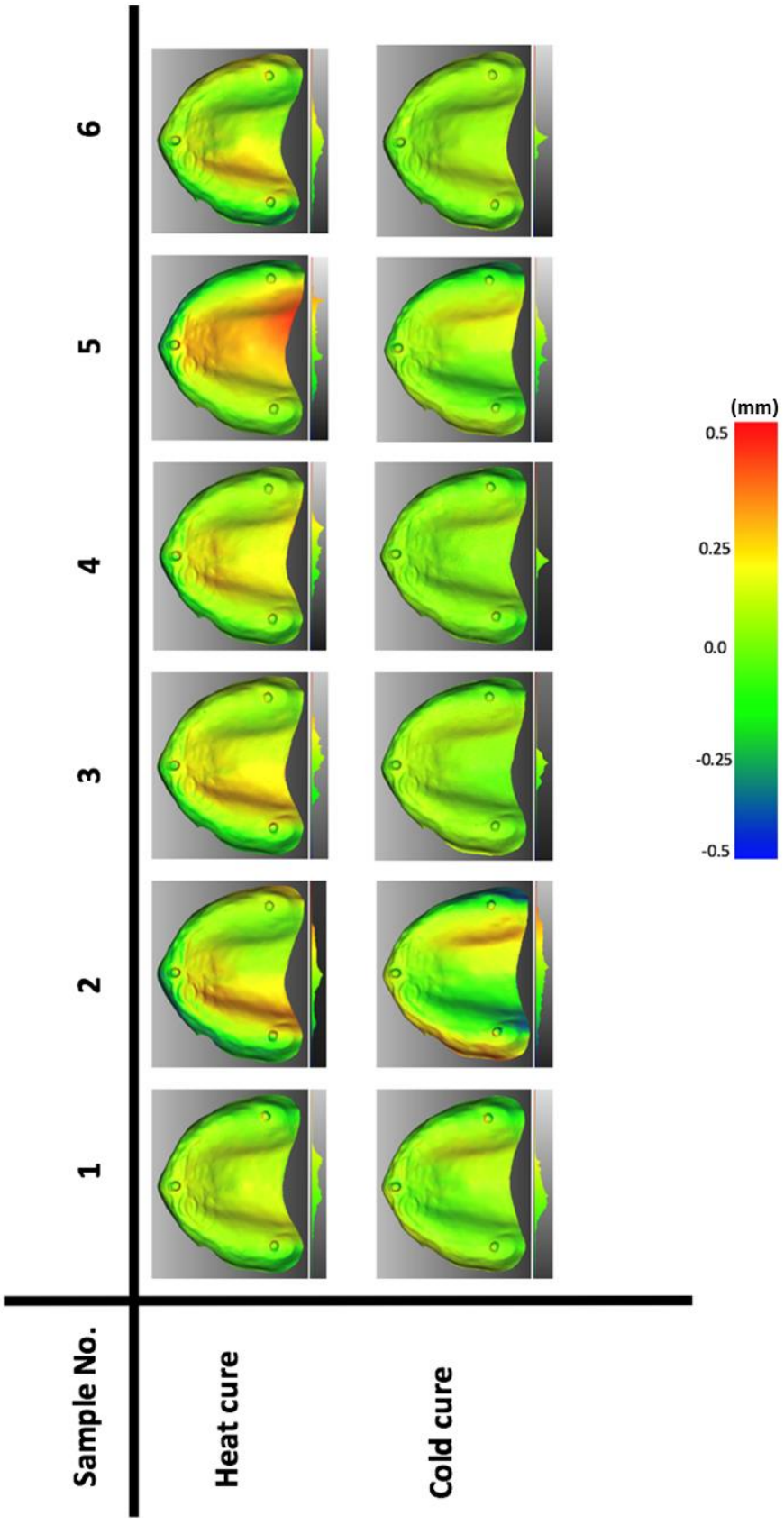


Figure 26. Colour coded maps for heat and cold cure samples.

Table 13: Mean ± SD of conventional samples (unit = mm).

Sample No.	Heat cure	Cold cure
1	-0.0035 ± 0.074	0.0065 ± 0.075
2	0.0219 ± 0.153	0.014 ± 0.173
3	0.0292 ± 0.121	0.0109 ± 0.055
4	0.0254 ± 0.110	-0.0127 ± 0.046
5	0.0274 ± 0.173	-0.0239 ± 0.098
6	0.0273 ± 0.112	0.006 ± 0.045

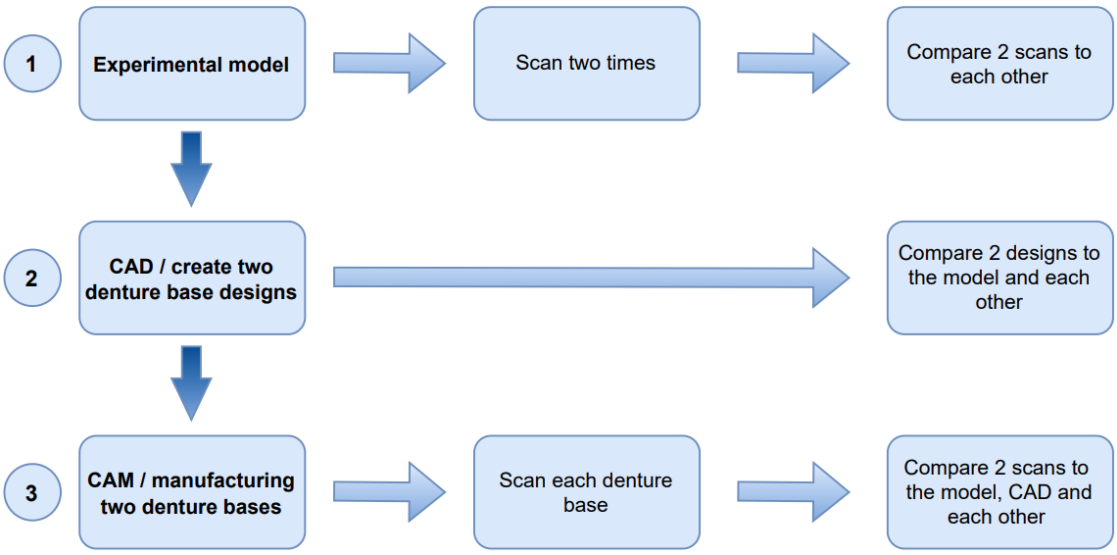


Figure 27. Chart illustrates the three steps in evaluating the accuracy of fit of CAD/CAM groups. In the first step, the experimental model is scanned two times then these 2 scans compared to each other. In the second step, one of the experimental model scans is used to create two denture base designs (CAD), then these designs are compared to the scan and to each other. In the third step, one of the denture designs is used to manufacture 2 denture bases through CAD/CAM techniques, then each denture base is scanned and compared to the model, CAD, and each other.

Table 14: Mean ± SD and colour coded map of the first and second steps (unit = mm).

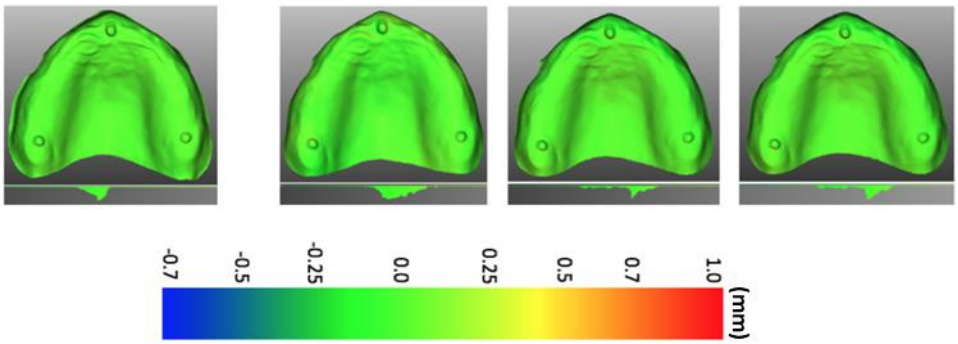
Results	The first step	The second step		
	2 scans of the model	#1 design Vs. The model	#2 design Vs. The model	#1 design Vs. #2 design
Colour map				
Mean ± SD	0.0063 ± 0.023	0.0053 ± 0.047	- 0.0092 ± 0.059	- 0.0011 ± 0.062

Table 15: Mean ± SD of CAD/CAM samples (the third step) (unit = mm).

The comparison description	#1 denture Vs. The design	#2 denture Vs. The design	#1 denture Vs. #2 denture	#1 denture Vs. The model	#2 denture Vs. The model
Milling	0.027 ± 0.078	0.0327 ± 0.072	- 0.0047 ± 0.022	- 0.0217±0.101	-0.0159 ± 0.083
X FDM	- 0.1203 ± 0.236	- 0.1306 ± 0.324	0.0484 ± 0.126	0.1226 ± 0.27	0.1501 ± 0.252
Y FDM	- 0.0236 ± 0.085	- 0.0166 ± 0.118	0.0342 ± 0.060	0.0079 ± 0.079	0.0267 ± 0.134
X SLA Grey resin	- 0.032 ± 0.094	- 0.019 ± 0.122	- 0.0086 ± 0.085	0.0357 ± 0.071	0.0386 ± 0.11
Y SLA Grey resin	- 0.0218 ± 0.083	- 0.018 ± 0.097	0.0206 ± 0.1	0.0123 ± 0.136	0.0315 ± 0.084
X SLA Denture resin	- 0.032 ± 0.113	- 0.0411 ± 0.089	0.0136 ± 0.07	0.0136 ± 0.145	0.0489 ± 0.109
Y SLA Denture resin	- 0.0687 ± 0.175	- 0.0414 ± 0.247	- 0.0448 ± 0.118	0.0727 ± 0.198	0.052 ± 0.188

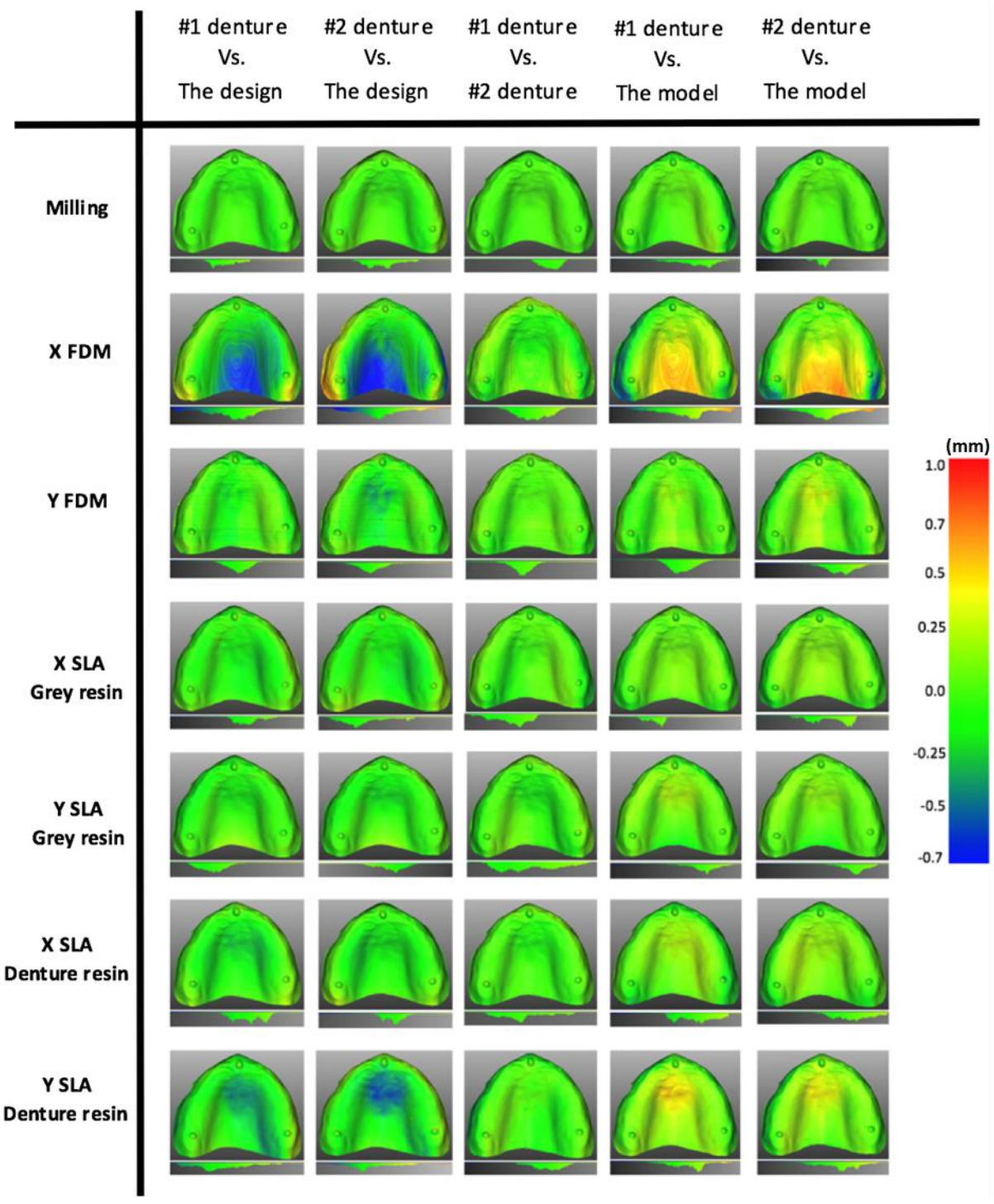


Figure 28. Colour map of surface matching for CAD/CAM samples (the third step). Each denture base of the tested groups was compared to the design (CAD), another denture base, and the model.

4. Discussion

This study aimed to evaluate the accuracy of fit of acrylic denture bases fabricated via different methods; conventional, milling and 3D printing techniques. This evaluation was carried out by measuring the gap between the fitting surface of the denture and the edentulous cast after superimposition of digital surfaces of these objects. The use of advanced techniques such as 3D printing for denture manufacturing allows to enhance the denture adaptation to underlying mucosa and to reproduce the same denture each time (Goodacre et al., 2016; Hsu et al., 2020). In order to obtain reliable and valid results for conventional groups, 6 samples were prepared for each group; it is important to notice that the conventional workflow of denture manufacturing contains sensitive laboratory procedures which are more likely to undercome human error. However, for CAD/ CAM groups, 2 samples were fabricated for each group since the digital workflow is automated and therefore involves less human intervention. Each processing phase on the digital workflow for denture manufacturing has been evaluated to locate the exact step of the faults where the discrepancies of fit might occur, as seen in Figure 7.

This laboratory study was carried out over an edentulous cast while the adaptation of the denture in the oral environment may depend on many effective factors such as saliva, soft tissue undercuts and limited access during scanning. The use of scanning spray on the denture is another limitation in this study. Applying the scanning spray which is required to enhance the quality of the scanned surface, might affect the outcomes by creating a variation in the density of the layer. Moreover, denture bases with 2 mm thickness without artificial teeth were used in this study for evaluating the accuracy of fit; Kanazawa has found that 3D printed dentures show fitting disparity when evaluated before and after teeth placement (Kanazawa et al., 2011). Furthermore, the PMMA filament and the grey resin that was used in this study to 3D print a denture show unesthetic appearance since it does not match the gingiva colour.

Based on the presented results, cold cure dentures are more accurate than heat cure ones and this finding is concurrent with literature: specifically, studies have concluded that autopolymerising resins show better accuracy of fit performance than other resins used for conventional fabrication of dentures (Al Elsheikh and Abdel-Hakim, 1995; Craig et al., 2004). Most cold cure samples show green colour in the colour map as seen in Figure 6, which

indicates less discrepancies between the denture and its corresponding cast. The evaluation of the accuracy of fit for CAD/CAM groups undergo multiple comparisons as shown in the Figure 7 and these comparisons are divided into three steps. The first step includes comparing between two scans of the model while the second step includes comparing each denture design (CAD) to the model and comparing two designs to each other. The comparison in the first and second step displayed almost identical results, as seen in Table 2, and this indicates that the process of the scanning and the denture designing (CAD) provide highly accurate outcomes (no faults associated with these procedures).

The third step of comparison contains a comparing between the CAD/CAM dentures and the denture design (CAD), all groups show less discrepancies except the X FDM group which means that using FDM technique to fabricate a denture with X orientation is unable to provide a denture that highly accurate to the denture design (CAD) file. Also, the comparison between the two manufactured dentures to each other was performed, and the milling group is more accurate among tested groups while the worst results were in the FDM group. This is evidence of higher repeatability and stability of the milling technique than other CAD/CAM techniques. When comparing CAD/CAM dentures to the model, all groups except X FDM group showed reasonable and similar results with green colour in the majority of a colour map while X FDM colour map showed yellowish colour which means more deviation between the denture and the model. All the previous multiple comparisons were performed to answer the question of if the error happened is it before or after milling or printing the denture? From the previous illustration of all the steps in the CAD/CAM workflow analysis, we can say that the misfit of the denture is more likely due to the process of manufacturing (printing errors).

Our findings indicate that milled denture shows better overall adaptation and reproducibility in comparison to other fabrication techniques of the denture, and this is consistent with previous studies. Many reports claimed that milled denture shows high adaptation since it is fabricated from pre-polymerized PMMA blocks where no more dimensional deformation takes place (Goodacre et al., 2016; Hsu et al., 2020; Yoon et al.,2018). However, polymerisation shrinkage is associated with conventional dentures and needs to be considered (Lee et al., 2019). Furthermore, unlike milling technique, SLA printing technique requires post processing procedures such as washing the denture with isopropanol and post

curing, where polymerisation deformation may exist, so the accuracy of fit of the denture is altered (Bennett, 2017; Wang et al., 2021; Wu et al., 2019). Unlike SLA technique, FDM technique does not require post processing procedures but many processing factors, such as layer height and extruder temperature, might impact the dimensional stability (Mahmood et al., 2018) and consequently affect the accuracy of fit of the denture. Distortion of the FDM printed object is more likely to happen since this object is subjected to frequent heating and cooling phases during the manufacturing (Azhikannickal and Uhrin, 2019).

Studies have reported that the acceptable clinical deviation between the denture and the mucosa is 0.3 mm (Deng et al., 2018; Wang et al., 2021) and our finding in all groups performed within this clinical requirement for adaptation of the denture to the mucosa. The finding of this study is specific to tested materials and might differ with other printing filaments or resins, 3D printers and milling machines. Future investigations should assess 3D processing parameters such as: the layer thickness, and conditions such as different hydration circumstances, and their effect on the accuracy of fit of the denture.

5. Conclusions

- Milling technique can provide a denture with a uniform adaptation and a best reproducibility among tested manufacturing techniques.

- Most of the faults / misfit occurs from the printing outcomes not from the denture design (CAD) file which is imported into the printer.

- FDM technique shows differences in the accuracy of fit between two printing orientations (X &Y) while SLA technique shows no significant differences between these orientations.

Chapter VIII. General discussion and conclusion

1. General Discussion

A critical objective of incorporating new techniques into the dental practice is to afford advanced treatment options for the patients. The shortcomings of conventional PMMA such as polymerisation shrinkage, allergic reaction and susceptibility to microbial adhesion have steered novel materials and manufacturing methods to emerge (Gautam et al., 2012; Akin et al., 2015). Digital advancement on prosthodontics started in the 1980s when CAD/CAM systems was first used to fabricate a complete denture prosthesis (Miyazaki et al., 2009) and has been successfully progressing since then. This comprehensive study focuses on assessing the feasibility of using filament-based additive manufacturing for the fabrication of denture bases and it specifically aims to characterise the properties of 3D-printed (FDM) PMMA denture base materials and compare them to conventional processed PMMA, milled PMMA, and photo-cured resins via stereolithography (SLA). For assessing the suitability of using 3D-Printing PMMA filament as a denture base material, key properties were considered in this study, and these included mechanical properties, water sorption and solubility, tooth bonding, microbiological characterisation and the accuracy of fit. Also, 3D printing orientation was assessed to test variabilities in these properties caused by the layer-by-layer manufacturing process.

The CAD/CAM workflow can provide many advantages such as digital storage of patient's data which means faster replacement of prostheses in case of loss or fracture and lowering the number of appointments required for denture manufacture. The dominant disadvantage of the milling technique is the waste material, as a part of the material block stays unused and is ignored through this process (Anadioti et al., 2020). However, 3D printing can provide a complex multicolour denture with less waste materials (Deng et al., 2018) and the cost of the desktop 3D printer is much lower than a milling machine (Anadioti et al., 2020). The financial implications of CAD/CAM removable prostheses are an obvious concern as compared to conventional techniques. However, a recently published study concluded that regardless of the high cost of establishment for a digital denture workflow, within the long term it is defined to be a less expensive method of fabrication complete removable prosthesis in terms of clinical appointment time and laboratory expenses (Srinivasan et al., 2019).

Based on results presented in this work, PMMA filament has been shown to be able to meet the ISO standards for flexural strength and impact strength; it shows good mechanical performance in comparison to other tested groups. Also, PMMA filament can withstand the effect of the wet environment since its water sorption and solubility results are within ISO specifications, while tooth bonding results of PMMA filament shows the worst performance among other tested groups. But PMMA filament shows poor surface finish which results on the growth of more candida colonies than other comparable materials. Regarding the accuracy of fit, dentures printed by PMMA filament show low adaptation as compared to other manufacturing methods (and this is dependent on printing orientation).

This study highlights some issues related to a denture manufactured by 3D printing technique. The FDM 3D printed PMMA filament is not yet approved to be used intraorally and it is not either available with aesthetic appearance matching gingiva colour. The final appearance of an FDM denture does not look like a conventional or a milled denture since the layers of printing impact/distort the final appearance of the denture. Also, this thesis has shown that the FDM denture presents more surface irregularities that can result in the retention of plaque and therefore development oral diseases. Regarding SLA technique, or to be specific the established Form 2 printer, the denture printed by this technique showed excellent properties in terms of ISO specifications and can achieve clinical requirements of the denture. However, the Form 2 has a high cost in terms of supplement materials such as: the denture base resin (£ 279 for 1 litre in May of 2021), the denture teeth resin (£ 369 for 1 litre) and the plastic tank (£ 75) which requires regular renewal. 4 shades of denture base resin and 6 shades of denture teeth resin are currently available, which means each shade should be in a separate tank and this requires a 'library' of resins tanks to be available. Because of this, the high cost of supplemental materials of this printer would reflect on higher treatment cost for patients. Furthermore, the texture and appearance of the plastic denture teeth is incomparable to printed denture teeth which are dull and rough, requiring post processing, while the plastic teeth are shiny and smooth, and have 3-dimensional morphology colours.

Although successful in different ways, this study shows a number of limitations; the common and shared limitation is related to the PMMA filament which is not approved to be used intraorally and is of unaesthetic appearance, being transparent in colour. Another limitation

is related to the resolution of printing and the layer thickness which is not consistent among FDM and SLA techniques. The 0.1 mm layer thickness has been determined to be a layer thickness in this study and FDM samples were manufactured within this thickness. The Form 2 denture base resin was released in the UK market recently and this resin is designed to be printed with one specific layer thickness which is 0.05 mm. So, the FDM samples were printed with 0.1 mm and the SLA samples were printed with 0.05 mm.

This study was carried out within laboratory conditions where clinical aspects have not been assessed over a long time. Our 3D printed denture prototypes have not been used intraorally for enough time to evaluate the resistance of this technique against intraoral factors such as the masticatory force and the wet environment. Therefore, it is hard to draw final conclusions regarding the materials and the techniques.

The future enhancement of 3D printing for denture manufacturing should focus on the following recommendations to make sure this technique is more valuable and practical for digital denture manufacturing. For PMMA filament to be approved by the FDA, it should show a minimum of clinical requirements for denture manufacturing. Also, the PMMA filament is printed with high temperature (100 °C), so the 3D printer that is used with this filament should bear the high temperature to get a denture with high quality. The surface finish of FDM printing should be investigated further to enhance the final appearance of the FDM denture without impacting other properties. In this study, the acetone vapour finishing technique was used to smooth the surface of the FDM sample and we succeeded in achieving this, but the dimensional accuracy was altered/compromised. This study used one 3D printer that complies with SLA technique which is the Form 2, so judgement on this technique would be unfair and needs to be more specific of issues that are related to the specific Form 2 printer. As mentioned earlier, the cost of supplement materials for this printer is relatively high and a dental laboratory needs to have all the shades of denture base resins and denture teeth resins available. So, is there a future way to mitigate that? Probably not because the colour is set into the resin. One suggestion that manufacturers could implement is to come up with a way of injecting the colour as you print like other printing methodologies such as Polyjet printing technique, but the cost of the printer can increase dramatically (about ten times what Form costs).

Although the FDM technique did not show good results in terms of accuracy of fit, surface finish and microbiological adhesion compared to other manufacturing techniques, there is still promise with this technique. Cost and accessibility represent the strength aspects within FDM technique. The 3D printing market is now supplied with low cost FDM machines because of the expiration of the FDM patent (Minetola et al., 2016). Furthermore, the FDM technique is a convenient form of 3D printing which can be used in many places such as schools, libraries and at home. A real example showing accessibility of FDM technique is during the COVID pandemic, where people could not get out, they printed masks at home (Oladapo et al., 2021).

The FDM technique is not yet ready to be used for denture base manufacturing and there must be further research to increase clinical usage by optimising the highlighted defects associated with this technique. However, the FDM technique can be used for applications that used one time and don't require properties meet the ISO specifications, such as applications of a custom tray, teaching models and surgical guides. This research showed that we are successful in printing a prototype denture with FDM technique and we characterise the properties that are required for acceptable clinical performance. This achievement would be the starting point for future research to enhance these properties to be clinically acceptable.

Impact

The main impact of this study, regardless of the materials tested, is the testing regime (ISO standards) which are aligned to conventional manufacturing techniques. They are not fit and updated to modern manufacturing techniques such as 3D printing. From this work, the following recommendations are made for 3D printed polymers for dental applications:

- Mechanical assessment should include testing with different printing orientations (X, Y&Z) due to potential variability of mechanical properties in each orientation from the layer-based deposition of material. 3D printed materials should be tested in different orientations to make sure they are not sufficiently weak in one orientation.

- Water sorption and solubility tests are critical for 3D printed materials assessment, so the test specimen needs to be changed to have one more readily 3D printed geometry and a representative of 3D printed structure for testing (to make sure multiple layers are present for water sorption and solubility tests).

- Free monomer of 3D printed materials is a crucial issue that needs to be considered and assessed since high presence of uncured polymer are associated with allergy and soft tissue irritation.

- Thermal properties of thermoplastic materials should be acknowledged since these materials would be used intraorally and get affected by heat and cool, so this behaviour needs to be considered when using this type of material (e.g., during postproduction or cleaning).

- The tooth bonding test should be operated based on clinical practice which include mechanical and chemical modifications. These modifications enhance the bonding strength between the artificial teeth and the 3D printed base.

- The assessment of candida adhesion of 3D printed materials needs to be standardised to get an accurate evaluation/ judgment of candida behaviour on 3D printed surfaces.

2. General conclusions

This study aims to evaluate the suitability of using 3D-Printing PMMA filament as a denture base material, and to attain this, we characterise the properties that required for acceptable clinical performance of denture base materials. These were successfully achieved, and the following points illustrate the obtained knowledge from each part of the study.

- The FDM technique provides good mechanical performance for a denture base material.

- The FDM technique shows excellent performance of denture base material in the wet environment and within ISO specifications when water sorption and solubility tests are applied.

- The tooth bonding test of FDM samples does not comply with ISO specifications so other modifications (for example retention grooves) should be used to enhance the bonding between the artificial teeth and the FDM denture base.

- The FDM technique provides samples with poor surface finish and leading to a high candida adhesion on the surface.

- The accuracy of fit of an FDM denture shows discrepancies which means that the FDM technique is unable to provide a denture highly accurate to the original denture design.

- A denture fabricated by the FDM technique does not demonstrate properties that comply with clinical requirements, so this technique is not yet ready to be used in such applications.

- The current ISO testing regime for denture base polymers does not meet the requirements of modern manufacturing techniques.

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Appendix

Table 1: Analysis of variance (ANOVA) of impact strength, flexural strength and Vickers hardness.

Test	Source	Sum of Squares	Df	Mean Square	F	Sig.
Flexural strength	Between groups	42596.057	11	3872.369	22.761	.00
	Within groups	8166.396	48	170.133		
	Total	50762.453	59			
Impact strength	Between groups	22.650	11	2.059	21.662	.00
	Within groups	10.266	108	.095		
	Total	32.916	119			
Vickers hardness	Between groups	2548.823	11	231.711	157.159	.00
	Within groups	159.232	108	1.474		

Table 2: Analysis of variance (ANOVA) of water sorption and solubility.

Test	Source	Sum of Squares	Df	Mean Square	F	Sig.
Water sorption	Between groups	8363.439	8	1045.430	278.134	.00
	Within groups	135.314	36	3.759		
	Total	8498.753	44			
Water solubility	Between groups	130.338	8	16.292	21.468	.00
	Within groups	27.321	36	.759		
	Total	157.660	44			

Table 3: Water sorption and solubility raw data for heat cure group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg					Weigh3, µg		
1	1147300			1147300					**1147300**		
2	1082800			1082800					**1082800**		
3	1013700			1013700					**1013600**		
4	106700			1067400					**1067200**		
5	1002900			1002900					**1002900**		
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	48.72	49.44	49.09	49.08	0.58	0.56	0.53	0.55	0.59	0.56	1058.92
2	49.77	49.96	49.48	49.73	0.56	0.58	0.43	0.53	0.56	0.53	1028.92
3	49.18	49.15	48.86	49.06	0.51	0.51	0.52	0.53	0.55	0.52	982.48
4	48.92	49.2	49.35	49.15	0.52	0.57	0.53	0.57	0.55	0.54	1024.02
5	49.61	49.76	50.05	49.8	0.56	0.53	0.4	0.51	0.61	0.52	1012.35

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1174700**	1	1147600	1147500	**1147400**
2	**1107500**	2	1082200	1082200	**1082400**
3	**1036600**	3	1013400	1013400	**1013400**
4	**1086900**	4	1066300	1066300	**1066400**
5	**1025000**	5	1002900	1002900	**1002900**

Table 4: Water sorption and solubility raw data for cold cure group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg						Weigh3, µg	
1	1042200			1041900						**1042100**	
2	943000			942800						**942600**	
3	1127500			1127300						**1127500**	
4	989700			989600						**989600**	
5	920000			919800						**919800**	
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	49.67	49.13	49.82	49.54	0.53	0.48	0.51	0.61	0.57	0.54	1040.34
2	48.77	49.08	48.98	48.94	0.57	0.51	0.48	0.46	0.45	0.49	912.28
3	49.6	49.52	49.73	49.61	0.52	0.53	0.48	0.55	0.59	0.53	1023.96
4	50.05	49.93	49.9	49.96	0.6	0.51	0.47	0.63	0.46	0.53	1038.46
5	49.26	48.99	49.2	49.15	0.46	0.43	0.46	0.46	0.47	0.45	853.35

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1062200**	1	1041500	1041300	**1041300**
2	**960600**	2	942200	942200	**942200**
3	**1153600**	3	1126800	1126800	**1126700**
4	**1008500**	4	989100	989000	**989000**
5	**935700**	5	919200	919100	**919100**

Table 5: Water sorption and solubility raw data for milling group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg					Weigh3, µg		
1	1110300			1110200					**1110200**		
2	1159000			1158900					**1159000**		
3	1078900			1079100					**1079000**		
4	1028600			1028600					**1028600**		
5	1210100			1210100					**1210200**		
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	50.01	49.97	49.94	49.97	0.5	0.55	0.53	0.45	0.41	0.48	940.86
2	49.94	49.98	50.04	49.98	0.54	0.57	0.45	0.6	0.53	0.53	1039.29
3	49.8	50.04	50.06	49.96	0.5	0.54	0.44	0.52	0.48	0.49	960.08
4	49.87	49.85	49.99	49.9	0.6	0.58	0.48	0.54	0.42	0.52	1016.42
5	49.97	49.94	49.83	49.91	0.58	0.49	0.52	0.59	0.55	0.54	1055.93

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1129700**	1	1109800	1109800	**1109800**
2	**1180400**	2	1158600	1158600	**1158600**
3	**1097200**	3	1078100	1078200	**1078200**
4	**1046400**	4	1028000	1027900	**1028000**
5	**1226200**	5	1204500	1204400	**1204400**

Table 6: Water sorption and solubility raw data for X FDM group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg					Weigh3, µg		
1	1090100			1090100					**1090000**		
2	940100			940100					**940000**		
3	1168500			1168300					**1168200**		
4	1262600			1262400					**1262200**		
5	1127500			1127400					**1127300**		
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	50.13	50.11	49.85	50.03	0.5	0.53	0.5	0.48	0.45	0.49	962.77
2	49.74	49.99	50.03	49.92	0.47	0.4	0.43	0.44	0.57	0.46	899.86
3	50.1	50.1	49.83	50.01	0.58	0.53	0.55	0.49	0.62	0.55	1079.8
4	49.79	50.12	50.02	49.97	0.55	0.57	0.6	0.55	0.57	0.56	1097.68
5	49.88	50.19	50.08	50.05	0.47	0.59	0.47	0.55	0.55	0.52	1022.54

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1119700**	1	1090100	1090000	**1090100**
2	**967300**	2	940100	940200	**940100**
3	**1196200**	3	1168300	1168300	**1168300**
4	**1293600**	4	1262300	1262400	**1262400**
5	**1154000**	5	1127400	1127400	**1127400**

Table 7: Water sorption and solubility raw data for Y FDM group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg						Weigh3, µg	
1	1323000			1323100						**1323100**	
2	1144200			1144000						**1144000**	
3	1145800			1145500						**1145500**	
4	1271300			1270900						**1271000**	
5	1280100			1279900						**1280000**	
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	51.11	50.83	50.69	50.87	0.56	0.58	0.61	0.58	0.64	0.59	1198.51
2	49.51	49.46	49.69	49.55	0.56	0.45	0.52	0.5	0.66	0.53	1021.48
3	49.61	49.57	49.31	49.49	0.54	0.46	0.5	0.56	0.62	0.53	1019.01
4	51.02	49.94	51.21	50.72	0.61	0.57	0.46	0.57	0.66	0.57	1151.07
5	50.76	50.5	51	50.75	0.52	0.6	0.55	0.53	0.6	0.56	1132.21

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1352000**	1	1323300	1323200	**1323100**
2	**1169100**	2	1144500	1144000	**1144100**
3	**1174000**	3	1146100	1145700	**1145800**
4	**1300500**	4	1271600	1271100	**1271300**
5	**1310300**	5	1280500	1280000	**1280200**

Table 8: Water sorption and solubility raw data for X SLA (Grey resin) group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg					Weigh3, µg		
1	1001700			10014					**1001300**		
2	858800			858400					**858200**		
3	1067800			1067500					**1067700**		
4	992100			991900					**992000**		
5	952000			952000					**951900**		
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	48.83	49.57	49.05	49.15	0.53	0.51	0.44	0.47	0.6	0.51	967.13
2	48.39	48.65	48.73	48.59	0.41	0.47	0.55	0.47	0.56	0.49	908.15
3	49.65	49.87	49.8	49.77	0.56	0.57	0.56	0.61	0.58	0.57	1108.35
4	48.85	49.26	48.95	49.02	0.57	0.52	0.4	0.57	0.62	0.53	999.75
5	48.7	49.84	48.87	49.13	0.41	0.45	0.48	0.41	0.61	0.47	890.55

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1051500**	1	998700	996700	**996700**
2	**899800**	2	853800	853000	**853100**
3	**1121600**	3	1063600	1061800	**1061800**
4	**1041000**	4	989300	987000	**986900**
5	**998800**	5	948900	947700	**947600**

Table 9: Water sorption and solubility raw data for Y SLA (Grey resin) group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg					Weigh3, µg		
1	1055800			1055200					**1055200**		
2	1225700			1224400					**1224300**		
3	1120200			1119000					**1118900**		
4	1120900			1119900					**1119800**		
5	1094900			1094300					**1094100**		
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	49.83	49.78	49.81	49.8	0.45	0.49	0.45	0.46	0.56	0.48	934.47
2	49.85	49.74	49.71	49.76	0.57	0.55	0.59	0.56	0.64	0.58	1127.34
3	49.74	49.83	49.76	49.77	0.52	0.47	0.55	0.54	0.63	0.54	1050.02
4	49.87	49.78	49.87	49.84	0.54	0.52	0.55	0.54	0.55	0.54	1052.97
5	49.82	49.77	49.84	49.81	0.49	0.54	0.49	0.48	0.58	0.51	993.28

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1105900**	1	1054700	1052600	**1052500**
2	**1283600**	2	1225300	1221800	**1221800**
3	**1175100**	3	1117700	1115300	**1115400**
4	**1173800**	4	1119900	1117200	**1117100**
5	**1147200**	5	1094200	1092100	**1092000**

Table 10: Water sorption and solubility raw data for X SLA (Denture resin) group.

Conditioned mass, M1											
Sample #	Weigh 1, µg			Weigh 2, µg						Weigh3, µg	
1	1242300			1241600						**1241700**	
2	1089400			1089000						**1089000**	
3	1269600			1269200						**1269300**	
4	1276300			1275600						**1275600**	
5	1324100			1323400						**1323600**	
Volume mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).											
Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	50.87	50.8	50.56	50.74	0.63	0.48	0.41	0.66	0.6	0.55	1111.56
2	50.48	50.94	50.87	50.76	0.54	0.44	0.41	0.52	0.51	0.48	970.85
3	51.03	51.05	50.76	50.94	0.6	0.43	0.44	0.66	0.64	0.55	1120.34
4	50.67	51.11	50.82	50.86	0.57	0.44	0.68	0.53	0.67	0.57	1157.43
5	50.88	50.91	50.92	50.9	0.54	0.51	0.53	0.62	0.63	0.56	1138.92

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1264400**	1	1243500	1242400	**1242400**
2	**1105700**	2	1087700	1087100	**1087000**
3	**1290800**	3	1269800	1269100	**1269100**
4	**1300000**	4	1277800	1276400	**1276200**
5	**1349300**	5	1325500	1324400	**1324200**

Table 11: Water sorption and solubility raw data for Y SLA (Denture resin) group.

Conditioned mass, M1			
Sample #	Weigh 1, µg	Weigh 2, µg	Weigh3, µg
1	1395300	1394200	**1394400**
2	1107100	1106300	**1106400**
3	1239300	1238400	**1238500**
4	1383600	1382800	**1382900**
5	1252500	1251200	**1251200**

Volume

mean of three diameter measurements x the mean of five thickness measurements (Centre and at four equally spaced locations around the circumference).

Sample #	3 diameter #			Mean diameter	5 thickness #					Mean thickness	Volume $\pi * r^2 * h$ (mm^3)
1	50.69	50.82	50.79	50.76	0.55	0.58	0.54	0.59	0.62	0.57	1152.88
2	50.69	50.64	50.72	50.68	0.49	0.5	0.44	0.51	0.56	0.5	1008.12
3	50.91	50.59	50.7	50.73	0.59	0.56	0.46	0.55	0.62	0.55	1111.12
4	51.05	50.79	50.83	50.89	0.59	0.53	0.63	0.58	0.64	0.59	1199.46
5	50.79	50.71	51.04	50.84	0.59	0.52	0.5	0.59	0.64	0.56	1136.23

Wet mass, M2		**Reconditioned mass, M3**			
Sample #	Weigh, µg	Sample #	Weigh 1, µg	Weigh 2, µg	Weigh 3, µg
1	**1423200**	1	1397000	1395200	**1395100**
2	**1128800**	2	1107300	1106900	**1106800**
3	**1262100**	3	1239400	1238600	**1238500**
4	**1410400**	4	1385000	1383600	**1383300**
5	**1276300**	5	1252800	1251800	**1251800**

SEM images

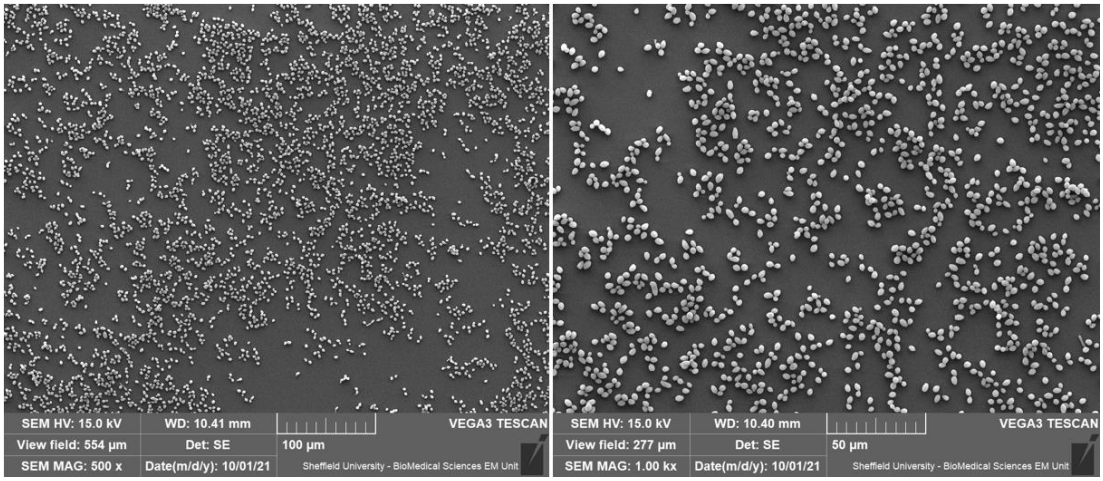

Figure 1: SEM images of C. albicans biofilm on the surface of glass disc (as a positive control) in different magnification, left: 500x, right: 1000x. SEM images

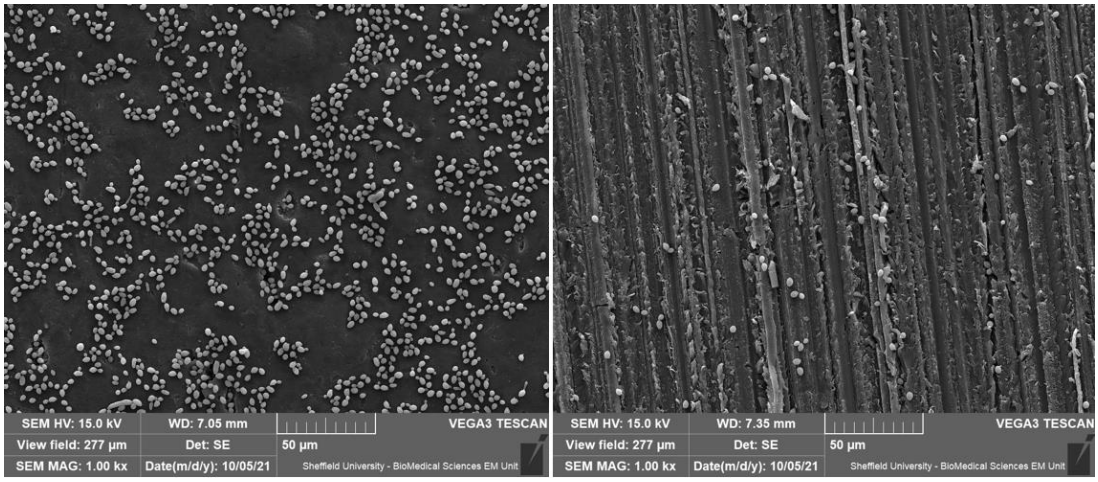

Figure 2: SEM images of C. albicans colonization on the surface of heat cured sample, (left) as processed, (b) standardised finished.

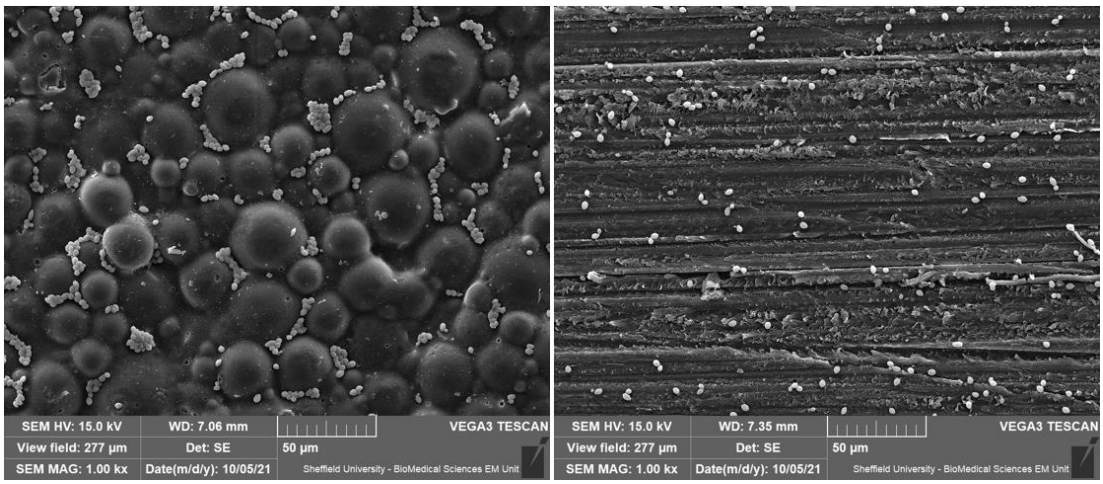

Figure 3: SEM images of C. albicans colonization on the surface of cold cured sample, (left) as processed, (b) standardised finished.

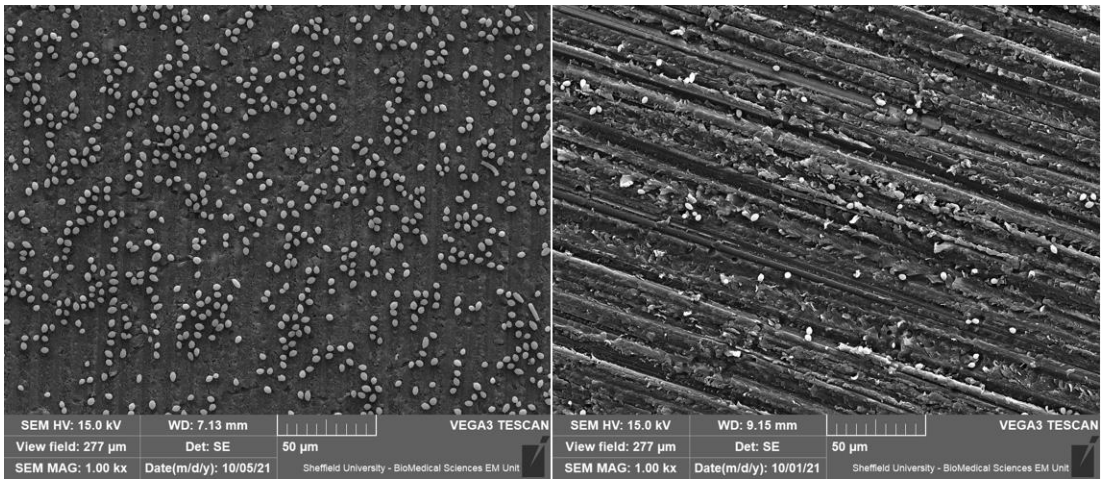

Figure 4: SEM images of C. albicans colonization on the surface of milled sample, (left) as processed, (right) standardised finished.

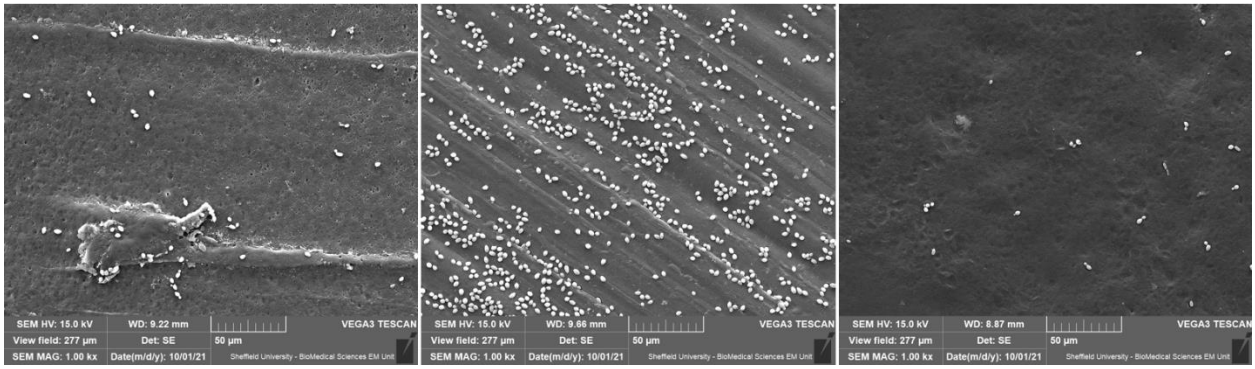

Figure 5: SEM images of C. albicans colonization on the surface of X FDM sample, (left) as processed, (middle) standardised finished, (right) acetone vapour finishing.

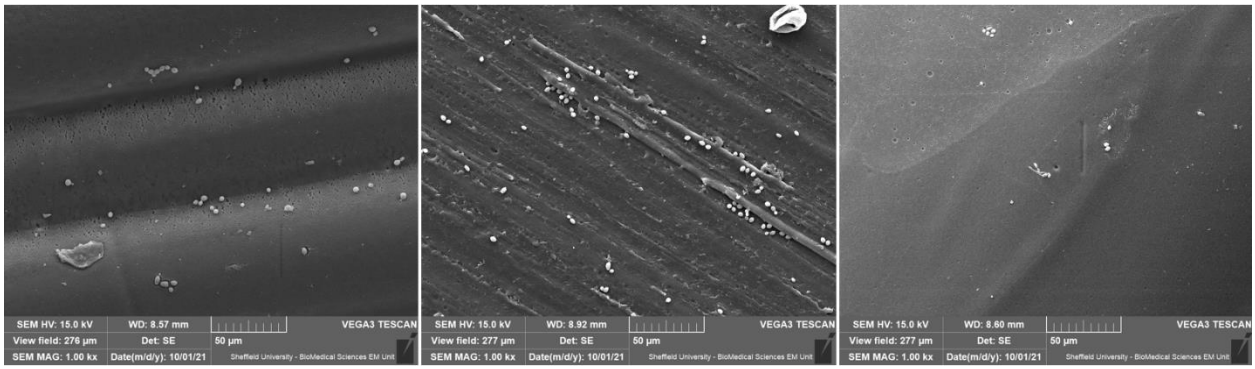

Figure 6: SEM images of C. albicans colonization on the surface of Y FDM sample, (left) as processed, (middle) standardised finished, (right) acetone vapour finishing.

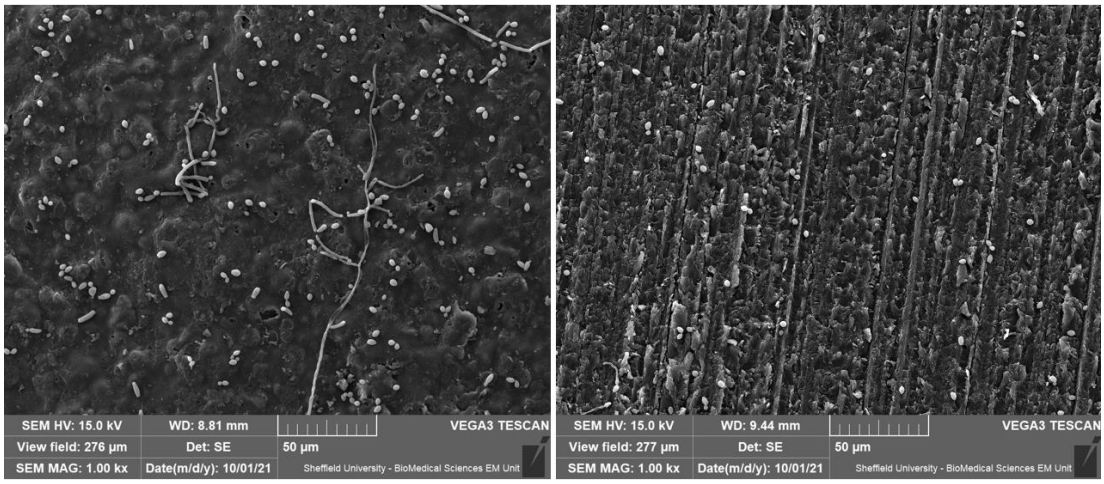

Figure 7: SEM images of C. albicans colonization on the surface of X SLA (Grey resin) sample, (left) as processed, (right) standardised finished.

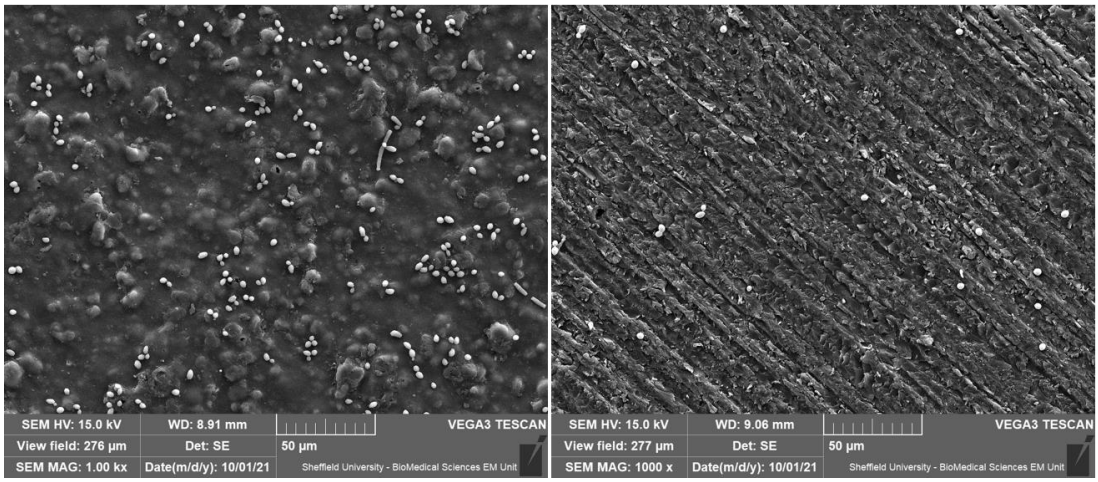

Figure 8: SEM images of C. albicans colonization on the surface of Y SLA (Grey resin) sample, (left) as processed, (right) standardised finished.

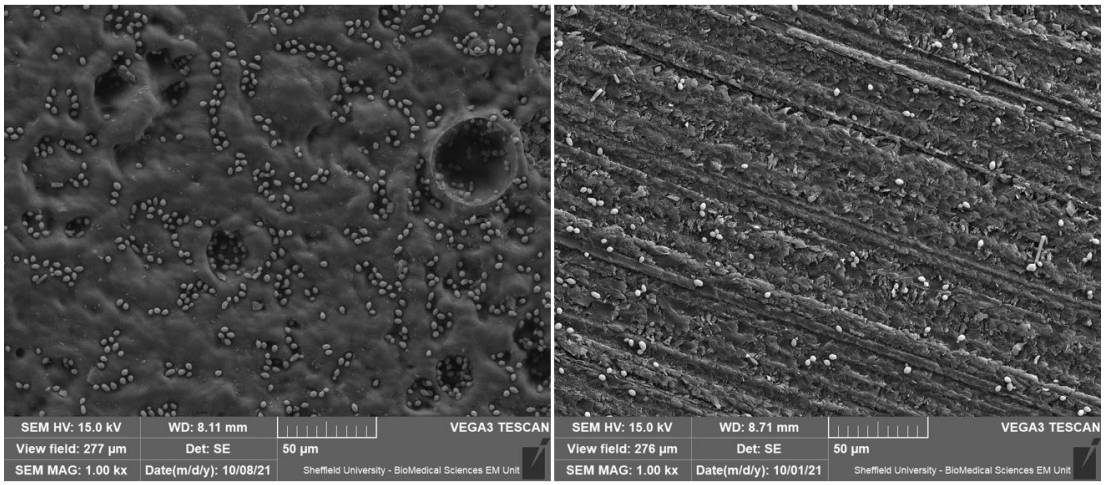

Figure 9: SEM images of C. albicans colonization on the surface of X SLA (Denture base resin) sample, (left) as processed, (right) standardised finished.

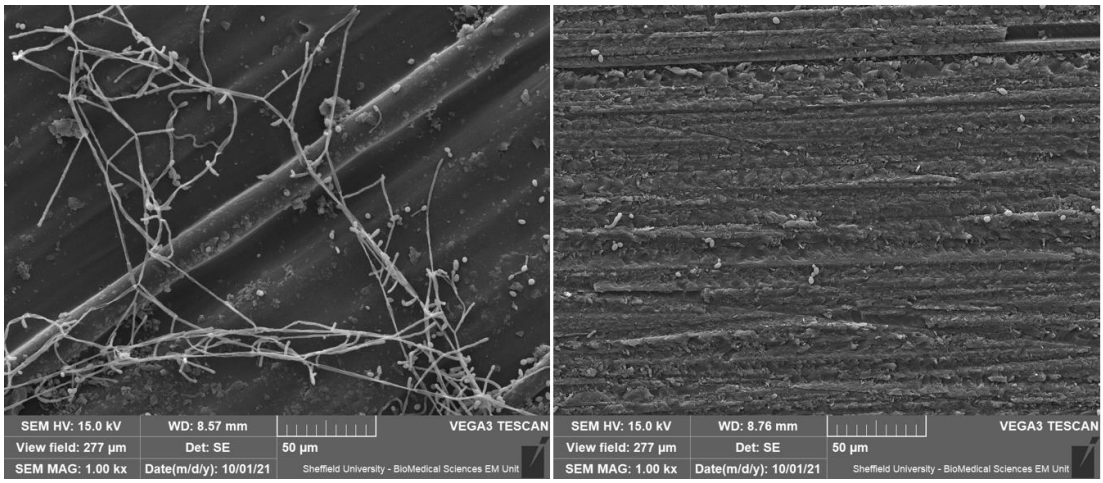

Figure 10: SEM images of C. albicans colonization on the surface of Y SLA (Denture base resin) sample, (left) as processed, (right) standardised finished.