Chemo-mechanical Characterisation and Biocompatibility of CAD/CAM Composite Blocks

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>°C</td>
<td>Centigrade</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>μg/ml</td>
<td>Microgram per millilitre</td>
</tr>
<tr>
<td>μg/mm³</td>
<td>Microgram per cubic millimetre</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometre</td>
</tr>
<tr>
<td>AB</td>
<td>Alamar blue</td>
</tr>
<tr>
<td>ADA</td>
<td>American dental association</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AS</td>
<td>Artificial saliva</td>
</tr>
<tr>
<td>Bis-EMA</td>
<td>Bisphenol A ethoxylate dimethacrylate</td>
</tr>
<tr>
<td>Bis-GMA</td>
<td>Bisphenol-A glycidyl methacrylate</td>
</tr>
<tr>
<td>BPA</td>
<td>Bisphenol-A</td>
</tr>
<tr>
<td>C–C</td>
<td>Carbon carbon single bond</td>
</tr>
<tr>
<td>C=C</td>
<td>Carbon carbon double bond</td>
</tr>
<tr>
<td>CAD/CAM</td>
<td>Computer aided design and computer aided manufacturing</td>
</tr>
<tr>
<td>CF</td>
<td>Caffeine</td>
</tr>
<tr>
<td>CMC</td>
<td>Carboxymethyl cellulose</td>
</tr>
<tr>
<td>CNC</td>
<td>Computer numeric controlled</td>
</tr>
<tr>
<td>D</td>
<td>Diagonal</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>DAPI</td>
<td>4′,6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DNH</td>
<td>Double network hybrid</td>
</tr>
<tr>
<td>ED₅₀</td>
<td>Effective dose</td>
</tr>
<tr>
<td>ELSD</td>
<td>Evaporative-light-scattering detector</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>ETD</td>
<td>Everhart-Thornley Detector</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FG</td>
<td>Functionally graded</td>
</tr>
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<td>FPD</td>
<td>Fixed partial denture</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>gf</td>
<td>Gram Force</td>
</tr>
<tr>
<td>GPA</td>
<td>Giga Pascal</td>
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<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HA</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-Hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>HF</td>
<td>Hydrofluoric acid</td>
</tr>
<tr>
<td>HGF</td>
<td>Human gingival fibroblasts</td>
</tr>
<tr>
<td>HGK</td>
<td>Human gingival keratinocytes</td>
</tr>
<tr>
<td>HP</td>
<td>High pressure</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HT</td>
<td>High temperature</td>
</tr>
<tr>
<td>INT</td>
<td>Iodonitrotetrazolium</td>
</tr>
<tr>
<td>ISO</td>
<td>International Standards Organization</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>Y-TZP</td>
<td>Yttria-stabilized tetragonal zirconia polycrystal</td>
</tr>
<tr>
<td>------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Significance level</td>
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Abstract

Composite with improved properties for CAD/CAM processed restorations can be produced using high temperature and/or high pressure polymerisation, and innovative filler microstructure and loading. Many new CAD/CAM composite materials have been investigated to evaluate their performance and durability. The overall aim of this research was to assess the mechanical, chemical and biological properties of CAD/CAM composite upon ageing in different oral and food simulating fluids at various time intervals, comparing them to ceramics and to other conventional resin-composite materials where appropriate.

The first part of this study evaluated hardness and elastic modulus of CAD/ CAM composite blocks in comparison to CAD/CAM ceramic and tooth structure. It also investigated the effect of filler weight on these properties. It was found that all resin-composite blocks (RCB) had hardness and elastic moduli closer to dentine than enamel. Polymer-infiltrated ceramic network (PICN) exhibited hardness and elastic modulus closer to that of enamel. These properties were positively correlated to filler loading. The hardness of CAD/CAM composite blocks (RCB and PICN) compared to CAD/CAM ceramic was then tested after storage in simulated oral fluids (water, artificial saliva and 75% E/W) for 30 and 90 days. It was found that CAD/CAM ceramic exhibited superior softening resistance (less hardness reduction) than CAD/CAM composite blocks with PICN showed superior softening resistance to RCB. The hardness reduction levels were negatively correlated to the filler loading, and were mostly influenced by 75% ethanol/water (E/W) storage.

The sorption and solubility of CAD/CAM composite blocks in comparison to CAD/CAM ceramic were assessed over eight months of storage in water and artificial saliva. It was found that CAD/CAM composite blocks were not as hydrolytically stable as CAD/CAM ceramic and exhibited varying degrees of sorption and solubility, influenced by their resin-matrix composition and the filler weight percentage. In addition, water and artificial saliva were considered comparable as storage media in relation to water sorption. The viscoelastic stability of CAD/CAM composite blocks was assessed under a constant static compressive stress at 24 h in dry conditions and after 3 months of water storage. The PICN material exhibited superior viscoelastic stability compared to RCB in both storage conditions, with predominantly elastic rather than viscoelastic deformation.

Monomer elution of CAD/CAM composite blocks and conventional resin-composites was assessed using high performance liquid chromatography in different storage media (water, artificial saliva and 75% E/W) for 3 months. There was minimal or no monomer elution from CAD/CAM composite blocks. Finally, and based on the monomer elution experiment, selected materials (one PICN, two RCB and a conventional resin-composite) were investigated in relation to their influence on human gingival fibroblasts (HGF) and gingival keratinocytes (HGK). All investigated materials influenced HGK proliferation and caused higher cytotoxicity than that of HGF. It was concluded that different manufacturing techniques of CAD/CAM composites had no significant effect on their biological properties and that PICN showed a cytotoxic effect in HGK.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Rasha A. Alamoush
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The Author

I graduated from the University of Jordan in 2009, gaining a DDS degree ranking in the top 10% with a GPA of 91%. I worked as a general practitioner at the Ministry of Health of Jordan for three years, and then as a teaching assistant in the Department of Removable Prosthodontics, Faculty of Dentistry in the University of Jordan during 2013-2014. I was awarded a scholarship to pursue my postgraduate studies. In 2015, I was awarded a MSc (merit) in Fixed and Removable Prosthodontics from the University of Manchester. In the same year, I enrolled in a full-time four-year clinical PhD course (Doctor of Clinical Dental Science in Fixed and Removable Prosthodontics).

In 2018, the School of Dentistry at the University of Manchester awarded me an IADR travel bursary. I am also a reviewer for the Journal of Dental Materials and the International Journal of Biomaterials.

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- British Society of Prosthodontics, Manchester, April 2016.

I have also presented aspects of my research at the following meetings:

- AIC 19th International Congress and Conseuro, Bologna (Italy), May 2016; “Nanoindentation versus Vickers hardness of Hybrid Ceramics”.
- Academy of Dental Materials (ADM) annual meeting, Nuremberg (Germany), October 2017; “Elution of Monomers from CAD/CAM Hybrid Ceramics Using HPLC”.
- IADR/PER General Session and Exhibition, London (UK), July 2018; “Long Term Hydrolytic Stability of Hybrid Ceramics”.

In addition, I have published the following papers during my studies:

Dedication

I would like to dedicate this work first and foremost to my father, who passed away this year, leaving a massive space behind him. He is the one who always encouraged me to look at every achievement as a starting point for a bigger one. May Allah grant him heaven for being always a peaceful, helpful and merciful person. I also express my deepest sadness and apologies for not being there for him over the last 5 years. He was always looking for the day I came back home with my PhD degree, but sadly he left early.

I also dedicate this work for my husband, and my daughter Toleen. Without their support and encouragement, I would not be here today. This thesis is dedicated to my mother and my siblings for their continuous prayers and support.
Acknowledgment

All praise is to Allah, the Almighty, for his blessings in my life and giving me the strength throughout the hard times and the good times.

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Chapter One:
Review of the Literature
1.1 Introduction

Prosthodontics is defined as the “dental specialty that includes diagnosis, treatment planning, rehabilitation, and maintenance of the oral function, comfort, aesthetics, and health of patients with clinical conditions associated with missing or deficient teeth and/or maxillofacial tissues using a biocompatible substitute mostly a prosthesis” (The Glossary of Prosthodontic Terms, 2005). Consequently, any prosthesis is expected to be durable, accurate and biocompatible with good aesthetics.

Alternative aesthetic restorations to conventional laboratory-processed restorations have been introduced due to advances in adhesive dentistry techniques and technological developments using computer aided design (CAD) and computer aided manufacturing (CAM) systems. The two main types of materials available for CAD/CAM aesthetic indirect restorations are glass-ceramics/ceramics and resin-composites (Fasbinde et al., 2005; Fasbinder, 2010; Ruse & Sadoun, 2014). Ceramics are considered the best aesthetic restorative material and are very strong and biocompatible (Barizon et al., 2014; Messer et al., 2003). However, there is still clinical concern regarding the abrasiveness of these materials to the opposing enamel (Fasbinder et al., 2010; Lee et al., 2014). Brittleness is another concern that might lead to crack introduction in construction, under functional load or upon adjustment of the restoration. Over time, these cracks might propagate, leading to restoration failure (Lamon & Evans, 1983).

The attractiveness of resin-composites as a CAD/CAM processed restorative material mainly attributed to them being less abrasive and brittle (Tsitrou et al., 2007) and easier to fabricate and repair than ceramics (Ruse & Sadoun, 2014). New polymerisation modes, different compositions and microstructures of resin-composites have been developed in order to improve their performance as a CAD/CAM processed restorative material (Awada & Nathanson, 2015; Nguyen et al., 2012). Another material that is gaining attention as a CAD/CAM material is polyetheretherketone (PEEK). It has similar tensile properties to those of bone, enamel and dentine (Martin & Ishida, 1989) and adequate mechanical properties, which make it suitable for prosthodontic applications (Mehta et al., 2019; Schimidlin et al., 2010).
The following sections will discuss, CAD/CAM technology, restorative dental materials mainly; CAD/CAM composite materials, and finally, the properties and techniques relevant to this research.

1.2 CAD/CAM technology

1.2.1 Introduction

CAD/CAM was first developed in the 1960s in the aircraft and automotive industries (Davidowitz & Kotick, 2011). Duret and Preston were the first to apply it in dentistry, using an optical impression and numerical milling machine to make crowns (Duret & Preston, 1991). In the 1980s, CEREC, the first commercial CAD/CAM system for dentistry, was developed (Miyazaki et al., 2009). Another commercial CAD/CAM system, Procera (now known as Nobel Procera, Nobel Biocare, Zurich, Switzerland) was developed by Dr Andersson in 1983, who was the first person to construct CAD/CAM composite veneer restorations (Andersson et al., 1996).

Dental indirect restorations fabricated using traditional techniques start with a conventional impression from which a stone model is then made, and a wax pattern is constructed. The wax pattern is then invested and replaced with the restorative material. This method requires considerable dental staff intervention and manipulation of materials. Consequently, time-consuming, costly and staff- or material-related errors are expected during the impression, waxing and casting steps (Beuer et al., 2008a; Sadan et al., 2005). With the introduction of CAD/CAM techniques in prosthodontics, many of the conventional steps such as waxing, investing and casting are not required. The application of CAD/CAM in dentistry has many advantages, generally: speed, ease of use, time-efficient and better quality of restorations (Miyazaki & Hotta, 2011). However, many conventionally produced prostheses show a better fit than milled ones (Han et al., 2011; Tan et al., 2008). Different materials including ceramics, metals, resins and waxes can be processed using the available CAD/CAM systems (Beuer et al., 2008b; Kollar et al., 2008; Miyazaki et al., 2009) and are increasingly used in prosthodontics, orthodontics, and oral and maxillofacial surgery (Farley et al., 2013).
1.2.2 CAD/CAM Components

The three main components of any CAD/CAM system are (Beuer et al., 2008b; Noort, 2012):

1. **Data acquisition:** This is the first step, where in the treatment site or a stone model is scanned using a digitalisation tool/scanner to acquire digital data that is then processed by a computer.

2. **Data processing:** Software processes the data to produce a data set for the desired prosthesis.

3. **Manufacture:** This production component transforms the digital data into the required prosthesis. After the design of the prosthesis is completed, the data is sent to the production unit, which is controlled by computer aided manufacturing software. Figure 1-1 shows the CAD/CAM versus conventional workflow for crown restoration.

![Figure 1-1: The CAD/CAM (grey) versus conventional workflow (blue) for crown restoration.](image-url)
1.2.3 CAD/CAM techniques

To date, there are two computer aided manufacturing techniques: subtractive and additive. They have differences in their basic processing protocol, materials used, and consequently, accuracy. Generally, a subtractive technique is more suitable for an intraoral prosthesis as it produces homogenous objects with acceptable accuracy. However, where high accuracy is required, especially with large prosthesis such as facial prostheses, an additive technique is preferred (Abduo et al., 2014).

1.2.3.1 Subtractive technique

Subtractive manufacturing uses a large material blank to construct the final prosthesis. The manufacturing procedure starts when the computer aided manufacturing (CAM) software transfers the computer aided design (CAD) to the milling machine. An essential component of the manufacturing procedure is the computer numeric controlled (CNC) machine, which is the construction part of the CAD/CAM system. This machine has power-driven machine tools that cut the material to produce the prosthesis. The CNC machine starts the manufacturing process once it gets the manufacturing data, which includes sequencing, milling tool choice, and tool motion direction and magnitude (Rekow et al., 1991). Different materials can be processed from industrial blanks by subtractive technique, including metals, ceramics, resins and waxes, with fewer manufacturing defects, such as porosities and inhomogeneous consistency are produced compared to conventional manufacturing (Abduo & Lyons, 2013; Denry & Kelly, 2008).

There are different milling devices based on the number of milling axes: 3-axis, 4-axis and 5-axis milling machines. The most common system is the 3-axis milling system with three path directions (X, Y and Z axes) based on calculated path values. It is unable to produce large prostheses because the milling movement is limited to the milling tool (Beuer et al., 2008b). 4-axis machines, which have an additional axis for a large blank movement, can mill larger blanks and long-span frameworks. 5-axis machines have a rotating path of the milling tool or the blank. This feature makes them able to fabricate very complex geometries and smooth external surfaces such as acrylic denture bases (Kanazawa et al., 2011). Nevertheless, the number of axes does not reflect the quality
of restoration; it instead describes the method of processing and the milling path (Beuer et al., 2008a). Figure 1-2 shows a schematic representation of the different milling axis systems.

![Milling bur](Image)

**Figure 1-2:** A schematic representation of the different milling axis systems, 3-axis (X, Y, Z), 4-axis (X, Y, Z, A), and 5-axis (X, Y, Z, A, B) systems. Adapted from Abduo et al., 2014.

### 1.2.3.2 Additive technique

Additive techniques have been used in dental and medical applications. The main principle is the formation of a three-dimensional (3D) object by a layering technique (adding the material layer by layer) based on the 3D design data (Davis, 2010; Noort, 2012). The final 3D design of the prosthesis appears as a multi-slice image. As processing starts, material is added as liquid or powder layers. Finally, the added layers are joined together, forming the final 3D prosthesis, which then needs refinement and removal of the supporting arms (Choi & Chan, 2004; Choi & Cheung, 2005). Different materials can be processed by an additive technique based on the prosthesis type and the manufacturing method. Variable shapes that can fit any biological site can be produced using an additive technique, which make it an attractive manufacturing option for dental applications (Abduo et al., 2014). There are different additive systems used in dentistry;
the most common three are selective laser sintering or melting, stereolithography, and 3D printing (Noort, 2012).

Compared to subtractive technique, an additive technique has many advantages including no material wastage and any excess material can be used in the future (Webb, 2000); the ability to manufacture large prostheses (Feng et al., 2010); the possibility of different consistencies and material characteristics of the processed material; passive production with no force needed; and higher accuracy (Abduo et al., 2014). Table 1-1 includes a summary of some advantages and disadvantages of the two techniques.

Table 1-1: Advantages and disadvantages of subtractive and additive techniques (Alghazzawi, 2016).

<table>
<thead>
<tr>
<th>Subtractive techniques</th>
<th>Additive techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>Can be used for all material types</td>
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<td></td>
<td>Ability to reproduce fine details</td>
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<td></td>
<td>More economical and faster</td>
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<td></td>
<td>Less material wastage</td>
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<td></td>
<td>Ability to produce large and/or complex prosthesis</td>
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<td></td>
<td>Passive production</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Manufacturing depends on the milling tool size, which might result in over milling and loose fit of the prosthesis</td>
</tr>
<tr>
<td></td>
<td>Cannot be used for all material types (i.e. titanium)</td>
</tr>
</tbody>
</table>
1.3 Restorative materials

Dental restorations can be divided based on their placement technique into two main categories, direct and indirect restorative materials. Indirect restorations involve fabricating the restorative material with extra laboratory steps; these steps can be conventional depending on the laboratory technician skills or digital fabrication depending on computer guided digital machines. The following sections will discuss ceramics, PEEK and resin-composite with emphasis on materials available for CAD/CAM systems.

1.3.1 Ceramics

1.3.1.1 Introduction

Ceramic is traditionally defined as: “a non-metallic, inorganic material usually processed by firing at a high temperature to achieve desirable properties” (Sakaguchi & Powers, 2012). However, the 2013 version of the American Dental Association (ADA) code on dental procedures and nomenclature defines the term porcelain/ceramic as “pressed, fired, polished, or milled materials containing predominantly inorganic refractory compounds including porcelains, glasses, ceramics, and glass-ceramics.” (American Dental Association, 2013).

Different manufacturing methods had been used for dental ceramics such as layering, slip casting, hot pressing and CAD/CAM (Griggs, 2007). Traditional manufacturing techniques have the disadvantages of low strength and porous ceramics, while ceramics produced for CAD/CAM restorations have improved mechanical properties and densities (Fasbinder, 2010; Griggs, 2007; Zhang & Kelly, 2017).

1.3.1.2 General properties

Ceramics are considered the best restorative material in terms of aesthetics and have many advantages, such as biocompatibility, high strength and colour stability (Barizon et al., 2014; Messer et al., 2003). However, their brittleness and high abrasiveness create some difficulties in terms of clinical performance and laboratory processing. Brittleness might lead to crack introduction during restoration processing, intraorally under functional load, or during adjustment or repair of the restoration. Over time, these
cracks can propagate, leading to ceramic restoration failure (Griggs, 2007; Lamon & Evans, 1983; Shenoy & Shenoy, 2010). Wear of the antagonistic tooth structure or less hard restorative materials is another concern regarding the clinical performance of ceramic (Fasbinder et al., 2010; Lee et al., 2014). Other restorative materials such as resin-composites are not as hard as ceramics and exhibit less abrasiveness and brittleness (Tsitrou et al., 2007); thus, fewer difficulties are encountered during their fabrication and clinical performance (Griggs, 2007; Ruse & Sadoun, 2014).

1.3.1.3 Ceramic classification

Ceramics can be considered as a composite of a glassy or polycrystalline matrix with fillers added to improve mechanical properties or stabilise the polycrystalline structure (Kelly, 2004; 2008). The glass matrix is responsible for aesthetic properties and ceramic translucency; however, it is brittle, which compromises the mechanical properties. The fillers added into the glass matrix are crucial to improve mechanical properties and reduce cracks development (Kelly, 2008; Li et al., 2014).

Glass-ceramics

Glass-ceramics have excellent translucency that mimics that of the natural tooth structure. As a result, they have been used for the construction of inlays, onlays, veneers, crowns, implant superstructures and fixed partial dentures (FPDs) (Sorensen et al., 1991). They can be classified into:

Silica-based glass-ceramics

Feldspathic and leucite-reinforced ceramics are composed mainly of a glass matrix, thus they are considered the best aesthetic ceramics. Yet, due to their high translucency, they are not the best option for discoloured teeth (Kelly, 2004; Vichi et al., 2014). Moreover, due to insufficient strength, they are unsuitable for restorations under high occlusal load (Bindl et al., 2006; Stawarczyk et al., 2013a).

Silica-based CAD/CAM blocks are available as monochromatic or multi-coloured layer blocks (Vitablocs TriLuxe [Vita], IPS Empress CAD Multi [Ivoclar Vivadent]). Vitablocs Mark II (Vita-Zahnfabrik) was the first feldspathic CAD/CAM ceramic that originated
from conventional feldspathic ceramic, and is still in use (Spitznagel et al., 2018). Empress CAD (Ivoclar-Vivadent) originated from CAD/CAM blocks with 40 % leucite crystals embedded in a feldspathic glass-ceramic (Höland et al., 2007). Glass-ceramic CAD/CAM restorations have shown excellent marginal integrity (Lu et al., 2018; Nejatidanesh et al., 2015; Otto & Mormann, 2015) with high survival rates (Federlin et al., 2014; Lu et al., 2018; Otto, 2017; Otto & Mormann, 2015), with leucite-based exhibiting a superior colour match to that of feldspathic-based ceramics (Nejatidanesh et al., 2015).

Lithium-based glass-ceramics

Lithium-based glass-ceramics have less glass matrix volume (30 %) than silica-based ceramics. Based on their fillers, they can be either lithium disilicate or lithium silicate glass-ceramics (Kelly, 2004; Kelly & Benetti, 2011). They have high flexural strength; good aesthetic with variable levels of translucency and shades available (Bindl et al., 2006). Both types are suitable for monolithic restorations such as veneers, inlays, onlays, endocrowns, anterior and posterior crowns. Lithium disilicate may be suitable for bridges in anterior teeth; however, neither type is suitable for bridge frameworks (Lambert et al., 2017).

*Lithium disilicate glass-ceramic* blocks have high stability values (Taskonak & Sertgoz, 2006; Tinschert et al., 2001), low thermal conductivity and high colour stability (Von Steyern et al., 2005). Nevertheless, lithium disilicate restorations are not the ideal in cases where there is a limited inter-occlusal distance or if the patient has a parafunctional habit (Kelly & Benetti, 2011). The brittleness of these ceramics and their low to moderate flexural strength and fracture toughness are the main disadvantages. Consequently, a ceramic thickness of at least 1 mm should be used to avoid fracture, and their use is usually restricted to low to moderate stress-bearing areas (Ozturk et al., 2008). Abrasion of the opposing tooth structure is also a disadvantage of lithium disilicate (Kelly & Benetti, 2011). Nevertheless, lithium disilicate CAD/CAM restorations have shown high clinical survival rates (Akin et al., 2014; Rauch et al., 2018; Seydler & Schmitter, 2015).
**Lithium silicate/Phosphate glass-ceramics.** This type has 10% dissolved zirconium dioxide in the glass matrix with very fine lithium metasilicate and lithium disilicate crystals (Rinke et al., 2015), hence it is also called zirconia-reinforced. Lithium silicate glass-ceramics (VITA Suprinity, VITA Zahnfabrik; Celtra, Celtra Duo, Dentsply-Sirona) were introduced in 2013 as monolithic CAD/CAM blocks. Two blocks, Suprinity and Celtra, have a similar microstructure (Belli et al., 2017), and they have shown a fracture toughness similar to lithium disilicate ceramics (Ramos Nde et al., 2016). No data on long-term survival rates is available, but some initial reports show a high success rate (Zimmermann et al., 2018).

**Glass-infiltrated ceramics**

These ceramics are porous and are infiltrated with lanthanum glass afterwards, producing high filled glass-ceramics. All grindable blocks of infiltration ceramics originate from the Vita In-Ceram system (Vita). Three varieties are available depending on the core material (Raigrodski & Chiche, 2002a; 2002b):

*Vita In-Ceram Alumina (Al$_2$O$_3$):* this type is suitable for crown copings, both in the anterior and posterior regions, and 3-unit FPD frameworks in the anterior region.

*Vita In-Ceram Zirconia (70% Al$_2$O$_3$: 30% ZrO$_2$):* this type has the same indication of the first variation. However, it can also be used for posterior 3-unit FPD frameworks, and for discoloured abutment teeth due to its superior masking ability.

*Vita In-Ceram Spinell (MgAl$_2$O$_4$):* this variation has the highest translucency of all oxide ceramics, which makes it recommended for high aesthetic demanding areas, such as anterior crown copings, particularly on vital abutment teeth and in young patients.

**Polycrystalline ceramics**

High performance oxide ceramics such as aluminium oxide and zirconium oxide are currently available for CAD/CAM systems. This group does not contain any glass as a non-metallic component and has a fine-grain crystalline structure as their main property (Sriamporn et al., 2014).
Aluminium Oxide (Al₂O₃) ceramics: the fabrication process of this type involves grinding in a pre-sintered phase, then sintering at a temperature of 1520°C. This ceramic type is indicated for anterior and posterior crown copings, primary crowns, and three-unit anterior FPD frameworks. Different colours can be gained by staining the ground frames individually with Vita In-Ceram colouring liquid (Dunn, 2007; May et al., 1998).

Zirconium oxide ceramics (ZrO₂): mainly yttria-stabilized tetragonal zirconia poly crystal (Y-TZP). Y-TZP has the highest strength and fracture toughness after machining and sintering (Sriamporn et al., 2014). Zirconia ceramic has superior mechanical characteristics to those of other dental ceramics, especially flexural strength and fracture toughness. Consequently, it is suitable as a framework material for both crowns and FPDs. It can also be used as an individual implant abutment in the appropriate indications (Stawarczyk et al., 2013b; Vagkopoulou et al., 2009). CAD/CAM zirconia restorations have shown high survival rates with no fractures, cracks, or chipping noticed (Bömicke et al., 2017; Seydler & Schmitter, 2015). Abrasion of antagonist teeth can be avoided if the zirconia surface is well polished and glazed (Lohbauer & Reich, 2017; Stober et al., 2014).
1.3.2 Polyetheretherketone (PEEK)

1.3.2.1 Introduction

Polyetheretherketone (PEEK) is a synthetic polymeric material that was first reported in the literature in the early 1980s (Attwood et al., 1981). It belongs to the chemical group of polyaryletherketone (PAEK), although its actual scientific name is polyoxy-1,4-phenylene-oxy-1,4-phenylenecarbonyl-1,4-phenylene). PAEKs are a group of high performance semi-crystalline thermoplastic resins with an aromatic backbone of aryl rings (Kurtz & Devine, 2007).

Different chemical structures are available according to the ratio of ketone- (PEKK) and ether- (PEEK) functional groups (Williams, 2008a). Figure 1-3 shows the chemical structure of PEEK and PEKK monomers. The monomer unit of etheretherketone polymerises via a step-growth diaalkylation reaction of bis-phenolates to form polyetheretherketone (Kurtz & Devine, 2007). PEEK can be modified in two ways: pre-polymerisation through the addition of functionalised monomers, or post-polymerisation modifications through chemical processes such as sulfonation, amination and nitration (Staniland et al., 1992). Three forms of PEEK are available: industrially pressed blanks for CAD/CAM milling, industrially pre-pressed pellets, or industrially pressed granular form. The latter two forms require a thermo-pressing or melting processing (Wagner et al., 2018).

![PEEK and PEKK Monomers](image)

**Figure 1-3:** The chemical structure of PEEK and PEKK monomers.
Invibio (Invibio, Ltd, Thornton-Cleveleys, United Kingdom) has commercially offered PEEK as an implant material since 1998 (Kurtz & Devine, 2007). Titanium and its alloys could be associated with stress shielding and failure of prostheses due to a higher elastic modulus than bone (Lee et al., 2012). Therefore, PEEK has been increasingly used as an implant material in many cases such as trauma, orthopaedic, dental, spinal and cranial implants (El Halabi et al., 2011; Kelsey et al., 1997; Liao, 1994). One major limitation of PEEK that it has lower inherent osteoconductive properties compared to titanium (Rabiei & Sandukas, 2013). Hence, many studies have proposed different methods to improve the bioactivity of PEEK, such as coating with synthetic osteoconductive hydroxyapatite (Barkarmo et al., 2013); increasing surface roughness and chemical modifications (Poulsson et al., 2014); and incorporating bioactive particles (Wang et al., 2014).

1.3.2.2 General properties

PEEK has low elastic modulus and hardness (Zok & Miserez, 2007) but it can be modified using other materials such as carbon fibres; carbon-reinforced PEEK has an elastic modulus of up to 18 GPa (Skinner, 1988), which is comparable to those of cortical bone and dentine (Rees & Jacobsen, 1993). In addition, the elastic properties of PEEK are relatively unaffected by changes in body temperature, which is below the glass transition (Tg, 143°C) of PEEK (Kurtz & Devine, 2007). However, the yielding and plastic flow properties of PEEK are affected by body temperature changes (Rae et al., 2007).

PEEK has been found to be a biocompatible material in both laboratory and clinical studies. It has no cytotoxic or mutagenic effects, and does not cause any significant tissue reactions (Katzer et al., 2002; Nieminen et al., 2008; Rivard et al., 2002; Wenz et al., 1990). The following factors make PEEK a highly biocompatible material: it has stable chemical and physical properties due to the unique chemical structure of an aryl ring connected with ketone and other groups (Williams, 2008a) and it is resistant to other substances except for concentrated sulfuric acid (Kurtz & Devine, 2007).
1.3.2.3 CAD/CAM PEEK

PEEK can be used for CAD/CAM restorations as a substitute for polymethylmethacrylate (PMMA) and has been used in many aspects of prosthodontic dentistry such as implants, provisional abutment, implant-supported bars and removable dental prostheses (Mehta et al., 2019; Schwitalla et al., 2015; Tetelman & Babbush, 2008). It has low solubility and water sorption compared to other polymer-based CAD/CAM materials (Liebermann et al., 2016) and favourable mechanical properties (Schwitalla et al., 2015). It has shown adequate wear resistance, low enamel abrasion and adequate bond strength to both resin-composites and teeth (Wimmer et al., 2016). PEEK has a low translucency and greyish pigmentation that necessitate veneering for aesthetic appearance (Stawarczyk et al., 2013c).

PEEK has recently gained more attention in CAD/CAM technology for fixed (Beuer et al., 2008a; Reich et al., 2005; Stawarczyk et al., 2015) and removable dentures (Schwitalla et al., 2015). For instance, three-unit PEEK fixed partial dentures manufactured using CAD/CAM have higher fracture resistance, stability and reliability compared to pressed granular- or pellet-shaped PEEK dentures (Stawarczyk et al., 2015). Furthermore, CAD/CAM milled PEEK fixed partial dentures were reported to have higher fracture resistance compared to those of lithium disilicate glass-ceramic, alumina (Beuer et al., 2008a) and zirconia (Kolbeck et al., 2008).
1.3.3 Resin-composite

In the 1950s polymethylmethacrylate resin (PMMA) was used for dental restorations. It had a good aesthetic appearance, was easy to manipulate and had a low cost. The main problems encountered were high polymerisation shrinkage, dimensional change, discolouration and low wear resistance (Anusavice et al., 2013). Resin-composites with improved mechanical properties and better clinical performance were then developed in the early 1960s. Over the years, resin-composite has been improved in different ways, especially with the incorporation of nanotechnology and development of adhesive dentistry. These improvements include alteration of monomer resins, initiation systems and fillers to form a composite material. These improvements developed a material with enhanced durability, wear resistance and tooth-imitating appearance (Sakaguchi & Powers, 2012).

1.3.3.1 Resin-composite composition

Resin-composite is mainly composed of three major components: the organic phase (resin-matrix); the inorganic or dispersed phase (filler particles); and the coupling agent (interfacial phase) (Randolph et al., 2018).

1.3.3.1.1 Resin-matrix

The resin-matrix forms a cross-linked structure in which the inorganic filler is dispersed. It is composed mostly of monomers. Methyl methacrylate (MMA) monomer was originally used in resin-composites, later to be replaced by an aromatic monomer (bisphenol A-glycidyl methacrylate, Bis-GMA) with molecular weight (MW)=512 g/mol), which was first developed by Bowen in 1962 (patent US3179623A) (Randolph et al., 2018).

Bis-GMA has better mechanical properties and less polymerisation shrinkage than MMA, due to the higher molecular weight and larger molecular size compared to MMA. Bis-GMA is highly viscous; therefore, it is difficult to handle and to incorporate fillers (Davy et al., 1998). A suitable viscosity for Bis-GMA can be obtained by mixing Bis-GMA with lower molecular weight monomers such as triethylene glycol dimethacrylate (TEGDMA, MW=286 g/mol ) (Feng & Suh, 2007). TEGDMA has a higher concentration of
double bonds, and when mixed with Bis-GMA, increases the degree of conversion and cross-linking compared to Bis-GMA alone (Dickens et al., 2003; Sideridou et al., 2002). However, this induces higher polymerization shrinkage (Goncalves et al., 2011), with decreased colour stability (Janda et al., 2004) and increased water sorption (Sideridou et al., 2007). Another way to dilute Bis-GMA is by substituting the hydroxyl (-OH) groups of Bis-GMA with ethoxy groups to give an ethoxylated Bis-GMA or so-called ethoxylatedbisphenol A dimethacrylate (Bis-EMA, MW=540 g/mol) (Feilzer & Dauvillier, 2003; Goncalves et al., 2010; 2011). The addition of Bis-EMA improves the degree of conversion (Froes-Salgado et al., 2015) and reduces polymerisation stress (Boaro et al., 2010).

The biocompatibility of Bis-GMA may be questionable due to the possibility, although unlikely, of bisphenol A (BPA) release (Polydorou et al., 2009; Sevkusic et al., 2014). Thus, other high molecular weight monomers have been introduced, such as urethane dimethacrylate monomers. Many resin-composites now contain urethane dimethacrylate (UDMA, MW= 470 g/mol). UDMA has a lower viscosity than Bis-GMA and high flexibility due to the urethane linkage compared to Bis-GMA (Floyd & Dickens, 2006). This increases the degree of conversion and reduces polymerisation shrinkage (Dickens et al., 2003; Goncalves et al., 2010). Consequently, this improves the mechanical properties of resin-composites composed of UDMA, such as flexural strength, elastic modulus and hardness (Tanimoto et al., 2005). In addition, UDMA shows less water sorption and solubility compared to Bis-GMA (Sideridou & Karabela, 2011). Unlike Bis-GMA, UDMA does not necessarily require the addition of TEGDMA as a diluent to lower the viscosity. Figure 1-4 shows the chemical structure of dimethacrylate monomers mostly used in resin-composite.
Figure 1-4: The chemical structure of dimethacrylate monomers mostly used in resin-composite.

1.3.3.1.2 Fillers

Fillers are inorganic and/or organic particles of resin-composite that improve the mechanical properties, decrease thermal expansion and polymerisation shrinkage, and reduce water sorption (Anusavice et al., 2013).

Silica is considered the first filler incorporated in the resin-matrix. It is also called quartz in crystalline form (Anusavice et al., 2013). Nanosilica and aggregates particles can be produced via superheating of silicon tetrachloride or quartz sand, a process called silica pyrogenesis, those nanoparticles could combine and form agglomeration (Kim et al., 2007; Nozawa et al., 2005). Silica is considered more opaque than other fillers; but it has
desirable mechanical properties (Habib et al., 2016). Silica can be integrated with alkaline oxides such as barium oxide (BaO) and strontium oxide (SrO) with different formulations available such as barium borosilicate, barium aluminosilicate, barium aluminium borosilicate, and strontium silicate (Habib et al., 2016). The main advantage of alkaline-oxides containing composites is the high radioopacity due to the presence of heavy metals such as barium and strontium (Watts, 1987) but it has lower hardness compared to pure silica (Osiewicz et al., 2015). Nevertheless, it has comparable wear and stiffness, and superior optical properties to that of silica (Habib et al., 2016).

Other glasses such as calcium, sodium or phosphorus oxides have been tested as resin-composite fillers. Various metal oxides, other than silicon dioxide, can be used as resin-composite fillers such as aluminium oxide (Al₂O₃) (Thorat et al., 2013), titanium dioxide (TiO₂) (Thorat et al., 2012; Yoshida et al., 2001a; 2001b), zinc oxide (ZnO) and zirconium oxide (ZrO₂). These metal oxides are not widely used commercially. However, 3M ESPE uses significant amounts of zirconia and hybrid zirconia-silica fillers in their resin-composite formulations (Habib et al., 2016; Suzuki et al., 1996; Yap et al., 2004).

Specific fillers with specific geometries and structures adjusted to improve resin-composite properties, such as fibres, clusters, or pre-polymerized fillers, have been used in resin-composite. Fibres or fibre-like particles fillers with SiO₂ are added to improve the fracture toughness of resin-composites. They improve the fracture toughness due to the high length-to-diameter ratio of these fibres within the resin-matrix (Bocalon et al., 2016; Garoushi et al., 2013). However, fibre-infiltrated resin-composites exhibit rough surfaces (Van Dijken & Sunnegårdh-Grönberg, 2006) and low wear resistance (Manhart et al., 2000). Clusters or nano-clusters defined as agglomerations of submicron filler particles via sintering or chemical binding are other variations of specific resin-composite fillers. They are claimed to improve the strength of resin-composites (Curtis et al., 2009). Moreover, pre-polymerised fillers (PPFs), defined as pre-cured resin-composites of more than 10 µm size, composed of nano and/or microsized filler particles, are another variation of specific fillers. They are proposed to improve the aesthetic properties of resin-composites as they have higher polishability, hence are usually used in anterior or aesthetic applications (Angeletakis et al., 2005). However, they reduce the elastic modulus of resin-composites and exhibit high water sorption
(Randolph et al., 2016). Figure 1-5 illustrate specific fillers: cluster, fibre and pre-polymerised fillers.

![Diagram of fillers: cluster, fibre and pre-polymerised fillers]

**Figure 1-5**: Specific fillers: cluster, fibre and pre-polymerised fillers. Adapted from Randolph et al., 2018.

Other types of composite fillers include organically modified ceramic (Ormocers) fillers which combine organic (polymer) with inorganic oxide (ceramic) constituents at the molecular level (Klapdohr & Moszner, 2005), forming an inorganic network by polycondensation (Kalra et al., 2012). This combination is claimed to reduce viscosity, volumetric shrinkage and shrinkage stress compared to conventional resin-composites (Pick et al., 2011).

Hydroxyapatite nanoparticles have been investigated as resin-composite filler but have been deemed unsuitable for use in their own unless mixed with HA microparticles to have sufficient mechanical properties (Arcis et al., 2002; Domingo et al., 2001). Urchin-like hydroxyapatite (UHA) is a novel HA preparation that has improved mechanical properties (Liu et al., 2014).
1.3.3.1.3 Coupling agent

Coupling agent is responsible for filler-matrix bonding. It is usually applied on the filler particle surface to ensure chemical bonding with the organic matrix. The most common resin-coupling agent is silane (Anusavice et al., 2013). Figure 1-6 shows the chemical structure of silane coupling agent (3-methacryloxypropyltrimethoxysilane (MPS)). In addition to the bonding function, coupling agents can improve the mechanical properties of resin-composite, provide stress distribution between filler particles and the organic matrix and reduce water sorption (Sakaguchi & Powers, 2012).

![Chemical structure of silane coupling agent (MPS)](image)

**Figure 1-6**: The chemical structure of silane coupling agent (MPS).

1.3.3.2 Degree of conversion

The degree of conversion is the percentage of carbon-carbon double bonds (C=C) converted into single bonds (C–C) due to the polymerisation process (Anusavice et al., 2013). The degree of conversion of dimethacrylate polymers usually ranges from 55% to 75% (Imazato et al., 2001; Ruyter & Oysaed, 1987). A higher degree of conversion positively influences mechanical properties, wear resistance (Ferracane et al., 1997), hardness (Chen et al., 2005), elasticity modulus (Lin-Gibson et al., 2009), colour stability and biocompatibility of resin-composites (Krifka et al., 2012).
1.3.3.3 Resin-composite classification

1.3.3.3.1 Classification of resin-composite based on filler content

Filler content usually comprises more than 50 volume percentage (vol%) of dental resin-composite (Adabo et al., 2003; Randolph et al., 2016). Based on filler vol%, resin-composites can be classified into ultra-low fill with less than 50 vol%, low-fill of more than 50 vol% and compact resin-composites of higher than 74 vol% (Randolph et al., 2016). When fillers are used at high percentages, there is a difficulty in their incorporation by mixing. This difficulty might be encountered less with industrial resin-composites (CAD/CAM blocks) as their manufacturing makes better incorporation of fillers possible (Mainjot et al., 2016).

Composites can also be classified based on their filler; size, distribution, geometry and composition (Randolph et al., 2018), however, filler size is the most commonly used. Macrofilled resin-composites have large, spherical or irregularly shaped glass or quartz particles of usually one size, but can range in size from 10-50 μm (Randolph et al., 2018). This material is very strong but it can not be polished. To overcome this disadvantage, microfilled resin-composite were introduced in the 1970s. These composites contain some nanoparticles (<100 nm) of amorphous spherical reinforcing silica particles; thus, would more precisely be called nanofills. Filler level incorporated in these composites was low, but this was increased using highly filled, pre-polymerised resin fillers (PPRF) within the resin-matrix (Ferracane, 2011). Later, hybrid composites were introduced utilising nano and microsized particles. They are mainly composed of nanoparticles (<100 nm) and submicron particles (Ferracane, 2011). Based on filler composition and size, they can be microhybrid or nanohybrid (Randolph et al., 2018). Figure 1-7, shows the chronological classification of resin-composite based on filler size.
Figure 1-7: Chronological classification of resin-composite based on filler size (Randolph et al., 2018).
1.3.3.3.2 Classification of resin-composite based on manufacturing

Resin-composites can be divided into two main categories, direct and indirect. Indirect resin-composite has the same composition and structure as a direct resin-composite; however, it has less polymerisation stress on the tooth structure as it is photopolymerised extraorally (Ferracane & Hilton, 2016) and it can be conventional that is fabricated and polymerised in dental laboratories or industrial that is available as pre-cured blocks for CAD/CAM systems.

The photopolymerisation efficiency of conventional resin-composite depends on the curing unit parameters (irradiation time and mode, irradiance, radiant exposure), the temperature, the material content (photoinitiator, monomers, fillers, shading pigment), the material viscosity and optical properties (Leprince et al., 2013). Consequently, varying degrees of conversion are expected with different parameters (Imazato et al., 2001). Hence, internal stresses within the material are introduced due to polymerisation heterogeneity from the surface to the depth that can negatively influence the mechanical properties and result in free monomer release (Nguyen et al., 2012), which is less encountered with extraoral polymerisation. Post-curing heat application (>100°C) has been reported to improve double-bond conversion, the mobility of monomers and the polymer chains, hence improving cross-linking (Bagis & Rueggeberg, 2000). This can enhance the degree of conversion and mechanical properties of the indirect resin-composite (Ferracane & Condon, 1992).

Industrial composite blocks polymerised under high pressure and/or high temperature result in higher homogeneity and reliability with fewer flaws and pores compared to the conventional indirect composite (Nguyen et al., 2012). This polymerisation mode allows for higher filler content incorporation without affecting the viscosity (Giordano, 2006). Therefore, CAD/CAM composites have improved mechanical properties compared to indirect resin-composites (Alt et al., 2011); they have better wear resistance (Mormann et al., 2013; Stawarczyk et al., 2013d), flexural strength (Nguyen et al., 2014), fracture toughness and fracture strength (Coldea et al., 2013a; Della Bona et al., 2014). Heat polymerised urethane dimethacrylate (UDMA) matrices have been recently used in most CAD/CAM composites. UDMA-containing composite has a high degree of
conversion and cross-linking (Sideridou et al., 2002) and consequently, low water sorption and solubility (Sideridou & Karabela, 2011), improved mechanical properties and better colour stability (Sideridou et al., 2002).

1.3.4 Composites for CAD/CAM restorations

Aesthetic restorative materials with high fracture resistance can be either high crystalline ceramics or resin-composites with high filler loading. The main problem faced with such materials is the elasticity mismatch with that of enamel and dentine, leading to fracture of the ceramic restoration, or sometimes tooth structure where resin-composite restoration is used (Zhang et al., 2016). Moreover, ceramics are more prone to damage during machining, especially thin sections (Giannetopoulos et al., 2010; Tsitrou et al., 2007). Newer formulations of CAD/CAM materials aim to make the best combination of resin-composite and ceramics utilising advantageous properties such as the durability and colour stability of ceramics, and the improved flexural properties and low abrasiveness of resin-composite (Coldea et al., 2013a; Schlichting et al., 2011), with a modulus of elasticity that is close to that of dentine when compared to traditional ceramics. Such combination also allow easier milling and adjustment and are easier to repair or modify (Gracis et al., 2015). Moreover, they might be a better option for implant-supported crowns or restorations under high occlusal load (He & Swain, 2011; Silva et al., 2017).

CAD/CAM composite materials are composed of polymer matrix with a high volume fraction of different fillers. Hence, they can be considered as ceramic-like materials based on the new definition of ceramic materials by ADA (American Dental Association, 2013). Based on their structures and industrial polymerisation, CAD/CAM composite can be heavily filled resin-composite polymerised under high temperature and/or pressure, that are marketed under different names such as resin-matrix ceramics, ceramic-like materials, nano-ceramics, hybrid ceramics, resin-based composites (RBCs) or resin-composite blocks (RCB) (Coldea et al., 2013a; Mainjot et al., 2016). Resin-composite blocks (RCB) might be more relevant to describe resin-composites designed for use with CAD/CAM systems, and covers a more extensive range of materials.
Polymer-infiltrated ceramic network (PICN) is a different type of CAD/CAM composite materials, which is a sintered ceramic network infiltrated with a resin (Coldea et al., 2013a; Mainjot et al., 2016). PICN is also described as resin-matrix ceramic, hybrid ceramic, double-network material or ceramic-based interpenetrating-phase composites (Denry & Kelly, 2014; Gracis et al., 2015; Mainjot et al., 2016; Swain et al., 2016). The term PICN seems more descriptive for this material. In addition, another CAD/CAM composite has been fabricated using filler press and monomer infiltration method (FPMI) (Okada et al., 2014). For simplicity, the term CAD/CAM composite blocks will describe both PICN and resin-composite blocks (resin-composite designed for CAD/CAM systems) for the rest of literature review and experimental chapters. In cases where distinction is required, they will be referred to as PICN or RCB.

1.3.4.1 Resin-composite blocks (RCB)

These materials are conventional resin-composites that are polymerised under high temperature and/or high pressure (HT/HP), resulting in enhanced mechanical and chemical properties (Nguyen et al., 2013; Phan et al., 2014; Tang et al., 2014). They are manufactured by incorporating filler particles in a monomer mixture. The first commercially released RCB was Paradigm MZ100 (3M ESPE, St. Paul, MN, USA) in 2000, with 85% ultra-fine zirconia-silica ceramic particles (0.6 μm) embedded in a polymer matrix of Bis-GMA, TEGDMA and an initiator system. Lava™ Ultimate (3M ESPE) with nanoparticle and nanocluster fillers was introduced in 2011. It is a highly cured resin-matrix reinforced with zirconia-silica filler particles of about 80% by weight (wt%), with better mechanical properties than Paradigm MZ100 due to different polymerisation temperature and pressure parameters (Thornton & Ruse, 2014). It also has similar modulus of elasticity (12 GPa) to that of dentine (Lauvahutanon et al., 2014). After the release of Lava™ Ultimate, many industrial materials were released by different manufacturers, with variable filler loadings and compositions, such as Shofu Block HC by Shofu (60% filler weight), Cerasmart by GC (70 wt%), BRILLIANT Crios by Coltene (71 wt%) and many other examples.
1.3.4.2 Polymer-infiltrated ceramic network (PICN)

PICN is manufactured through two stages, first: production of a porous, pre-sintered ceramic network that is then conditioned by a coupling agent; second: polymer infiltration into this network by capillary action (Coldea et al., 2013a; Della Bona et al., 2014). Management of polymerisation shrinkage stress effects on the ceramic-network was problematic when PICN was first formulated (Swain et al., 2015). However, use of high temperature (HT of about 180-200°C) (Nguyen et al., 2012; Sadoun, 2011) and high pressure polymerisation (HP of about 300 MPa) has overcome this problem (Sadoun, 2011). HT decreases shrinkage and defects both in number and size by increasing the chain mobility and thereby polymerisation (Nguyen, et al., 2012).

This material can also be called double network hybrid (DNH) as it is composed of two interpenetrating networks of ceramic and polymer (Dirxen et al., 2013), imitating the natural interlocking teeth prism bands (Zhang & Kelly, 2017). This network of ceramic and polymer results in a three-dimensional scaffold that improves stress distribution throughout the polymeric network (Feng et al., 2003), and material resistance to breakdown and deformation through ceramic network (Mainjot et al., 2016; Swain et al., 2016). In early 2013, VITA (VITA Zahnfabrik, Bad Säckingen, Germany) introduced Enamic, the first and only commercially available PICN. Enamic is mainly composed of a dual network of a pre-sintered glass-ceramic network (86 wt%, 75 vol%) infiltrated with a monomer network (14 wt%, 25 vol%) which is then polymerised. The ceramic part is composed of 58% to 63% SiO₂, 20% to 23% Al₂O₃, 9% to 11% Na₂O, 4% to 6% K₂O, 0.5% to 2% B₂O₃, and less than 1% of Zr₂O and CaO. The polymer network is composed of UDMA and TEGDMA (Coldea et al., 2013a). Figure 1-8, shows the networking principle of PICN (Enamic) and Figure 1-9 shows schematic representations and SEM images demonstrating the internal structure of RCB and PICN.
Figure 1-8: The networking principle of PICN (Enamic). Adapted from (www.vitazahnfabrik.com, 2019).

Figure 1-9: Schematic diagram (A) and SEM image (B) demonstrating the internal structure of RCB (Lava™ Ultimate) and schematic diagram (C) and SEM image (D) demonstrating the internal structure of PICN (Enamic). Adapted from Aboushelib & Elsafi, 2016; Swain et al., 2016.
1.3.4.3 Experimental PICN

Experimental PICN blocks have shown promising results regarding mechanical and biological properties (Nguyen et al., 2013; 2014). Differences in the ceramic manufacturing process (pressed or slipped); variations in HT and HP parameters; and the nature of the monomers and initiators are all factors that affect the final microstructure of the materials and consequently their performance (Nguyen et al., 2014; Ruse & Sadoun, 2014). A study on an experimental HT (180°C) and HP (300 MPa) polymerised PICN using two different manufacturing techniques resulted in two different microstructures with different mechanical properties. Slip casting and infiltration of a sintered glass-ceramic network (73.8 vol%) with UDMA (without initiator) was used to make a PICN that showed a flexural strength around 288 MPa, while conventional filler mixing of the same components, i.e. RCB (65 vol%), showed a flexural strength of only around 122 MPa (Nguyen et al., 2014). PICN made with an initiator had more improvement in the flexural strength, up to 300 MPa, a value that is very close to the most resistant glass-ceramics (Ruse & Sadoun, 2014). Moreover, freeze casting can be used to produce a highly aligned porous ceramic-network that can be infiltrated with polymer to produce PICN (Algharaibeh et al., 2019; Al-Jawoosh et al., 2018).

Polymer infiltration was found to improve the fracture toughness of PICN, with similar indentation creep to that of enamel, and lower hardness than ceramics. Subsequently, PICN has the potential to mimic the properties of enamel and dentine (He & Swain, 2011). Also, the strength and microstructure of PICN were greatly influenced by pressure and heating, for instance, defect-free PICN microstructure with improved strength, Weibull moduli and fracture toughness were produced due to the even distribution of defects mediated under pressure (300 MPa) polymerisation (Franco Steier et al., 2013). Further, using high density and high sintering temperature for ceramic networks of an experimental PICN (of zirconia ceramic network) resulted in flexural strength, elastic modulus, fracture toughness and hardness similar to that of tooth structure (Li & Sun, 2018).
Different experimental PICN have been manufactured adopting the concept of a biomimetic composite material. This concept proposes a composite material with different layers of alumina lamellae infiltrated with epoxy resin in a way that reproduces the anisotropy of tooth tissues (Petrini et al., 2013). Applying the same concept of producing a biomimetic material, an experimental functionally graded (FG) polymer-infiltrated ceramic network (PICN) block was fabricated using a slurry of glass-ceramic powder that was centrifuged and sintered. The central concept was to introduce a gradient of mechanical and optical properties throughout the thickness of the block. After that, the block was infiltrated with UDMA and polymerised under high temperature and pressure. This material exhibited superior flexural strength and flexural load energy to that of CAD/CAM ceramics (EMX, IPS e.max CAD; HT-ZIR, translucent zirconia) and similar hardness and elastic modulus to that of tooth structure (Eldaafrawy et al., 2018a).

1.3.4.4 General properties

The mechanical properties of PICN (Enamic) are superior to those of RCB (Lava™ Ultimate) (Coldea et al., 2013a; Thornton & Ruse, 2014) but both PICN and RCB have shown higher fracture and fatigue resistance under high loads compared to CAD/CAM ceramics (El Zhawi et al., 2016; Magne & Knezevic, 2009; Magne et al., 2010; Schlichting et al., 2011). These restorations are therefore more likely to survive heavy loads, even at a low thickness, and would be suitable for molar restorations (Furtado de Mendonca et al., 2019; Okada et al., 2018), especially RCB composed of a higher fraction of nanofillers in their resin-matrix (Yamaguchi et al., 2018). However, CAD/CAM composites mechanical properties are more affected by ageing conditions than CAD/CAM ceramics (Facenda et al., 2018; Porto et al., 2019; Thornton & Ruse, 2014; Yin et al., 2019); with CAD/CAM composite blocks having higher hardness and indentation moduli are less likely to degrade due to artificial tooth brushing or water storage (Flury et al., 2017).

PICN crowns (Enamic) have shown better resistance to crack initiation and propagation than glass-ceramics (Coldea et al., 2015; 2013b; Swain et al., 2016). Unlike ceramics, PICN does not cause any enamel wear (Coldea et al., 2013a). In terms of self-wear, PICN is similar to lithium disilicate glass-ceramics (Mormann et al., 2013) and inferior to
enamel (Lawson et al., 2016; Xu et al., 2017). The wear behaviour of RCB is similar to that of enamel (Swain et al., 2016), in that it does not roughen the opposing enamel surface (Sripetchdanond & Leevaloj, 2014), but it has a higher self-wear level compared to CAD/CAM ceramics (Mormann et al., 2013). PICN has similar abrasiveness and high flexural strength than that of dentine, with close elasticity to that of tooth structure, attributable to the fine particle feldspar ceramic and polymeric networks (Dirxen et al., 2013). With such properties, PICN can be a biomimetic alternative to conventional indirect resin-composite composites and ceramics (Zhang & Kelly, 2017).

In terms of bonding, surface treatment by sandblasting for RCB, and HF etching for PICN (Vita Enamic), followed by silanization to promote chemical adhesion, have been recommended (Eldafrawy et al., 2018b; Mine et al., 2019). Direct repair of resin-composite block restorations is simple following surface treatment protocol (alumina air abrasion, mechanical abrasion) then bonding and direct composite application (Wiegand et al., 2015). PICN restorations can also be repaired by direct resin-composite using hydrofluoric acid or sandblasting surface treatment followed by silanization (Bello et al., 2018). However, feldspathic ceramic restoration repair using direct resin-composite is not as successful and produces inferior aesthetics due to the differences in optical properties and discoloration susceptibility between the two materials (Ruse & Sadoun, 2014). This highlighted one of advantages of CAD/CAM composite blocks over ceramics, in that they can be repaired intraorally and are easily polished (Ruse & Sadoun, 2014).

CAD/CAM composite blocks have been found to be more susceptible to staining (Muhlemann et al., 2019) or may be comparable to ceramics in terms of staining susceptibility (Alharbi et al., 2017; Stawarczyk et al., 2012) but they have shown more colour stability than direct and indirect resin-composite materials (Quek et al., 2018). Hence, ceramic is considered the material of choice when a highly aesthetic restoration is required (Mainjot et al., 2016). PICN shade range is narrow, and their colour stability and durability, especially in cervical areas, are still questioned (Dirxen et al., 2013). However, in 2017, multicolour blocks with gradient six layers from cervical to incisor area were produced (www.vitanorthamerica.com, 2017).
CAD/CAM composite blocks have many advantages over ceramics in terms of milling, including better machinability, as they are easier to mill and repair in case of failure (Zaghloul et al., 2014). CAD/CAM composite blocks are milling damage tolerant, as they need less milling time, so result in better tool lifetime, i.e. less damage and abrasion of the milling tool (Lebon et al., 2015). They are not as hard as CAD/CAM ceramics and are less brittle, so they are less likely to chip during CAD/CAM milling procedure. Also, a better marginal quality can be obtained in less time (Giordano, 2006). One more advantage is that CAD/CAM composites do not need post-milling firing, which is typically used for ceramic staining or crystallisation (Lambert et al., 2017; Lebon et al., 2015; Zaghloul et al., 2014).

1.3.4.5 Restorative indications

CAD/CAM composite blocks can be used for veneers, inlays/onlays, anterior and posterior single crowns, and anterior and posterior bridges. PICN have the same indications of RCB, in addition to implant-supported crowns, as the ceramic-polymeric networking allows stress distribution and prevent plastic deformation (Feng et al., 2003; Mainjot et al., 2016; Swain et al., 2016; Zhang & Kelly, 2017). CAD/CAM composite materials are proposed for many restorative indications, including full-coverage crowns (Rosentritt et al., 2017) and posterior occlusal veneers (Johnson et al., 2014). Based on different case reports, CAD/CAM composite blocks, especially PICN, could be a suitable treatment option for dental erosion or bruxism (Dirxen et al., 2013), amelogenesis imperfecta (Guth et al., 2014), severe dental erosion (Mainjot, 2018; Peampring, 2014) and primary teeth restorations (Bilgin et al., 2016). Clinical evidence on these materials use and longevity is still scarce (Colombo et al., 2019), and these indications are provisional. Because of this, some manufacturers have removed some indications (full coverage crowns) from the material information (Lava™ Ultimate, 3M ESPE) due to debonding, or have even withdrawn materials from the market (Paradigm MZ100, 3M ESPE) (Lambert et al., 2017; Yoshihara et al., 2016).
1.4 Properties of Resin-composites

1.4.1 Mechanical properties

1.4.1.1 Hardness and Elastic modulus

Hardness is defined as the resistance of a material surface to indentation or penetration. It has been used as an indication of many properties such as wear resistance, polishability and the abrasiveness of the material itself or to the opposing dentition (Sakaguchi & Powers, 2012). Microhardness measurements can also be used to assess material softening upon storage in different solvents (Leprince et al., 2014; Marghalani & Watts, 2013). This softening usually results from hydrolytic degradation of the resin-matrix (Ferracane, 1994; Ferracane et al., 1998). Microhardness is influenced by the filler composition (Curtis et al., 2008; Kim et al., 2007), monomer system, coupling agent (Curtis et al., 2008; Ferracane et al., 2014; Kim et al., 2007), ageing and water sorption (Martos et al., 2003; Sonmez et al., 2018).

There are different elastic properties that reflect the elastic strain or plastic strain behaviour of dental materials. The most commonly used one is the modulus of elasticity (Elastic modulus or Young’s modulus); other elastic properties include the dynamic elastic modulus, flexibility, resilience, and possion’s ratio. The elastic modulus reflects how rigid the material is. In other words, it measures the material stiffness. The elastic modulus of a particular material is constant and it is not influenced by any elastic or plastic stress applied to that material (Anusavice et al., 2013). It is represented by the slope of the elastic part of the stress strain curve of a particular material (Anusavice et al., 2013).

The longevity and performance under masticatory forces of restorative material, is greatly influenced by the elastic modulus. Stiffer materials, such as ceramics, tend to crack under stress, leading to restoration failure, whereas in composites, which are less stiff than tooth structures, the stress might be transferred to the tooth structure, leading to tooth structure fracture (Zhang et al., 2016). Restorative materials with comparable elasticity or stiffness to that of tooth structure tend to distribute occlusal stress without restoration failure (Feng et al., 2003; Wang et al., 2017; Zhang & Kelly, 2017).
This is important in implant-supported crowns, as a less stiff restorative material such as resin-composite, rather than ceramic, allows stress distribution, especially that the periodontal ligament is lost (Feng et al., 2003; Zhang & Kelly, 2017). Both hardness and elastic modulus are important in predicting the material wear resistance, viscoelastic stability, water sorption and other mechanical properties; and both are influenced by the filler loading of resin-composite (Chung, 1990; Lin-Gibson et al., 2009).

CAD/CAM blocks have better mechanical properties than conventional resin-composites, due to polymerisation methods (including HT and HP) allowing higher filler content and higher conversion of the polymeric matrix (Alt et al., 2011; Coldea et al., 2013a; Della Bona et al., 2014; Giordano, 2006; Mormann et al., 2013; Nguyen et al., 2012; 2014; Stawarczyk et al., 2013d). Also, monomer composition shifting toward UDMA has a role in that improvement (Sideridou et al., 2002; Sideridou & Karabela, 2011). Some of the available CAD/CAM blocks exhibit comparable mechanical properties to that of dentine and enamel (Coldea et al., 2013a; Ruse & Sadoun, 2014).

1.4.1.2 Viscoelastic behaviour (creep)

Creep can be defined as the strain that a material exhibits in response to load application (Vaidyanathan & Vaidyanathan, 2001). Based on the load, creep can be classified into static creep, where a constant load is applied, or dynamic creep, where a cyclic load is applied (El Hejazi & Watts, 1999). Both types can be positively correlated (El Hejazi & Watts, 1999; Kaleem et al., 2012; Odén et al., 1988; Vaidyanathan & Vaidyanathan, 2001). Creep in conventional resin-composites has been extensively investigated (Al-Ahdal et al., 2015; Baroudi et al., 2007; El Hejazi & Watts, 1999; El-Safty et al., 2013; Wei et al., 2011). However, a few studies have investigated the creep of PICN with different filler loadings (Coldea et al., 2014; He & Swain, 2011). The viscoelasticity of a dental restorative material can be investigated utilising various techniques including indentation (Coldea et al., 2014; He & Swain, 2011), three-point bending (Alrahlah et al., 2018) and compression of a cylindrical specimen (Al-Ahdal et al., 2015; Baroudi et al., 2007; El Hejazi & Watts, 1999; El-Safty et al., 2013). The latter method has been used to investigate the creep behaviour of CAD/CAM composite materials in this research. Factors influencing viscoelastic behaviour (creep) are:
**Resin-composite composition:** The filler microstructure (volume percentage, size and distribution) and matrix composition can both influence creep behaviour (Al-Ahdal et al., 2015; Vaidyanathan & Vaidyanathan, 2001). Filler microstructure and loading improve mechanical properties such as tensile and compressive strength, hardness, and elasticity modulus (Chung, 1990; Lin-Gibson et al., 2009) and consequently, improve creep resistance (El-Safty et al., 2012).

**Degree of polymerisation:** This is more influential for conventional rather than CAD/CAM resin-composites, which have adequate polymerisation under high pressure and temperature (Nguyen et al., 2012) resulting in 95% degree of conversion (Phan et al., 2014). Another reason for this improved degree of conversion is UDMA being used in most CAD/CAM composites (Sideridou et al., 2002). Consequently, improved polymerisation and degree of conversion resulted in more viscoelastic stability or, in other words, more creep resistance (Al-Ahdal et al., 2015; Watts, 1987).

**Temperature and humidity:** Restorative materials are subjected to variable acidity levels, temperatures and masticatory forces that affect longevity and clinical performance (Musanje & Darvell, 2003; Sarrett, 2005). Intraoral changes in temperature and moisture can lead to degradation and elution of composite components over time (Ferracane, 1994; Ferracane et al., 1998) and lower the glass transition temperature (Kildal & Ruyter, 1997). This jeopardises the mechanical and viscoelastic stability of composite materials (Druck et al., 2015; Drummond et al., 1991; El-Safty et al., 2013; Mair & Padipatvuthikul, 2010; Musanje & Darvell, 2003; Tuna et al., 2008).

**Applied load:** The force mode and magnitude also influence the degree of creep. For instance, a tensile load is more likely to cause fracture more than a compressive one. The load magnitude and loading time can transform linear viscoelastic behaviour into nonlinear one as moving from low to high magnitude load (Ruyter & Øysæd, 1982).
1.4.2 Chemical properties

1.4.2.1 Sorption and solubility

Water sorption in resin-composites is defined as a water diffusion process that mainly takes place into the polymer matrix and to a lesser extent on the surfaces of the fillers (Sideridou, 2011). The polymer matrix composition and volume along with the bonding between the filler and resin-matrix influences water sorption (Ferracane et al., 1998; Mortier et al., 2004). Different factors related to the organic matrix can influence water sorption, such as polarity and hydroxyl group presence (as they can form hydrogen bonds with water), and the cross-linking of the organic matrix (Mortier et al., 2004). Some polymers also have hydrolytically susceptible groups, which also influence sorption such as ester, ether, and urethane groups (Ferracane, 2006). Among resin-composite monomers, Bis-EMA shows the least sorption, followed by UDMA then Bis-GMA (Ertas et al., 2006; Sideridou & Karabela, 2011), while TEGDMA has the most sorption (Beatty et al., 1993; Ertas et al., 2006; Sideridou et al., 2003; Sideridou & Karabela, 2011). Current dental composites are mostly glassy polydimethacrylate-based, namely Bis-GMA, Bis-EMA, UDMA and TEGDMA (Sideridou, 2011). Water sorption by these polymers can be explained by the dual-mode theory. This theory assumes that the solution absorbed is entrapped either in the polymer matrix by ordinary dissolution according to Henry’s law, or in polymer microvoids (Sideridou et al., 2003; 2004).

The absorbed water can lead to filler-matrix de-bonding and subsequently affecting physical, mechanical, aesthetic and bonding properties (Drummond et al., 1991; Fonseca et al., 2017; Mair & Padipatvuthikul, 2010; Mortier et al., 2004; Tuna et al., 2008; Um & Ruyter, 1991). Water sorption can lead to polymer plasticisation, which can reduce both hardness and the glass transition temperature (Ito et al., 2005; Kildal & Ruyter, 1997). Moreover, components of the composite, such as unreacted monomers and fillers, may leach out into the oral environment, leading to weight loss or solubility (Boaro et al., 2013; Ferracane, 1994). The unreacted monomer can be trapped during polymerisation, either in microgels between the polymer chains, or into micropores (monomer pool). Monomer trapped in micropores is more susceptible to be leached out (Sideridou et al., 2003).
Like sorption, the organic matrix composition and presence of unreacted monomers along with the solvent chemistry (saliva, water, acids, bases, salts, alcohol, and oxygen) and exposure time are all factors influence solubility (Boaro et al., 2013; Ferracane, 1994; 2006; Zhang & Xu, 2008). In addition, filler loading and surface area, silane coupling agent, monomer structure and degree of cross-linking are all critical factors in solubility (Ortengren et al., 2001). The solubility parameters of the polymer and solvent are also very important factors to consider. The closer the solvent and polymer solubility parameters are, the more soluble the material is (Ferracane, 2006; Marghalani & Watts, 2013). 75% ethanol, for instance, has a close solubility parameter to that of dimethacrylate-based resin-composites, resulting in more solubility compared to water-based solvents (Manojlovic et al., 2013; Sideridou et al., 2007). Water sorption and solubility of dental composite materials can be assessed by weighing the material before and after storage in various media over various periods of time (International Standards Organization (ISO) FDIS 4049, 2009; Sideridou et al., 2003; Sideridou et al., 2007; Wei et al., 2011).

1.4.2.2 Monomer elution

As mentioned previously, unreacted monomers and other components can leach out from dental composites. Monomer elution can compromise the biocompatibility, as well as mechanical properties, of a material. Elution depends on several factors, such as the degree of conversion, solvent type, chemical structure of eluted molecules, and filler composition and microstructure (Ferracane, 1994; Polydorou et al., 2009). Dental composites can release low molecular weight monomers such as HEMA and TEGDMA, and high molecular weight monomers such as Bis-GMA and UDMA. TEGDMA has been shown to be the most released monomer from dental composites in water (Alshali et al., 2015a; Geurtsen et al., 1998; Ortengren et al., 2001). In addition, free radicals and photoinitiator molecules can be released (Kingman et al., 2012; Leprince et al., 2013; Van Landuyt et al., 2011). A particularly important factor in monomer elution is the molecular size of the monomer; small molecules such as TEGDMA are more easily mobilised and eluted than large ones such as Bis-GMA (Krifka et al., 2013, Pelka et al., 1999). Monomer elution is also highly correlated with the surface area of resin-composite restorations (Pelka et al., 1999). Most monomers are usually eluted in the
first few days, and then the material reaches equilibrium (Alshali et al., 2015a; Van Landuyt et al., 2011; Łagocka et al., 2018) or they may still be eluted in small amounts for longer periods up to one year (Alshali et al., 2015a; Polydorou et al., 2007; 2009; 2012).

Some toxic substances such as formaldehyde and methacrylic acid may be leached out, compromising materials biocompatibility (Gupta et al., 2012; Pearson & Longman, 1989). Although such substances are not constituents of dental composite, they can be produced by the degradation of the organic resin-matrix, particularly hydrolytically susceptible groups (Ferracane, 2006). Enzymatic activity in oral saliva can also liberate methacrylic acid from dimethacrylate-based dental composite (Gupta et al., 2012; Munksgaard & Freund, 1990).

1.4.3 Biocompatibility and toxicity

Biocompatibility can be defined as “ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response to that specific situation, and optimising the clinically relevant performance of that therapy.” (Williams, 2008b). The release of monomers such as bisphenol-A raises concerns about the biocompatibility of resin-based materials (Goldberg, 2008; Goldberg et al., 2014). This release is mainly due to incomplete polymerisation or degradation of components over time (Ferracane, 1994; Gupta et al., 2012; Van Landuyt et al., 2011). The two main components of resin-based composite that are claimed to cause adverse effects are:

Bisphenol A: In 1996, Olea et al. highlighted the fact that the well-known endocrine disruptor bisphenol A (BPA) was present in dental materials (Olea et al., 1996). BPA is not released directly from Bis-GMA based restorations, but rather from the degradation of Bis-DMA, which is a component of the sealant for pits and fissures. Unlike the ether bonds in Bis-GMA, the ester bonds in Bis-DMA are prone to degradation by salivary enzymes, such as esterase, that may result in the release of BPA. It is worth mentioning that there are many sources of BPA. Hence, it is complicated to evaluate BPA release from dental composites (Bakopoulou et al., 2009).
**Methacrylate monomers:** As mentioned earlier, different monomers can be leached out from resin-composite. There is a strong relation between the structure of the organic matrix of dental composites and their cytotoxicity levels. For instance, UDMA is considered less toxic than Bis-GMA, as it is not synthesized from bisphenol A (BPA), and it displays a lower cytotoxic effect on human gingival and pulp fibroblasts *in vitro* (Gupta et al., 2012). In addition, Bis-GMA has been found to inhibit DNA-synthesis, reflecting its mutagenic properties (Geurtsen et al., 1998; Sideridou & Achilias, 2005).

TEGDMA have many cytotoxic and genotoxic effects due to its small molecular size, which enhances diffusion processes (Krifka et al., 2013). It was reported to be moderately mutagenic at subtoxic concentrations, and might promote the proliferation of the cariogenic microorganisms such as lactobacillus acidophilus and streptococcus sobrinus (Geurtsen et al., 1998; Sideridou & Achilias, 2005). Also, it can induce DNA damage and caspase activation (Huang, et al., 2015).

Nanoparticles can also present hazardous risks due their ability to penetrate cellular membranes and organelles (European Commission Scientific Committee on Emerging and Newly Identified Health Risks, 2015). However, nanoparticles are usually bound in the matrix and are less likely to be released from dental materials (Rupf et al., 2015). Nevertheless, other exposure routes such as inhalation, swallowing or direct tissue contact should be evaluated (Schmalz & Galler, 2017; Van Landuyt et al., 2014). Figure 1-10 shows possible material tissue interactions. It is currently believed that nanoparticles are less risky, although more research is needed in this area. Wet grinding of dental restorations is recommended, as fewer particles are produced (Schmalz & Galler, 2017; Schmalz et al., 2017).
In theory, CAD/CAM composite blocks should exhibit superior biocompatibility to that of conventional indirect resin-composites due to the high degree of conversion, less monomer release of HT and HP polymerised blocks (Phan et al., 2014) and the fact that fewer toxic monomers and no photoinitiators are used in their manufacture (Gupta et al., 2012; Krifka et al., 2013). Currently, UDMA monomer, which is more biocompatible, is used in CAD/CAM composite blocks rather than Bis-GMA (Gupta et al., 2012). Some PICN blocks had been developed without the addition of TEGDMA and initiator, which makes them less cytotoxic (Grenade et al., 2016; 2017; Krifka et al., 2013; Nguyen et al., 2013). Finally, CAD/CAM blocks exhibit better resistance to breakdown and subsequently less toxic components release (Coldea et al., 2015; Swain et al., 2016; Van Landuyt et al., 2011).
1.5 Mechanical characterisation

1.5.1 Assessment of hardness

Hardness indentation can be classified into macroindentation tests (with a load of more than 2 N) and microindentation tests (with a load of less than 2 N). Microindentation tests are commonly used in comparative studies of dental materials (Ilie et al., 2017; ISO 14577-1, 2015). Knoop and Vickers microindentation tests are the most common tests used to investigate the hardness of dental restorative materials (Darvell, 2009; Ilie et al., 2017). The most common hardness tests are discussed below in their prevalence of use for dental composites (Ilie et al., 2017):

**Vickers hardness test:** A Vickers indenter is a square-based pyramid diamond with opposite sides meeting at the apex at an angle of 136°. The hardness in this test is calculated from the surface area of a square-shaped indentation. This indentation is produced when the indenter is forced into the tested surface for a specific dwell time (typically 10-15 seconds). The average indentation length of both diagonals (D) is measured to determine the indentation surface area. This indentation has the same geometry for different tested materials and loads (Darvell, 2009; ISO 6507-1, 2006; Ilie et al., 2017), and can be used to measure microhardness for a wide range of materials (Darvell, 2009).

**Knoop hardness test:** A Knoop indenter is a rhombic-based pyramidal diamond indenter. It is suitable for testing of thin plastic or metal sheets or brittle materials. The main advantage of this test that it can be used to measure a wide range of hardness values by using variable loading forces (Ilie et al., 2017; Sakaguchi & Powers, 2012). Both Vickers and Knoop test are classified as microhardness tests and they are strongly correlated (Poskus et al., 2004). Figure 1-11 shows a schematic diagram of Knoop and Vickers indenters and their corresponding indentation shape.
Rockwell hardness test: This test uses indenters with different shapes (spherical or conical) and materials (steel, tungsten, carbide, or diamond). The calculation of hardness in this test relies on the depth of the indentation. The main disadvantage of this test that the indentation might disappear in a short time before the depth is measured (Sakaguchi & Powers, 2012).

Brinell hardness test: In this test, a small spherical steel indenter (Brinell ball) is used, producing a circular indentation; the diameter of which indicates the material hardness (Darvell, 2009; ISO 6506-1, 2014; Ilie et al., 2017). The main limitation of this test is that it produces a relatively large indentation area. Therefore, it can only be used to measure average hardness values, not localised values (Sakaguchi & Powers, 2012). Both Brinell and Rockwell hardness tests are classified as macrohardness tests, and neither of them can be employed in brittle materials and are less frequently used for testing dental composites (Ilie et al., 2017).
1.5.2 Assessment of hardness and elastic modulus using Nanoindentation

Various techniques have been used to measure elastic modulus such as uniaxial (Chabrier et al., 1999; Masouras et al., 2008a), biaxial (Higgs et al., 2001; Leone et al., 2007), and flexure tests (Choi et al., 2018). Also, elastic modulus can be determined using an optical technique (digital image correlation (DIC), that is able detect surface deformation of variety of biomaterials and dentine using images (Choi et al., 2018; Palamara et al., 2000; Wang et al., 2015). Nanoindentation is a well-documented and accurate method to measure elastic modulus and hardness of both dental materials and teeth (Doerner & Nix, 2011; Masouras et al., 2008b). This method allows a load as small as 30 mN, with the resulting indentation size less than 5 µm, compared to an indentation size of about 100 µm with microhardness testing (Broitman, 2016). A wide range of materials can be tested with nanoindentation, ranging from soft polymers to hard diamond-like thin carbon films. A lot of information can be gained from the load-displacement curve of the tested material, mainly hardness and elastic modulus, but also phase transformation, cracking and delamination of films (Fischer-Cripps, 2011).

The three essential parameters that can be measured using the load-displacement (P-h) curve (Figure 1-12) are: the maximum load (Pmax); the maximum displacement (hmax) which is determined using the applied load (P) and the contact area of indentation, which depends on the geometry of the indentation; and the elastic unloading stiffness (S =dP/dh), defined as “the slope of the upper portion of the unloading curve during the initial stages of unloading.” It is also referred to as contact stiffness (Oliver & Pharr, 2004; Fischer-Cripps, 2011). If these parameters are accurately measured during nanoindentation, the final hardness and elastic modulus values can be obtained. Also noted in figure 1-12 is the final depth (hf), which is the final and permanent depth of the indenter penetration in the unloading cycle (Oliver & Pharr, 2004).
Despite being a simple testing technique, nanoindentation is very sensitive to thermal changes, mechanical vibrations and acoustic noise. In addition, despite the diamond indenter being very hard, it is brittle and care must be taken not to chip it. Also, during testing, it is recommended to pre-set a hold time (pause) which gives both the indenter and the tested material time to stabilise before commencing another indentation (Fischer-Cripps, 2011). One of the major limitations relates to material homogeneity; although nanoindentation can measure the microstructural parts of materials, it is not the ideal testing method for biphasic materials such as dental composites. This limitation can be faced when the indenter tip is smaller than the filler particle size (Alcala et al., 1998), or when the maximum load used is too small and would not provide sufficient information about the bulk of the material (Masouras et al., 2008b). However, choosing the appropriate indenter size along with appropriate load will provide sufficient information about the material properties.
1.6 Chemical characterisation (assessment of monomer elution) using HPLC

Chemical analytical methods are usually used to measure the released components such as monomers, initiators or metal ions. These methods involve the separation and redistribution of the mixture of constituents; separation can be complete or partial. In complete separation, all the constituents are individually separated, while in partial separation, only particular constituents are separated (Skoog et al., 2004). Figure 1-13 presents a schematic explanation of the basic principle of both types of separation.

Common analytical separation methods include chemical or electrical precipitation, distillation, solvent extraction, ion exchange, chromatography, electrophoresis and field flow fractionation (Skoog et al., 2004). Chromatography and particularly high performance liquid chromatography (HPLC) is the most commonly used method for monomer separation in the dental field (Ortengren et al., 2001; Van Landuyt et al., 2011). HPLC is widely used to separate particular constituents of biological, organic and inorganic materials for either preparatory or analytical purposes. It can be used to separate a variable range of chemical and biological compounds such as proteins (Wei et al., 2019), enzymes (Busch et al., 2018), natural products (Pourasghar et al., 2019) and unstable and non-volatile compounds (Donald et al., 2006).

Figure 1-13: Schematic diagram of complete separation (A) and partial separation (B). Adapted from Skoog et al., 2004.
1.6.1 HPLC History and principle

Chromatography is a chemical analytical separation technique in which the constituents of a mixture are separated based on the different rate at which they are carried through a stationary phase by a mobile phase; this phase can be liquid or gaseous. Tswett was the first to describe this separation method in 1903. He separated different plant pigments into coloured bands using calcium carbonate (CaCO₃). In his experiment, he used a CaCO₃ column (the stationary phase) and dissolve the plant pigments into petroleum ether (the mobile phase). After that, the plant pigments were separated according to their absorption differences into CaCO₃ column (Li et al., 2013). Figure 1-14, illustrate Tswett’s experiment for separating plant pigments.

![Plant extract in solvent](image)

**Figure 1-14**: Tswett’s experiment for separating plant pigments. Adapted from Waters, 2016.

Chromatography can be classified according to the method of contact between the two phases into column chromatography, where the stationary phase is held in a narrow column, and the mobile phase moves through that column by gravity or under pressure (Li et al., 2013). Figure 1-15 presents a schematic demonstration of column chromatography. The second type is planar chromatography in which the stationary phase is coated on a flat glass or porous paper through which the mobile phase moves.
by capillary action or gravity (Li et al., 2013). Figure 1-16 shows thin layer chromatography, which is a form of planer chromatography.

**Figure 1-15**: A schematic demonstration of column chromatography, illustrates the separation process of two components A and B, the tube represent the stationary phase while the liquid inside is the mobile phase; over time, separation starts, component A is separated at \(t_3\), and finally at \(t_4\) component B is separated (Li et al., 2013).

**Figure 1-16**: Thin layer chromatography, illustrate the separation process of the black sample at time \((t_0)\), into three separate analytes after 10 minutes \((t_{10})\). Adapted from Waters, 2016.
1.6.2 Basic components and operation

The basic components of HPLC are shown in figure 1-17. HPLC consists of six major components: solvents, pump, sample injection port, analytical column, detector and data processor.

![Flow chart of HPLC mechanism and basic components](image)

**Figure 1-17**: Flow chart of HPLC mechanism and basic components. Adapted from Waters, 2016.

The solvent or mobile phase is usually a mixture of polar and non-polar liquid; their concentration is selected depending on the sample composition. It is crucial to choose appropriate eluting solvents and to take into consideration the adsorption power of the stationary phase as well as the polarity of solutes. If too strong solvent is used this may result in the entire sample passing through the column without separation (Li et al., 2013).

The pump pushes the liquid at a flow rate of 1 to 2 ml/min under high pressure (400 to 600 bar) through the column; with either a constant mobile phase composition (isocratic) or an increasing mobile phase composition (gradient) (Agilent, 2015).

The injector injects the liquid sample (1 to 25 μL) into the flow stream of the mobile phase. Automatic injection using an auto-sampler can be used when many samples are to be analysed (Agilent, 2015; Moldoveanu & David, 2013a).

The column has the stationary phase that separates the sample components using various physical and chemical parameters. Most HPLCs employ a liquid mobile phase and a very finely divided stationary phase. The mobile phase (eluent) is always a liquid...
that contains the sample as a mixture of solutes. The stationary phase is typically a
column that is packed with 1-5 µm diameter porous particles. High pressure is applied
to ensure a satisfactory flow rate of liquids through the column (Moldoveanu & David,
2013a). There are different aspects to consider when choosing the column: (1) Nature
and type of the active stationary phase and physical characteristics of particles as the
retention of the analytes is strongly associated with the column packing particles and
the mobile phase that pushes analytes through the packed column. (2) Column
dimensions (length, diameter); large diameter used usually for preparative rather than
analytical purposes. (3) The mechanical construction of columns; stainless steel columns
used usually to withstand the high pressures during HPLC. However, when inert surfaces
acquired, less pressure-tolerant materials such as polyetheretherketone (PEEK) or glass
can also be used (Agilent, 2015; Moldoveanu & David, 2013a; 2017a; Waters, 2016).

The detector detects and quantifies the separated compounds and provides this
information to a computer to create the liquid chromatogram. UV detectors are usually
used and these can either be set to detect a specific wavelength or a broad range of
wavelengths (diode-array detector). Other detectors, such as a fluorescence detectors
and evaporative-light-scattering detectors (ELSD), can also be used depending on the
analyte to be detected (Waters, 2016).

Finally, the computer has software that uses the information from the sensors to
determine the time of elution or retention time; defined as the time (usually measured
in minutes) taken for a particular compound to go through the column after injection,
from which the sample components (qualitative analysis) and amount (quantitative
analysis) can be known (Figure 1-18) (Agilent, 2015; Moldoveanu & David, 2013b).
1.6.3 HPLC separation mechanisms

HPLC can be classified according to the stationary phase into normal phase, reverse phase, ion chromatography, size exclusion chromatography, affinity chromatography and chiral chromatography (Moldoveanu & David, 2013a; Skoog et al., 2004). The primary interaction in normal phase and reverse phase chromatography is hydrophobic. Normal phase liquid chromatography is when the stationary phase is more polar than the mobile phase (Moldoveanu & David, 2013a). Reverse phase liquid chromatography (RPLC) involved the stationary phase being less polar than the mobile phase. It is considered the most commonly used HPLC mechanism due to its versatility, reliability and ease of use (Dolan, 2008). The most commonly used stationary phases in RPLC contain octadecyl groups (C18 or ODS) or octyl groups (C8) bonded on silica particles, and the mobile phase is usually water with an organic solvent such as methanol and acetonitrile (Moldoveanu & David, 2013a; 2017a). The optimum ratio between organic to inorganic solvents should be determined based on the polarity of the sample in order to achieve a reliable RPLC separation. Accordingly, two elution methods are available: isocratic elution, where the ratio between the weaker solvent (inorganic) and the
stronger solvent (organic) in the mobile phase remains constant throughout the separation. In gradient elution, this ratio is increased gradually throughout the separation (Dolan & Snyder, 2017; Moldoveanu & David, 2017a).

Gradient elution is commonly used in RPLC with a mobile phase of water, methanol and acetonitrile. It usually starts with a low-content organic solvent (0%) that is gradually substituted with pure organic solvent (100%), or an organic solvent containing some additives. Less polar compounds can be separated from the stationary phase as the organic solvent content is increased (Dolan & Snyder, 2017; Moldoveanu & David, 2017a). The main advantages of gradient over isocratic methods are the shorter retention time and better peak separation (resolution). Nevertheless, the gradient changes may affect the sensitivity and requires a highly reliable HPLC device to perform (Moldoveanu & David, 2017b; Schellinger & Carr, 2006).

**1.6.4 Qualitative and quantitative analysis of the separated compounds**

Retention time is considered the most important parameter for identification of the separated compounds. The separated compounds can be identified using the initial calibration or reference runs of specific compounds with certain retention times. Quantification of the separated compounds can be inferred from the peak area and height, which are proportional to the concentration of the analytes (Dolan & Snyder, 2017; Moldoveanu & David, 2013a; 2017a).

Quantitative analysis can be validated using two standard calibrations. The first is external standard calibration, which is the initial calibration of a specific compound with different concentrations. Usually, a linear relationship is obtained between the peak area or height and the compound concentration (Stuart, 2009). The other standard calibration is internal standard calibration. Internal standards are compounds that are not part of the sample and are added in a constant amount to all the samples and the external standards. Internal standards should not interfere with the analysis and have an easy-to-integrate peak. Internal standard calibration is advantageous for verifying reproducibility even when injection volume errors occur, and it enhances the precision of the HPLC analysis (Moldoveanu & David, 2013a).
1.7 Assessment of biocompatibility

1.7.1 Biocompatibility test requirements

There are three main factors to consider in the assessment of the biocompatibility of a material. First, a simulated body condition to test the relevant material. Second, measurement of cellular and tissue reactions over appropriate exposure time. Third, the effect of stress applied to the relevant material and how that affects overall tissue and cellular reactions (Anusavice et al., 2013). These tests can be performed in vitro (cellular experiments) and in vivo (animal models and clinical trials). Due to its simplicity, in vitro testing is the most commonly used. The primary strengths of in vitro testing are 1) the ability to control cellular environment and conditions as well as their interface with the investigated material, 2) the ability to assess cells responses and interactions with the material. Additionally, in vitro experiments are considered more cost and time effective and more reproducible than in vivo tests (Wataha, 2001; 2012). For in vitro tests, the relevant material is placed either in direct or indirect contact with simulated biological conditions outside of an organism. Different simulated biological conditions can be used such as mammalian cells, cellular organelles, tissue, body fluids, bacteria and certain enzymes (Anusavice et al., 2013). The design of an in vitro test depends on the intended outcomes and clinical relevance (Wataha, 2012).

1.7.2 Cell viability and cytotoxicity

Cell viability and cytotoxicity assays are good indicators of the condition of cells. Cell viability is a method of quantifying the number of healthy cells and cell growth (proliferation) over time. It can be done with either individual cells or whole populations; the latter needs less time but also give less information than the former (Stoddart, 2011). Substances released from materials could have different cytotoxicity mechanisms, such as cellular membrane destruction, protein synthesis inhibition, irreversible binding to receptors and enzymatic reactions (Ishiyama et al., 1996; Soenen et al., 2012). According to ISO 10993-5, three main methods of cytotoxicity testing are available (ISO 10993-5, 2009):
1) Extract (a solvent or solution in which the sample is stored and releases some substances), which is suitable to evaluate the toxicity of released substances from medical devices and has comparable results to animal toxicity tests.

2) Direct contact, where the relevant material is directly exposed to the simulated biological conditions and is considered the most sensitive method as it can measure low cytotoxicity levels (De Melo et al., 2013), and

3) Indirect contact tests, in which a barrier such as agar, a membrane filter or dentine is placed between the relevant material and the simulated biological conditions. Agar overlay assays are usually used to evaluate medical devices with large toxicity and bulk filtering (Sjogren et al., 2000; Wataha, 2012), while filter diffusion is usually suitable for evaluating small molecular weight medical devices (Jin et al., 2008).

Direct and indirect methods are correlated to each other but not to the extract method (Gao et al., 2013). Figure 1-19 shows the *in vitro* tests designs with various material–cells interfaces.

**Figure 1-19:** *In vitro* tests designs with various material–cells interfaces. Adapted from Wataha, 2012.
1.7.3 Cell viability and cytotoxicity assays

Various classifications are available for cell viability and cytotoxicity assays. The appropriate test depends on cell type, cell culture conditions and the purpose of the study (Stoddart, 2011). Additional factors to consider are laboratory settings, the material under investigation and detection mechanisms (Aslantürk, 2017). Cell viability and cytotoxicity assays can be classified based on measurement forms into (Aslantürk, 2017):

1) Dye exclusion: this category includes Trypan blue, eosin, Congo red and erythrosine B assays (Aslantürk, 2017). This method evaluates membrane integrity and is based on dye exclusion by viable cells but not by dead cells (Aslantürk, 2017)

2) Colorimetric assays: this category includes Alamar Blue (AB) assay, MTT assay, MTS assay, XTT assay, WST-1 assay, WST-8 assay, LDH assay, SRB assay, NRU assay and crystal violet assay. The mechanism of this method is based on cell metabolism evaluation via a biochemical marker. These assays usually change/develop colour in response to the viability of cells. This change in colour can be measured by spectrophotometer. Colorimetric assays can be used for adherent or suspended cell lines, are simple to use and are cost effective (Präbst et al., 2017).

3) Fluorometric assays: this category includes Alamar Blue assay and CFDA-AM assay. As for colorimetric assays, these assays can be used for both adherent and suspended cell lines, and are simple to use. Fluorescence changes can be evaluated via fluorescence microscope, fluorimeter, fluorescence microplate reader or flow cytometer (O’Brien et al., 2000; Page et al., 1993; Riss et al., 2016).

4) Luminometric assays: this category includes ATP assay and real-time viability assay. These assays are easy to use and fast to evaluate cell proliferation and cytotoxicity in mammalian cells. In addition, they can be performed in 96-well and 384-well microtiter plates and analysed using a luminometric microplate
reader (Duellman et al., 2015; Mueller et al., 2004; Riss et al., 2016). The main distinguishable feature is the long-lasting and stable glow-signal resulting from reagent addition. In addition, they can be used to evaluate both viability and cytotoxicity values from the same well (Niles et al., 2009).

1.7.3.1 Lactate dehydrogenase (LDH) assay

LDH is a cytosolic enzyme that is found within cytoplasm in many different cell types. When a cell membrane loses its integrity, LDH will be released into the culture medium. The LDH cytotoxicity assay is a colorimetric assay used to evaluate cell mediated cytotoxicity and chemical compound mediated cytotoxicity by measuring the level of LDH enzyme. This assay measures LDH using a coupled enzymatic reaction that converts tetrazolium salt (iodonitrotetrazolium (INT)) into red formazan diaphorase. First, LDH catalyses the conversion of lactate to pyruvate and thus NAD (Nicotinamide adenine dinucleotide is a cofactor found in all living cells) is reduced to NADH/H⁺. Then, the catalyst (diaphorase) transfers H/H⁺ from NADH/H⁺ to the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT), which is finally reduced to red formazan (Decker & Lohmann-Matthes, 1988; Lappalainen et al., 1994), Figure 1-20, shows a schematic diagram of LDH cytotoxicity assay mechanism.

![Diagram of LDH cytotoxicity assay mechanism](image)

**Figure 1-20:** Schematic diagram of LDH cytotoxicity assay mechanism (Thermo Fisher Scientific, 2014).

Red formazan is absorbed maximum at 492 nm and measured quantitatively at 490 nm wavelength and it is proportional to the LDH amount released into the medium, thereby indicating cytotoxicity levels (Thermo Fisher Scientific, 2014). A positive (high) control is usually used to determine the maximum LDH release from the cells and this can be
either a detergent such as Triton X-100 or membranolytic particles such as crystalline silica (Schins et al., 2002).

LDH has many advantages, including reliability, time efficiency and simplicity to perform and evaluate. It is also considered as an indicator of irreversible cell death due to the cell membrane damage and the release of intracellular LDH (Fotakis & Timbrell, 2006). The main disadvantage of this assay is that serum and some other compounds have inherent LDH activity, thus; LDH assay is more suitable to use with serum-free or low-serum conditions (Kumarasuriyar, 2007).

1.7.3.2 Alamar Blue (AB) assay

The Alamar Blue assay or resazurin reduction assay can be either colorimetric or fluorometric. The latter is more sensitive than, yet not as reproducible as, the colorimetric method (O’Brien et al., 2000). Resazurin is a phenoxazin-3-one dye and a non-toxic, cell permeable redox indicator that can be used to measure viable cell numbers, similar to the tetrazolium compounds (Ahmed et al., 1994; Riss et al., 2016). This assay is based on the conversion of blue non-fluorescent dye resazurin to the pink fluorescent resorufin by mitochondria and other enzymes such as diaphorases (O’Brien et al., 2000). Figure 1-21, shows the mechanism of reduction of resazurin to resorufin. Upon entering the cell, conversion takes place, resulting in colour change. The produced quantity is proportional to the number of viable cells. Thereafter, the ratio of viable cells can be quantified using a microplate reader where fluorescence is monitored at 530-560 nm excitation wavelength and 590 nm emission wavelength; colorimetric absorbance is monitored at 570 nm and 600 nm (Riss et al., 2016; Thermo Fisher Scientific, 2018).

![Figure 1-21](image)

**Figure 1-21**: The mechanism of reduction of resazurin to resorufin (Stoddart, 2011).
AB assay is relatively cheap and more sensitive than tetrazolium assays such as the MTT assay. It can be combined with other assays, such as measuring caspase activity to get a better evaluation of the cytotoxicity mechanism (Ahmed et al., 1994). One major disadvantage is that AB assays require a long incubation period (1-4 h); thus chemical interference and toxicity to cells are possible (Riss et al., 2016; Stoddart, 2011).

1.7.4 Apoptosis

Cell death is a primary part of cell studies, especially the determination of the mechanism of cell death, which can be either apoptosis or necrosis. Apoptosis is a programmed sequence of events to destroy unwanted cells that is initiated during healthy development or in response to irreversible cell damage caused by pathology or toxins (Schweikl et al., 2006). Necrosis, on the other hand, is cell death by tissue inflammation processes associated with clinical symptoms in response to infection, trauma or toxins (Majno & Joris, 1995; Zhivotovsky, 2004). Caspase activity, by cleaving specific cellular proteins, is considered a key marker of apoptosis, distinguishing it from necrosis (Stoddart, 2011). Besides, non-apoptotic caspases take part in inflammation, innate immunity, tissue regeneration, cell-fate determination, stem-cell differentiation and neural activation (Kuranaga, 2012; Yi & Yuan, 2009).

Caspases initially exist as inactive zymogens that can be either initiators or effectors based on the timing of activation. As the name implies, initiator caspases (mainly 8 and 9) are activated earlier following adaptor-mediated oligomerisation, and are called monomers. This process leads to activation of other caspases, known as effector caspases (mainly 3, 6, and 7) via cleavage-mediated activation, called dimers. The effector caspases are then activate selected death substrates and other caspases, which cause cell fragmentation (Pop & Salvesen, 2009; Yi & Yuan, 2009). There are two apoptotic pathways responsible for caspase activation: the extrinsic pathway, which is initiated by activation of a group of death receptors at the cell surface and controls the activation of Caspase-8; and the intrinsic pathway, which is initiated via the release of killer mitochondrial proteins, which in turn trigger the activation of Caspase-9 (Kurokawa & Kornbluth, 2009). Caspase-3 is considered a critical apoptotic caspase because it can be activated by both extrinsic and intrinsic pathways (Jin & El-Deiry, 2005).
1.8 Oral mucosa

This section briefly describes the relevant anatomy of HGK and HGF used in this research.

1.8.1 Epithelium

There are two types of epithelium present, keratinising and non-keratinising epithelium. For keratinising epithelium, the principal cells are the epithelial cells (keratinocytes) which differentiate into corneocytes, which are densely packed with keratin fibres. These cells are anucleate and tightly bind together to form the stratum corneum. Keratinocytes typically contain keratins, keratin-associated proteins such as filaggrin and involucrin, and cell surface carbohydrates (Dale et al., 1990; Presland & Dale, 2000). This structure forms the primary barrier to mechanical, bacterial and chemical injuries. Non-keratinising epithelium has similar differentiation and maturation processes but with less randomly distributed tonofilaments (Deo & Deshmukh, 2018). The epithelium barrier is mediated through its physical structure, epithelial cell turnover and desquamation (Cruchley & Bergmeier, 2018).

1.8.2 Lamina Propria (LP)

The oral epithelium is supported by connective tissue (the lamina propria) that consists of cells, blood vessels, neural elements and support fibres that are arranged in an amorphous ground structure. It can be divided into two layers: the superficial papillary layer, which has thin and loosely distributed collagen fibres; and the netlike reticular layer, which has collagen fibres that are bundled together and arranged parallel to the surface (Cruchley & Bergmeier, 2018). The key cells in LP are:

1. **Fibroblasts** are stellate or elongated cells with the abundant rough endoplasmic reticulum (ER) that are distributed throughout LP. Their main function is elaboration and turnover of fibre and ground substance. Thus, fibroblasts have a crucial role in maintaining tissue integrity, including wound healing (Atamas, 2002; Kataoka et al., 2005; Rinn et al., 2008).

2. **Macrophages** are round cells that contain lysosomes and phagosome vesicles, typically present in areas of chronic inflammation. The main function of these cells is ingestion of damaged cells/tissues and foreign material (Atamas, 2002; Atamas et al., 2002).
3. **Mast cells** are round cells with basophilic granules, typically present throughout LP. The primary function of these cells is related to allergic reactions, particularly, the transition from an acute to a chronic inflammatory reaction (Walsh, 2003).

4. **Inflammatory cells** such as lymphocytes and plasma cells are typically present throughout the LP in small numbers. However, they increase in number in pathological conditions such as infection or injury. The pathological conditions will influence the composition and distribution of inflammatory cells. In acute inflammation, polymorphonuclear leukocytes are considered the primary cell type, while in chronic conditions, macrophages, monocytes and lymphocytes infiltrations will take dominant place (Dongari-Bagtzoglou & Fidel, 2005).
Chapter Two:
Statement of the Problem and General Aims and Objectives
2.1 Statement of the problem

Despite improvements in CAD/CAM composite and PEEK, ceramics are still superior in many aspects including aesthetics, biocompatibility and strength (Barizon et al., 2014; Messer et al., 2003). However, ceramics have some drawbacks, such as abrasiveness of the opposing enamel (Fasbinder, et al., 2010; Lee et al., 2014) and brittleness (Tsitrou et al., 2007). CAD/CAM composite and PEEK materials, being less abrasive and less brittle, may have the potential to overcome some of these drawbacks (Coldea et al., 2013b; Sripetchdanond & Leevailoj, 2014; Swain et al., 2016; Tsitrou et al., 2007). They are also easier to fabricate and repair than ceramics (Ruse & Sadoun, 2014).

Mechanical properties such as flexural strength, flexural modulus, elastic modulus and hardness can be used to predict material clinical success (Charlton et al., 2008). Various mechanical properties of CAD/CAM composite blocks have been investigated (Awada & Nathanson, 2015; Coldea et al., 2013a; Lawson et al., 2016; Yin et al., 2019). However, there are limited studies exploring different ageing solvents that simulate the intraoral conditions and how they affect the surface and bulk properties of CAD/CAM composites. Furthermore, there is limited research on monomer elution and the biocompatibility of these materials and their influence on the adjacent gingival tissue.
2.2 General aims and objectives

The overall aim of the project was to characterise and compare different mechanical, chemical and biological aspects of CAD/CAM composite materials (RCB and PICN) and PEEK to CAD/CAM ceramic and, where appropriate, to the tooth structure and conventional resin-composites, with the following specific objectives:

1- To assess the effect of filler loading on mechanical properties (hardness, elastic modulus) of different CAD/CAM composite blocks and compare these to ceramic, enamel and dentine using two indentation techniques (nanoindentation and Vickers hardness).

2- To assess the effect of different storage media (distilled water, artificial saliva and 75% E/W) on the Vickers hardness of CAD/CAM composite blocks.

3- To assess water sorption and solubility of CAD/CAM composite blocks compared to CAD/CAM ceramics after 8 months of storage in water and artificial saliva.

4- To study the effect of water storage (3 months) on creep deformation and recovery of CAD/CAM composite materials in order to determine their viscoelastic stability.

5- To assess monomer elution from CAD/CAM composite blocks compared to that from conventional resin-composite using high performance liquid chromatography (HPLC) after storage in different media (distilled water, artificial saliva, and 75% E/W) for 3 months.

6- To investigate the influence of CAD/CAM composite materials on human gingival keratinocytes (HGK) and gingival fibroblasts (HGF).

Figure 2-1 shows an outline of the different studies conducted.
Assessment of CAD/CAM composite materials in terms of the following properties

- **Chapter 4**: Effect of the composition of CAD/CAM composite blocks on mechanical properties
- **Chapter 5**: The effect of different storage media on Vickers hardness of CAD/CAM composite blocks
- **Chapter 6**: Long-term hydrolytic stability of CAD/CAM composite blocks
- **Chapter 7**: Viscoelastic stability of pre-cured hybrid CAD/CAM composite structures
- **Chapter 8**: Analysis of monomer elution from CAD/CAM composite blocks using HPLC
- **Chapter 9**: Biocompatibility of CAD/CAM composite blocks

* Published

**Chapter 10**: General discussion, conclusions and future recommendations

**Figure 2-1**: Outline of different studies conducted
Chapter Three: Methodology
3.1 Introduction

CAD/CAM composite blocks were assessed in different aspects through six in vitro studies based on the available standard techniques. This chapter provides a detailed description of the techniques used in this research. As well as tests being conducted on a range of restorative materials, tooth structure was also used as substrate for one study (teeth were obtained from extracted wisdom teeth from young adults and ethical approval was granted by NHS, Health Research Authority, London - Harrow Research Ethics Committee (15/LO/1545)).

3.2 Sample preparation

Specimens of each CAD/CAM block, enamel and dentine were sectioned using a diamond blade (MK 303; MK Diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Saw; Buehler Co, IL, USA) under constant water irrigation. Figure 3-1 shows the Isomet 1000 Precision Saw used in the study.

Conventional resin-composite materials were prepared using a polytetrafluoroethylene (PTFE) mould, and were cured according to manufacturers’ recommendations using a LED light-curing unit with an output irradiance of 1200 mW/cm² (EliparTM; 3M ESPE, USA).

Figure 3-1: The Isomet 1000 Precision Saw used in the study.
Specimens for most of the studies conducted were wet ground and polished with a series of silicon carbide paper (SiC) (P320, P500, P1200, P2400, and P4000 grit (Buehler Co, IL, USA)) under water cooling and then polished with 0.25 μm diamond suspension (Meta Di Supreme, Buehler Co, IL, USA) using a lapping machine (MetaServ 250, Buehler Co, IL, USA). However, for cell viability and cytotoxicity specimens (Section 3.8); no diamond suspension was used and viscoelastic stability specimens (Section 3.6) were wet-ground and polished with silicon carbide papers (SiC) P500, P1200. Finally, specimens were cleaned in an ultrasonic bath (Ultrasonic Cleaning System; L&R Co, NJ, USA) with distilled water for 5 min. Specimen size varies according to each study, and the dimensions of specimens were confirmed, to an accuracy of ± 0.1 mm, using an electronic digital calliper (PDC150M, Draper tools Ltd, Hampshire, UK). Any specimens not within this range were rejected and discarded.

### 3.3 Artificial saliva preparation

Artificial saliva (AS) was prepared using the formula proposed by Macknight-Hane and Whitford (1992) (McKnight-Hanes & Whitford, 1992). However, sorbitol was not added as it increases the solution viscosity above that of natural saliva when mixed with sodium carboxymethyl cellulose (CMC) (Levine et al., 1987). All materials used and their concentrations are shown in Table 3-1.

**Table 3-1:** The compounds and their amounts used for artificial saliva preparation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound amount g/l (distilled water)</th>
<th>Manufacturer</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 4-hydroxybenzoate</td>
<td>2.00</td>
<td>Sigma-Aldrich</td>
<td>99-76-3</td>
</tr>
<tr>
<td>Sodium carboxymethyl cellulose (CMC)</td>
<td>10.00</td>
<td>Sigma-Aldrich</td>
<td>9004-32-4</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.625</td>
<td>BHD Chemicals Ltd, Poole, England</td>
<td>7447-40-7</td>
</tr>
<tr>
<td>Calcium chloride (CaCl₂)</td>
<td>0.166</td>
<td>Sigma-Aldrich</td>
<td>10043-52-4</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH₂PO₄)</td>
<td>0.326</td>
<td>May &amp; Baker LTD, Dagenham, England</td>
<td>7778-77-0</td>
</tr>
<tr>
<td>Magnesium chloride (MgCl₂)</td>
<td>0.059</td>
<td>Sigma-Aldrich</td>
<td>7786-30-3</td>
</tr>
<tr>
<td>Potassium hydroxide (KOH)</td>
<td>To adjust pH 6.75</td>
<td>May &amp; Baker Ltd, Dagenham, England</td>
<td>1310-58-3</td>
</tr>
</tbody>
</table>
Artificial saliva was prepared according to the following steps:

1) 2 g of methyle 4-hydroxybenxoate was dissolved in 800 ml of distilled water of which 20 ml of the solution was stored to be used as a solvent for other chemical reagents (solution 1), and the remaining solution was stored in the refrigerator (solution 2).

2) 200 ml water was boiled then 10 g of CMC was sprinkled into it and stirred using a magnetic stirrer until CMC was completely dissolved. Then this solution was poured into solution 2 and mixed on a stirring hot plate with a magnetic stirrer (Bibby HB502) (Figure 3-2, A) until a gel form solution formed (solution 3).

3) 0.625 g of KCl was dissolved into 5 ml of solution 1 and then this solution was mixed with solution 3.

4) 0.166 g of CaCl₂ was dissolved into 5 ml of solution 1 and then this solution was mixed with solution 3.

5) 0.326 g of KH₂PO₄ was dissolved into 5 ml of solution 1 and then this solution was mixed with solution 3.

6) 0.059 g of MgCl₂ was dissolved into the remaining 5 ml of item 1 and then this solution was mixed with solution 3.

7) Finally, KOH was added until the pH of artificial saliva was 6.75 using a digital microprocessor pH meter (Mettler Toledo, model DELTA 340), Figure 3-2, B.

Figure 3-2: The prepared artificial saliva on stirring hot plate with magnet stirring (A) and pH adjustment afterwards (B).
3.4 Nanoindentation, hardness, filler content and SEM imaging assessment

3.4.1 Assessment of hardness and elastic modulus using nanoindentation

Sixty-six specimens were prepared (Section 3.2) of 2 mm thickness, comprising 6 of each of the 11 materials (1 ceramic, 1 PICN, 5 RCB, 2 PEEK, enamel, and dentine). Elastic modulus and hardness (n=6, 5 readings per specimen) measurements were obtained using a nanoindenter (M3 Nanovea; Nanovea Co, CA, USA) equipped with a Berkovich three-sided pyramidal diamond tip with indenter cone angle 130.54° and elastic modulus of 1140 GPa. Calibration indents were made on a fused silica sample with an elastic modulus of 71.3 GPa and hardness of 8.9 GPa. The machine was set for the chosen parameters: a load of 20 g, a dwell time of 20 s (He & Swain, 2011) and material type; ceramics, which set the poisson’s ratio. The machine calculated the elastic modulus and nanohardness using the force-displacement curves generated from nanoindentation testing, based on the Oliver-Pharr method (Oliver & Pharr, 2004). Figure 3-3 shows the Nanovea nanoindenter with the pre-set parameters and the sample ready for testing.

Figure 3-3: The nanoindenter (M3 Nanovea; Nanovea Co, CA, USA), A: LCD, Touch screen with pre-set parameters (arrow), B: The diamond indenter holder, with the indenter projecting downward (arrow), C: Sample holder-working table, D: The sample is mounted and ready to test.
3.4.2 Assessment of Vickers hardness

Sixty-six specimens were prepared (Section 3.2) of 2 mm thickness, comprising 6 of each of the 11 materials (1 ceramic, 1 PICN, 5 RCB, 2 PEEK, enamel, and dentine). Microhardness of each material (n=6, 5 readings per specimen) was measured using Vickers indenter tester (FM-700; Future Tech Corp., Japan) under a load of 300 g and a dwell time of 20 s. For each indentation, both diagonals (D1, D2) were measured using a microscope at ×50 magnification. Five indents were undertaken for each sample in a straight line with 0.5 mm from the sample margins and the hardness values were averaged. The distance between the indentations was calculated by multiplying the average indentation diagonal length by four (4×D) to ensure sufficient distance between the indentations. The machine then automatically calculated the corresponding hardness value and presented it as Vickers hardness number (VHN). Figure 3-4 shows Vickers indenter tester with the pre-set parameters.

![Vickers Indenter Tester](image)

**Figure 3-4:** The Vickers indenter tester (FM-700; Future Tech Corp., Japan), A: A built-in microscope, B: Object lens, C: Vickers indenter, D: Working table, E: LCD touch panel with pre-set parameters (arrow).
3.4.3 Assessment of Vickers hardness reduction

Forty-eight specimens were prepared (Section 3.2) of 16×4×2 mm dimensions, comprising 6 of each of the 8 materials (1 ceramic, 1 PICN, 5 RCB and 1 PEEK blocks). Microhardness of each material (n=6, 5 readings per specimen) was measured using a Vickers indenter tester (FM-700, Future Tech Corp., Japan) under a load of 300 g, and a dwell time of 20 s under dry conditions at day 0. Then 2 samples of each material were immersed in 10 ml of either water, artificial saliva (Section 3.3), or 75% E/W, and stored at 37°C in individual glass vials. Sample size was based on previous research (Albero et al., 2015; Egilmez et al., 2018; Lauvahutanon et al., 2014; Lawson et al., 2016). Microhardness was measured after 30 and 90 days. The Vickers hardness number (VHN) was automatically calculated by the machine.

The hardness reduction (as a percentage) after 90 days storage was calculated as follows:

$$\text{HR\%} = \frac{\text{VHN}(d0) - \text{VHN}(d90)}{\text{VHN}(d0)} \times 100\%$$

(1)

Where VHN (d0) and VHN (d90) are the Vickers hardness numbers at day 0 and day 90, respectively.
3.4.4 Filler content assessment

The mass percentage of inorganic filler content of the CAD/CAM blocks was measured by elimination of the organic part of the CAD/CAM blocks. This was achieved by heating the sample at a constant temperature (Ash technique) in accordance to ISO standard 1172:1996 (ISO 1172, 1996). Three samples of each material (n=3) were kept in an electric furnace (Programat EP 5000; Ivoclar Vivadent, Liechtenstein, Austria) set at 625°C for 30 min, then cooled in a desiccator. The samples were then weighed to an accuracy of 0.01 mg using a calibrated electronic analytical balance (Ohaus Analytical Plus; Ohaus Corporation, USA). Figure 3-5 shows the Programat EP 5000 furnace used for samples heating with a burned-out sample at the top. The percentage of inorganic fillers by weight was then determined using the following equation:

$$\text{Filler weight\%} = \left[100 - \left(\frac{m_1 - m_2}{m_1}\right)\right] \times 100\%$$

(2)

Where $m_1$ is the mass before heating and $m_2$ the mass after heating and cooling.

**Figure 3-5**: Programat EP 5000 furnace used for samples heating with a burned-out sample at the top.
3.4.5 SEM imaging

The samples (n=1) of each CAD/CAM composite block were prepared as in section 3.2. The surfaces of the specimens were wet polished using SiC papers (P600 up to P4000) and diamond solutions of 9, 3, 1, and 0.25 μm and then cleaned in an ultrasonic bath (Ultrasonic cleaning system, L&R Co, NJ, USA) with acetone for 5 min. The specimens were dried, mounted on aluminium stubs and sputter-coated with carbon, Figure 3-6. The surface of each specimen was examined using a scanning electron microscope (SEM; FEI Quanta 200, OH, USA) and SEM images were obtained using the following parameters: 1000x and 5000x magnifications, accelerating voltage of 10 kV, detector: Everhart-Thornley Detector (ETD), spot size of 3.5, and working distance (WD) of 10 mm.

Figure 3-6: The SEM specimens sputter-coated with carbon.
3.5 Sorption and solubility

3.5.1 Sample preparation

Eight CAD/CAM blocks were investigated: (1 ceramic, 1 PICN, 5 RCB and 1 PEEK blocks). 112 specimens were prepared (Section 3.2) with dimensions of $14 \times 12 \times 3$ mm, comprising 14 of each of the eight materials, and divided into two groups (n=7). A sample size of 5 is recommended by ISO standards ISO FDIS 4049:2009 (ISO FDIS 4049, 2009). The specimens were dried according to ISO, FDIS 4049:2009 to a constant mass then specimens of each material (n=7) were randomly immersed in 10 ml of either water or artificial saliva (Section 3.3), stored at $37 \pm 1^\circ C$ in individual glass vials. The specimens were weighed periodically over eight months at various intervals (0, 7, 14, 28, 90, 180 and 240 days) and the mass at each point was recorded ($m_2$) ($t$). At each point, the specimens were dried on filter paper, agitated in air for 15 s, and finally weighed after 1 min and returned then to the individual glass vials. The pH of the storage media was checked by pH meter at the time of each measurement; if it had changed the storage medium was replaced. At the final time point (8 months), the specimens were dried according to ISO, FDIS 4049:2009 to a constant mass ($m_3$). Figure 3-7 shows the samples with silica gel before and after drying until a constant mass ($m_3$) was reached.

![Figure 3-7: samples with silica gel before and after drying procedure. Procedure was complete upon attainment of a constant mass ($m_3$).](image)
3.5.2 Sorption and solubility

The percentage mass change (weight loss) of each material during storage was calculated using the following equation:

\[ Mg\% = \frac{m_2(t) - m_1}{m_1} \times 100\% \]  

(3)

The sorption of each material in micrograms per cubic millimetre (μg/mm³) at the end of the eight-month (8m) storage period was calculated as follows:

\[ SP = \frac{m_2(8m) - m_3}{V} \]  

(4)

The percentage quantity of water or artificial saliva absorbed by each material was calculated as follows:

\[ SP\% = \frac{m_2(8m) - m_3}{m_1} \times 100\% \]  

(5)

The percentage amount of water or artificial saliva absorbed by the polymer matrix (where absorption mainly occurred) was calculated using the following equation (Sideridou et al., 2011):

\[ SP_{pm}\% = \frac{SP\%}{a} \]  

(6)

The solubility of each material was calculated using the following equation:

\[ SL = \frac{m_1 - m_3}{V} \]  

(7)

The percentage solubility, defined as the total weight of components extracted by the storage medium, was calculated as follows:

\[ SL\% = \frac{m_1 - m_3}{m_1} \times 100\% \]  

(8)

where \( m_1 \) is the initial-recorded mass, \( m_2(t) \) the mass recorded after each time interval, \( m_3 \) the final-recorded mass, \( V \), the volume in mm³, and \( a \) is the proportional weight of the polymer matrix of CAD/CAM blocks as measured in a previous study (Alamoush et al., 2018).
3.6 Assessment of viscoelastic stability (creep)

3.6.1 Sample preparation:

Five resin-composite blocks (RCB) and one polymer infiltrated ceramic network (PICN) were investigated. CAD/CAM blocks were sectioned and shaped into cylinders of 4 mm diameter and 6 mm height. In total 36 cylindrical specimens were prepared comprising 6 specimens of each material that were divided into two groups (three specimens in each group, n = 3) for storage, as follows: Group one: 24 h dry at 23 °C; Group two: 3 months in 37 ± 1 °C distilled water. Sample size (n=3) was based on previous research (Al-Ahdal et al., 2015; Baroudi et al., 2007).

3.6.2 Creep measurements

The static creep deformation of the CAD/CAM composite blocks was measured using a creep apparatus as described previously (Baroudi et al., 2007) and shown in Figure 3-8. A constant compressive stress of 20 MPa was applied on each specimen via a loading pin for 2 h, followed by load removal for a further measurement period of 2 h (total: 4 h). The creep strain and recovery were monitored continuously in real time using a linear variable displacement transducer (LVDT) system (Baroudi et al., 2007; El Hejazi & Watts, 1999).

Figure 3-8: A diagram of the creep apparatus.
The maximum creep-strain (%) and permanent set (%) were obtained after loading and recovery, respectively. Maximum creep strain, maximum creep recovery, percentage creep recovery and permanent set were obtained using LVDT calibration coefficient and the creep/time plots, Figures 3-9 and 3-10 respectively. The strain changes as percentage versus time were obtained using the following equations:

(1) Displacement change (µm) = LVDT strain changes (mV) / LVDT coefficient (mV/µm)  
(2) Strain changes as percentage versus time = Displacement change (µm)/the specimen original height (µm)

Then all parameters were calculated based on the values of creep strain versus time plots and data as follows (figure 3-10):

I) Maximum creep strain = A + B  
II) Maximum creep recovery = C + D  
III) Percentage creep recovery = [(C + D) / (A + B)] × 100  
IV) Permanent set (E)

**Figure 3-9:** LVDT calibration curve
**Figure 3-10:** Schematic representation of typical creep stages of the resin-composite material upon stress application and stress removal, where A is the elastic deformation, B is the viscoelastic deformation, C is the initial recovery, D is the time-dependent recovery and E is the permanent set.
3.7 Assessment of monomer elution

3.7.1 Sample preparation

Samples of CAD/CAM blocks (1 PICN, 5 RCB and 1 PEEK blocks) and conventional resin-composite materials (2 direct and 2 indirect) were prepared. One hundred and sixty-five specimens were prepared (Section 3.2) with 10×10×3 mm dimensions, comprising 15 of each material, and divided into three groups (n=5) immersed in 3 ml of either water, artificial saliva (Section 3.3) or 75% E/W solution. A sample size of 3 is recommended by ISO standard, 10993-13 (ISO 10993-13, 2010). Each specimen had a surface area of 320 mm² and a volume of 300 mm³. Caffeine (CF) was used as an internal standard, and 0.1 mg/ml was added to all storage media. The monomers of interest (Bis-GMA, UDMA, TEGDMA, Bis-EMA) were dissolved in methanol with CF as an internal standard at different concentrations and were assessed by HPLC to identify each eluted monomer retention time and to obtain a calibration curve to quantify each monomer, shown in Table 3-2. Also, control samples of only storage media were assessed by HPLC.

The specimens were stored in the dark at 37°C, and after 1 month, all storage solutions were collected for analysis and replaced with fresh solutions. After 3 months, the storage solutions were collected again for analysis and the total eluted monomer quantity in 3 months was calculated by adding the values obtained at the 2 time points.

Table 3-2: The calibration monomer solutions prepared in the study

<table>
<thead>
<tr>
<th>Calibration solution number</th>
<th>Amount of stock solution (10 mg/ml monomer+ 0.1 mg/ml CF)</th>
<th>Amount of solvent (with 0.1 mg/ml CF)</th>
<th>Final concentration (mg/ml)</th>
<th>Final concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1 ml</td>
<td>100 ml</td>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>0.5 ml</td>
<td>25 ml</td>
<td>0.2</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>0.5 ml</td>
<td>12.5 ml</td>
<td>0.4</td>
<td>400</td>
</tr>
<tr>
<td>4</td>
<td>0.5 ml</td>
<td>8.3 ml</td>
<td>0.6</td>
<td>600</td>
</tr>
<tr>
<td>5</td>
<td>0.5 ml</td>
<td>6.3 ml</td>
<td>0.8</td>
<td>800</td>
</tr>
<tr>
<td>6</td>
<td>0.5 ml</td>
<td>5 ml</td>
<td>1.0</td>
<td>1000</td>
</tr>
<tr>
<td>7</td>
<td>0.5 ml</td>
<td>4.2 ml</td>
<td>1.2</td>
<td>1200</td>
</tr>
</tbody>
</table>
3.7.2 Analysis of eluted monomers

The collected solutions at each time point were placed in HPLC vials and assessed by HPLC (Agilent 1100 series, Agilent Technology, Germany) to identify and quantify the eluted monomers using the calibration curves and retention times of the monomers of interest. Chromatography was performed using a reverse-phase column with isocratic separation. HPLC samples of 1 µL were injected into Phenomenex SphereClone 5 µm ODS (2) column of dimensions 4.6x250 mm (Phenomenex, USA). Chromatographic separation was achieved using a mixture of acetonitrile and water (65%:35%) at a flow rate of 0.5 ml/min. The column temperature was set at 22°C with a run time of 70 min for each sample, and the UV detector was set at 205 and 210 nm. Figure 3-11, 3-12 show the HPLC device, and method control window respectively.

Figure 3-11: The HPLC device used for the monomer elution study (Agilent 1100 series, Agilent technology, Germany).
Figure 3-12: The HPLC method control window with the chromatography conditions used in this study, Mobile phase: 65% Acetonitrile, flow rate 0.5 ml/min, column temperature 22 °C, injection volume 1 µl, UV detector set at 205 and 210 nm, and sample running time 70 min.
The calibration curve was obtained by plotting the HPLC peak area of monomer to peak area of CF against the concentration of monomer to the concentration of CF, of the calibration solutions as follows:

\[
\left( \frac{\text{Monomer peak area}}{\text{CF peak area}} \right) \text{ versus } \left( \frac{\text{Monomer concentration}}{\text{CF concentration}} \right)
\]  

(11)

Then, a linear regression analysis of the plotted ratio was carried out and the linearity, slope (b), and intercept (a) were obtained. The following equation was used to calculate the eluted monomer concentration (µg/ml):

\[
\text{Eluted monomer concentration (µg/ml)} = \left( \frac{(\text{Monomer peak area}) + (\text{CF peak area}) - a}{b} \right) \times \text{CF concentration}
\]  

(12)

Where the intercept (a), the slope (b) resulting from calibration curves, monomer and CF peak areas were obtained from HPLC, and the CF concentration (µg/ml) is known as an internal standard (100 µg/ml).

The eluted monomer amount (nmol/mm²) was calculated using the following equation (Van Landuyt et al., 2011):

\[
\text{Eluted monomer amounts (nmol/mm²)} = \text{conc} \times \text{solvent volume} \times \left( \frac{1}{\text{Mm}} \right) \times \left( \frac{1}{\text{sample surface area}} \right)
\]  

(13)

Where conc is the eluted monomer concentration in µg/ml units, solvent volume is in ml, Mm is the molecular mass in nmol/µg, and the sample surface area is in mm².
3.8 Assessment of cell viability and cytotoxicity

3.8.1 Samples preparation

Four materials: two resin-composite blocks (RCB), Grandio Blocs (GR) and Block HC (HC); one polymer-infiltrated ceramic network (PICN), Enamic (EN); and one conventional resin-composite, Grandioso (GND) were prepared (section 3.2) with 5×5×5 mm dimensions. In total, 96 specimens were prepared comprising 24 of each material divided into 12 for each cell line (HGK and HGF) of each material that were divided into four samples (n=4) for each of three replicas. All experiments were performed using appropriate controls with biological and instrumental triplicates. Specimens were cleaned in an ultrasonic bath (Ultrasonic cleaning system, L&R Co, NJ, USA) with phosphate-buffered saline solution, rinsed four times with purified cell culture water and then ultrasonically cleaned in 80% ethanol. Finally, they were sterilized by UV exposure for 1 hour per side.

3.8.2 Cell culture preparation

Commercially available cells were obtained and cultured according to a standard protocol for cell culture, maintenance, freezing and thawing. Two cell lines, primary human gingival fibroblast (Lot-201018) and primary human gingival keratinocytes (Lot-201014) (ATCC, VA, USA), were grown in their relevant growth media.

The cells were cultured in a T75 flask then kept in an incubator (5 % CO₂ and 95 % air) at 37°C. Then cells were passaged at regular periods based on their growth characteristics using 0.25% trypsin. Once the T75 flask was confluent, cells were detached and seeded into a 24-well culture plate, 5x10⁴ cells in 500 µl of complete growth medium. Once the cells attached, composite specimens were placed in the centre of each well signifying the start of the experiment according to ISO standard 10993-5 (ISO 10993-5, 2009). Figure 3-13 shows a diagrammatic representation of a 24 well plate with materials.
Figure 3-13: Diagram of 24 well plate set up, C – cells; M – material; HC – high control; LC – low control; BG (background) – only media; Composite material present in ‘C+M’ and ‘BG’ only.

3.8.3 Cell viability

Cellular viability of 100% was attributed to control wells, where cells were cultured with no composite blocks (LC or positive growth control). Cellular viability was quantified via a colorimetric assay using AlamarBlue™ cell viability reagent, DAL1100 (Thermo Fisher Scientific, IL, USA). At least one biological replica (24-well plate) was used for each assay at each time point (1, 3, 5 and 10 days).

HGF and HGK at each time point were exposed to AlamarBlue™ (1:10) for 1 hour at 37°C. Then 100 µl of supernatant was transferred into a 96-well plate for analysis at each time point. Cell viability was measured at the four-time points 1, 3, 5 and 10 days of cell growth. The 96-well plate was read with a UVM 340-microplate reader at 570 nm and 600 nm (ASYS, Scientific laboratory supplies).
Cell viability was calculated according to equation (14) (Thermo Fisher Scientific, 2018):

\[
\text{Cell viability}\% = \frac{A_{570} - (A_{600} \times R_0) \text{ for test well}}{A_{570} - (A_{600} \times R_0) \text{ positive growth control}} \times 100\% \tag{14}
\]

Where \(A_{570}\) and \(A_{600}\) are absorbance at 570 and 600 nm respectively and \(R_0\) is the correction factor calculated from \((A_{570}/A_{600})\) of the positive growth control.

### 3.8.4 Cytotoxicity

The cytotoxic potential of the tested materials was investigated using a Pierce™ LDH cytotoxicity assay kit, 88954 (Thermo Fisher Scientific, IL, USA). At least one biological replica (24-well plate) was used for each assay at each time point.

Cytotoxicity in HGF and HGK at each of the four time points (day 1, 3, 5 and 10) were measured using 50 µl of the supernatant and 50 µl of LDH cell reaction solution incubated for 30 minutes at room temperature in the dark. The reaction was stopped using the LDH “stop” solution. Appropriate controls were used as per the manufacturers’ protocol; maximum LDH release from the cells was set by adding membranolytic-particles, and was considered the positive (high) control, and the spontaneous LDH release control (water-treated) was considered the low control.

The 96-well plate was read with a UVM 340-microplate reader at 490 nm subtracted from 680 nm (ASYS, Scientific laboratory supplies) and cytotoxicity was calculated according to equation (15) (Thermo Fisher Scientific, 2014):

\[
\text{Cytotoxicity}\% = \frac{\text{Specimen-treated LDH activity} - \text{Spontaneous LDH activity}}{\text{Maximum LDH activity} - \text{Spontaneous LDH activity}} \times 100\% \tag{15}
\]

Where Specimen-treated LDH activity is the LDH expressed by cells cultured with composite materials; maximum LDH activity, is the LDH expressed by cells treated with membranolytic-particles; and the spontaneous LDH activity is the LDH expressed by cells treated with water.
3.8.5 Cell morphology and immunostaining

Cell morphology was evaluated using light microscopy imaging at days 1, 3, 5, 7, and 10, at ×10 magnification (Olympus IX51 inverted fluorescence microscope). Indirect immunostaining was used to assess the Caspase 3 activity. At the last time point, the growth medium was aspirated, and cell cultures in the plate were fixed using 4% paraformaldehyde (PFA) for 20 min and then permeabilization was done using 0.2% Triton-X for 30 min – both at room temperature. Blocking of Fc receptors (to prevent non-specific antibody binding) was done using normal goat serum with 0.1 BSA (Dilution factor 1:20) for 1 h at room temperature. Specimens were incubated with 1:250 primary antibody, mouse monoclonal Caspase 3 (Santa Cruz Biotechnology, Inc) at 4°C for 24 h. Secondary antibody 1:500, Alexafluor Goat anti-mouse (Life Technologies Corporation) was added after 24 h and kept in the dark for 1.5 h at room temperature. Cell nuclei were stained using DAPI (Vector Laboratories, CA, USA). Specimens were then observed using an Olympus IX51 fluorescence microscope connected to a digital camera at ×10 magnification (Olympus IX51 inverted fluorescence microscope).
Chapter Four:
Effect of the Composition of CAD/CAM Composite Blocks on Mechanical Properties

R. Alamoush, N. Silikas, N. A. Salim, S. Al-Nasrawi, and J. D. Satterthwaite.

Research Article

Effect of the Composition of CAD/CAM Composite Blocks on Mechanical Properties

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The aim of this study was to evaluate the effect of the composition of CAD/CAM blocks on their mechanical properties. Nine different CAD/CAM blocks, enamel and dentine, were tested. Sixteen samples of each material were separated for Vickers microhardness test (n=6, 5 readings per specimen), nanohardness test (n=6, 5 readings per specimen), filler weight (n=3), and SEM imaging (n=1). Data were statistically analysed using one-way ANOVA. Vita Mark II ceramic showed significantly higher values of hardness (in both nano- and microscale) and elastic modulus (6.83 GPa, 502 kg/mm², and 427 GPa), respectively, than other materials. CAD/CAM composite blocks showed comparable values of hardness and elastic modulus to those of dentine but lower than those of enamel and ceramics. SEM images highlighted different filler-matrix microstructure of CAD/CAM composite blocks.

It was concluded that (1) hardness and elastic moduli are positively correlated with ceramic filler percentage and microstructure and (2) CAD/CAM composite materials have comparable hardness and elastic moduli to tooth structure.

1. Introduction

Alternative aesthetic restorations have been introduced, as the digital technologies of CAD/CAM systems have been developed. Ceramic and composite have been used as indirect restorations using CAD/CAM systems as promising restorations [1]. In addition, PEEK has been proposed for CAD/CAM prosthodontics applications [2].

Ceramic has favourable properties for use as an indirect restorative material; it is a very biocompatible and strong material [1–3]. However, it is very stiff, hard, and brittle; these properties affect their clinical performance, durability, and machinability. In terms of their hardness and stiffness, ceramics are considered highly abrasive. This affects the material performance in two aspects: first, clinically, ceramic might cause opposing enamel wear and roughness [4, 5]; second, it is difficult to machine; for instance, in CAD/CAM systems ceramic hardness causes milling tool damage over time; also it takes longer to mill compared with composite [6, 7]. The other main disadvantage of ceramics is brittleness. Again, it affects the material in two main aspects: First, ceramic clinical durability is highly related to their brittleness; most ceramic restorations fail because of crack propagation due to the material brittleness [8]. Second, in terms of machinability, ceramic might chip or crack during processing. Hence it is difficult to manufacture even with CAD/CAM systems [9].

Over the years, indirect composite restorations have improved in relation to their mechanical properties in different ways: alteration of the composition (monomer resins, initiation systems); incorporation of high percentage filler particles; and polymerization modes (using high temperature and pressure for polymerization) [10, 11]. These have improved both tensile and compressive strength, hardness, elastic modulus [12], and wear resistance [13, 14]. CAD/CAM technology allows for many of these alterations in manufacturing to result in improved indirect composite restorations.

CAD/CAM composite has the following main advantages compared to ceramic: it has less hardness and stiffness, so
the opposing enamel exhibits less wear clinically. In addition, it is easily fabricated and repaired. It is also less brittle [15]. Consequently, less catastrophic failure is expected as well as less chipping and crack introduction during manufacturing [9]. In addition, they are more compatible with milling machine and exhibit better marginal quality [9, 10, 16].

Different formulations have been introduced recently with different material classifications such as ceramic-like materials, polymer infiltrated ceramics, CAD/CAM resin based blocks, or nanoceramics [3, 17]. CAD/CAM composites can be classified based on their microstructural geometry into two main types, resin with dispersed fillers and polymer infiltrated ceramic networks [18].

PEEK has favourable mechanical properties [19]. It has similar tensile properties to those of bone, enamel, and dentine [20]. Therefore, it has been proposed for use in fixed [21] and removable prostheses [22]. Further investigation of CAD/CAM composites in many aspects such as mechanical properties, bonding, and biocompatibility is highly needed. Most importantly, their mechanical properties such as flexural strength, flexural modulus, modulus of resilience, and hardness that can predict the material clinical success and performance are important to be evaluated [23–25].

In the view of limited research on CAD/CAM composite blocks and the need to evaluate their clinical success and performance, this study aimed to test the mechanical properties (hardness, elastic modulus, and microstructure) of different CAD/CAM blocks and compare them to ceramic, enamel, and dentine using two indentation techniques (nanoindentation and Vickers hardness). The null hypotheses were that (1) there is no difference in the tested mechanical properties between materials and (2) the mechanical properties of the tested materials will not be affected by the their composition.

2. Materials and Methods

2.1. Study Design. Nine different CAD/CAM blocks were tested (n=16 each group). Enamel and dentine discs were prepared from extracted wisdom teeth (n=12, each group). Samples of each material were allocated into two groups: Vickers microhardness test (n=6) and nanohardness test (n=6). In addition 4 samples (of each CAD/CAM block) were used for filler weight test (n=3) and SEM imaging (n=1). The microhardness was measured by means of a Vickers indenter tester (FM-700, Future Tech Corp., Japan). The test parameters were with load of 300 g and 20 s dwell time. Nanoindentation measurements (elastic modulus, hardness) were undertaken using a nanoindenter (M3 Nanoeva, Nanoeva Co., CA, USA) equipped with a Berkovich three-sided pyramidal diamond tip. The machine was set for the chosen parameters: load of 20 g and pause of 20 s. Thirty indentations on 6 samples (5 for each) were made for each material for each test. SEM images at 1000x and 5000x magnifications were obtained to assess filler-matrix microstructure of hybrid ceramics. Data were statistically analysed using one-way ANOVA.

2.2. Materials and Sample Preparation. The nine CAD/CAM blocks used in this study were resin composite CAD/CAM block (Lava Ultimate, Shofu, Cerasmart, Brilliant Crios, Grandio Blocks); polymer infiltrated ceramic network (PICN) ceramic block (Enamic); pure PEEK (Ceramill PEEK); ceramic filled PEEK (Dentokeep); and feldspathic ceramic block (Vitablocs Mark II). Enamel and dentine discs were prepared from extracted wisdom teeth. A list of materials studied, with details of filler percentage and polymer, is given in Table 1.

Six specimens of each of the II materials were prepared (9 CAD/CAM blocks, enamel and dentine). Each CAD/CAM block was sectioned into rectangular bars of 2 mm thickness using a diamond blade (MK 303, MK diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Cutter; Buehler Co, IL, USA) under constant water irrigation (ISO 6872:2008) [26]. Discs of 2 mm of enamel and dentine were prepared from extracted wisdom teeth from young adults (ethical approval was granted by NHS, Health Research Authority, London, Harrow Research Ethics Committee IS/LO/1543) and disinfected with 20 ml of 5% sodium hypochlorite for 10 min (3035965, BDH Chemicals Ltd., Poole, BH15, England) and then wrapped in cotton gauze saturated with physiologic saline (59300C, Dulbecco’s Phosphate Buffered Saline, Sigma-Aldrich Inc., St. Louis, USA) and kept at 4°C to be prepared and tested within a week.

All specimens were wet ground and polished with a lapping machine (MetaServ 250, Buehler Co, IL, USA) with a series of silicon carbide papers (SiC) and paper disks P320, P500, P1200, P2400, and P4000-grit (Buehler Co, Illinois, USA) under water cooling and then polished with 0.25 um diamond suspension (Meta Di Supreme, Buehler Co, IL, USA) and cleaned in an ultrasonic bath (Ultrasonic Cleaning System, L&R Co, NJ, USA) with distilled water for 5min. The specimens were stored dry for 24 hr at room temperature.

2.3. Nanoindentation. Elastic modulus and hardness measurements (nanoindentation measurements) were obtained using a nanoindenter (M3 Nanoeva, Nanoeva Co., CA, USA) equipped with a Berkovich three-sided pyramidal diamond tip which was used with indenter cone angle 130.54 and elastic modulus of 140 GPa.

Calibration indents were made on a fused silica sample with an elastic modulus of 71.3 GPa and hardness of 8.9 GPa. The machine was set for the chosen parameters, load of 20 g, and pause of 20 s. Poisson’s ratio for all tested materials was assumed to be 0.3. Thirty indentations were undertaken (5 for each sample) for each material at room temperature. The maximum load applied by the nanoindenter to examine the specimens was 20 g. The machine calculated the elastic modulus (E) and nanoindentation (H) by using the generated force-displacement curves from nanoindentation testing, based on the Oliver-Pharr method [27], using the following equations, respectively:

\[
E = \frac{1}{2} \times \frac{1}{\sqrt{\pi}} \times \frac{d}{\sqrt{h}} \times dP
\]
where A is the projected contact area; dh is the change in depth; dP is the difference in load.

\[
H = \frac{P_{\text{max}}}{A}
\]  

(2)

where A is the projected contact area, P_{\text{max}} is the maximum load.

2.4. Vickers Microhardness. Surface microhardness was measured by means of a Vickers indenter tester (FM-700, Future Tech Corp., Japan) under a 300 g loading and 20 s dwell time. For each indentation, both diagonals (DL, D2) were measured using the microscope. Five indents were undertaken for each sample in a straight line. The distance between the indentations was calculated by multiplying the average indentation diagonal length by four \((4 \times D)\) to ensure sufficient distance between the indentations. Five indentations were undertaken on each specimen and the hardness values were averaged. Thirty determinations on 6 samples were made for each material. The machine then automatically calculated the corresponding hardness value and presented it as VHN. Vickers microhardness can also be calculated using the following equation [28]:

\[
\text{VHN} = 1.854 \frac{P}{D^2}
\]  

(3)

where P is the applied load in kg and D is the indentation diagonal length in mm.

2.5. Filler Content. The mass percentage of inorganic filler content of the CAD/CAM blocks was measured by elimination of the organic part of the CAD/CAM blocks by heating at a constant temperature (ash technique) in accordance with ISO 1172:1996 [29]. Thermogravimetric analysis (TGA) is an alternative method to measure the filler content and is possibly more accurate. Three samples of each material \((n=3)\) were kept in an electric furnace (Programat EP 5000, Ivoclar Vivadent, Liechtenstein, Austria) set at 625°C for 30 min and then cooled in a desiccator. The samples were then weighted to an accuracy of 0.01 mg using a calibrated electronic analytical balance (Ohaus Analytical Plus, Ohaus Corporation, USA). The percentage of inorganic fillers by weight was then determined using the following equation:

\[
\text{Filler weight} \% = \left(100 - \left(\frac{m_1 - m_2}{m_1}\right) \times 100\right)
\]  

(4)

with m1 being the mass before heating and m2 the mass after heating and cooling.

2.6. Microstructure. The surfaces of the specimens were wet polished using SiC paper P600 up to P4000 and diamond solutions of 9, 3, 1, and 0.25 μm and subsequently ultrasonically cleaned with acetone for 5 min. The specimens were dried, mounted on aluminium stubs, and sputter-coated with carbon. The surface of each specimen was examined using a scanning electron microscope (SEM; FEI Quanta200, OH, USA) and SEM images at 1000x and 5000x magnifications at 10 kV were obtained.

2.7. Statistical Analysis. All results were tested using Levene’s test for homogeneity of variance \((P < 0.05)\), following the assumption of equal variances. Equal variances were confirmed \((P > 0.05)\); hence the Bonferroni post hoc test was used to determine the differences in the mechanical properties.
Table 2: Mean (SD) Vickers microhardness, nanohardness, elastic modulus, and the measured and manufacturers’ filler percentages by weight (wt%) for all tested materials. Values with the same superscript letters per column represent nonsignificant statistical difference for each individual property (p=0.05).

<table>
<thead>
<tr>
<th>Material type</th>
<th>Material (code)</th>
<th>Microhardness (GPa)</th>
<th>Nanohardness (GPa)</th>
<th>Elastic Modulus (GPa)</th>
<th>Manufacturers’ Filler (wt%)</th>
<th>measured filler (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin composite CAD/CAM blocks</td>
<td>Lava™-Ultimate (LU)</td>
<td>112.6 (0.44)y</td>
<td>1.25 (0.05)z</td>
<td>12.14 (0.76)z</td>
<td>80</td>
<td>74.8 (0.1)</td>
</tr>
<tr>
<td></td>
<td>Shofu (SH)</td>
<td>73.12 (1.04)z</td>
<td>0.775 (0.03)z</td>
<td>8.79 (0.35)z</td>
<td>61</td>
<td>63 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Cerasmart (CS)</td>
<td>80.06 (0.76)z</td>
<td>0.80 (0.006)z</td>
<td>10.36 (0.17)z</td>
<td>70</td>
<td>66.1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>BRILLIANT CriOS (BC)</td>
<td>82.61 (0.49)z</td>
<td>0.85 (0.008)z</td>
<td>10.98 (0.6)z</td>
<td>71</td>
<td>70 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Grandio Blocks (GR)</td>
<td>121.8 (2.1)z</td>
<td>1.3 (0.08)z</td>
<td>14.8 (0.4)z</td>
<td>86</td>
<td>84.6 (0.01)</td>
</tr>
<tr>
<td>Polymer infiltrated ceramic network (PICN) ceramic</td>
<td>Vita Enamic (EN)</td>
<td>203.1 (0.43)b</td>
<td>3.1 (0.17)b</td>
<td>34.56 (1.4)b</td>
<td>86</td>
<td>85 (0.3)</td>
</tr>
<tr>
<td>Pure PEEK</td>
<td>Ceramill PEEK (PE)</td>
<td>25.7 (0.05)a</td>
<td>0.337 (0.008)a</td>
<td>2.53 (0.15)a</td>
<td>0</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Ceramic filled PEEK</td>
<td>Dentcokeep (DK)</td>
<td>27.24 (0.19)a</td>
<td>0.34 (0.03)a</td>
<td>3.43 (0.29)a</td>
<td>20</td>
<td>27.5 (0.06)</td>
</tr>
<tr>
<td>Feldspathic ceramic block</td>
<td>Vitablocs Mark II (VM)</td>
<td>502.4 (2.28)a</td>
<td>6.83 (0.379)a</td>
<td>47.7 (3.47)a</td>
<td>100</td>
<td>100 (0)</td>
</tr>
<tr>
<td>Enamel</td>
<td>EM</td>
<td>313.3 (22.7)b</td>
<td>4.03 (0.35)f</td>
<td>59.7 (13)f</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dentine</td>
<td>DN</td>
<td>62.3 (3.5)y</td>
<td>0.76 (0.13)y</td>
<td>16.5 (2.3)f</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(hardness, elastic modulus, and microstructure). For the filler weight percentage measurement, Bland and Altman test was used to compare the measured values with the manufacturers’ values where a minimal variation was detected with high level of reproducibility.

3. Results

Mean microhardness, nanohardness, and elastic modulus for all tested materials are shown in Table 2 and Figure 1. A statistically significant difference in the means of microhardness, nanohardness, and elastic modulus between the tested materials was revealed.

The values of nanohardness ranged from 0.31 (SD.008) GPa for pure PEEK to 3.1 (SD.017) GPa for Vita Mark II ceramic. The values of microhardness ranged from 25.7 (SD 0.05) Kg/mm² for pure PEEK to 502.4 (SD 2.28) Kg/mm² for Vita Mark II ceramic. The values of elastic modulus ranged from 2.53 (SD 0.15) GPa for pure PEEK to 59.7 (SD 13) GPa for enamel.

The measured and manufacturers’ filler percentages by weight are presented in Table 2. Measured values of filler content ranged from 100% (SD 0.0) weight for Vitablocs Mark II to 0.00 for pure PEEK. The measured filler percentage by weight was compared to the manufacturers’ filler percentage. A Bland and Altman test shows minimal variation between results and high reproducibility. Elastic modulus, microhardness, and nanohardness were correlated with filler weight percentage and the results showed a positive correlation with linear regression: for filler weight percentage and microhardness (VHN), R²=0.43, P=0.05; for nanohardness (GPa), R²=0.38, P=0.07; for elastic modulus (GPa), R²=0.51, P=0.03 (Figure 2). In addition, the nanohardness and elastic modulus values were highly correlated where R²=0.93.

SEM images showed different microstructures of the tested CAD/CAM composite blocks (Figure 3). SH contained two varieties of spherical particles, CS contained relatively large and small and uniformly distributed particles, BC contained small and uniformly distributed particles, GR contained two varieties of particle, large and small irregularly shaped particles, LC contained a wide range of particle sizes, and EN exhibited a dense ceramic network structure with resin matrix. It can be noticed that CAD/CAM composite blocks had versatile microstructural constituents as well as variable filler weight percentages.

4. Discussion

The results of the present study show that the tested materials were significantly different in their mechanical properties (microhardness, nanohardness, and elastic modulus). CAD/CAM composite was significantly different from ceramics, enamel and dentine. Consequently, both null hypotheses were rejected.

It was noticed that CAD/CAM composite blocks had different microstructure as well as variable filler weight percentages and hence differences in the tested mechanical properties. However, it seems that the filler percentages have a more considerable role in these properties than do the microstructural constituents. In fact, PICN (EN) might be an exception as it exhibited higher values of hardness and elastic modulus compared to other CAD/CAM composite blocks,
which could be attributed to the manufacturing technique of polymer and ceramics networking.

Of the tested materials, Vita Mark II ceramic had the highest value of hardness (in both nano- and microscale) and elastic modulus, and it was significantly higher than the values of enamel and dentine rendering VM as very hard and stiff material. This might be considered a disadvantage in terms of machinability and durability [9, 25]. Enamic showed higher hardness and elastic modulus values compared to other resin composite CAD/CAM blocks, which might be attributed to the robust microstructural geometry of PICN as compared to other resin composite CAD/CAM blocks which are basically a resin with dispersed ceramic fillers. The PICN VHN, nanohardness, and elastic modulus were in between the enamel and dentine values but closer to enamel values. The tested resin composite CAD/CAM blocks were closer in their characteristics to dentine rather than enamel.

The values of the elastic moduli of PICN (Enamic) 34.56 GPa and resin composite CAD/CAM blocks are close to the values of enamel and dentine when compared to CAD/CAM ceramic which is very hard and stiff. This means they are closer to the tooth structure stiffness. Nanofilled and low filled ones (PEEK and Dentokeep) had lower hardness and elastic modulus values. These results were comparable with similar studies [10, 30–32].

The aim of any dental restorative material is to have similar characteristics to that of the root structure [25, 33]. Hence, resin ceramic combination in a network structure exhibits the positive characteristics of ceramics and resin [24]. This material has low rigidity, hardness, and stiffness but high flexibility and fracture toughness [34, 35]. Resin with dispersed ceramic fillers has good fracture and wear resistance and high compressive strength [36].
Figure 2: A scatter plot showing a positive correlation and linear regression between filler weight percentage and (a) microhardness (VHN), $R^2=0.43$, $P=0.05$, (b) nanohardness (GPA), $R^2=0.38$, $P=0.07$, and (c) elastic modulus (GPA), $R^2=0.51$, $P=0.03$.

Figure 3: SEM images of CAD/CAM blocks of six tested materials at 5000x and 10000x magnifications at 10 KV detector, ETD, spot size 3.5, WD: 10 mm.
PEEK showed favourable mechanical properties [19]. It has similar tensile properties to those of bone, enamel, and dentine [20]. Therefore, it has been proposed for use in fixed [21] and removable prostheses [22]. Although PEEK is increasingly used in fixed prosthodontics, the values of hardness and elastic modulus in this study were considerably low. However, for low percentage ceramic filled PEEK, the values were higher than pure PEEK; this is obviously attributed to the ceramic fillers. Low filled PEEK and PEEK have comparable hardness and stiffness values with PMMA. Hence, they might be a good choice for long-term restorations [32].

Nanoindentation is a well-documented method to measure the mechanical properties of both dental materials and teeth [37]. This test was used in this study to measure the elastic modulus as well as the hardness values of the tested materials at nanoscale. Using the microhardness test usually creates a relatively large indentation size of about 100 μm, while nanoinindentation allows applying load as low as 30 mN and indentation size less than 5 μm [38].

Nanoindentation might be a more precise method to investigate materials with microstructural constituents, such as microfilled or nanofilled composites [39]. However, it has limitations, mainly that the test is very sensitive to thermal changes and mechanical vibration and acoustic noise [40]. Also the indenter tip size in relation to the filler particle size [41] and the maximum load used is relevant; i.e., if the indenter tip size or load were too small it would not provide sufficient information about the bulk material properties [39]. However, the appropriate indenter size along with appropriate load will provide sufficient information about the material properties. Also, there is no microscope linked to the Nanovea machine, so the location of the tested point can only be determined by naked eyes.

Vickers microhardness test is a versatile method that can be used to measure hardness for a wide range of materials and easy to employ. The main advantage is that the indentation geometry does not change due to different loads or different tested materials. However, there is an operator subjective variation as the indentation surface area is determined according to the average length of both diagonals (d) which can be determined microscopically by naked eyes [42].

Although both tests give hardness values, their values cannot be directly or simply compared due to the different testing mechanisms such as indenter type, test settings, and loading force. In nanoinindentation the hardness depends on the applied load and indentation depth which is being measured as a function of the applied load (P) and the size of the contact area of indentation which depends on the geometry of the indenter [40]. However, in Vickers micro-hardness test, the hardness relies on the surface area of a square-shaped indentation. The indentation surface area is determined according to the average length of both diagonals (d) of the square-shaped indentation [28].

5. Conclusions

Within the limitations of this study, the following conclusions can be drawn:

(i) The hardness and elastic moduli are positively correlated to ceramic filler percentage and microstructure.
(ii) CAD/CAM composite materials have comparable hardness and elastic moduli to tooth structure.
(iii) CAD/CAM composites combine ceramic good strength with composite lower hardness. But further in vivo work is warranted to determine its clinical relevance and serviceability.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References


Chapter Five:
The Effect of Different Storage Media on the Vickers Hardness of CAD/CAM Composite Blocks

R. Alamoush, J. D. Satterthwaite, & N. Silikas
5.1 Abstract

Objectives: This study aimed to assess the effect of different storage media on the Vickers hardness of CAD/CAM composite blocks.

Materials and methods: Eight CAD/CAM blocks were investigated: Five resin-composite blocks (RCB) (Grandio blocs (GR), Lava™ Ultimate (LU), Cerasmart (CS), BRILLIANT Crios (BC), Block HC (HC)), one polymer-infiltrated ceramic network (PICN) block (Enamic (EN)), one ceramic-filled poly ether ether ketone (PEEK) block (Dentokeep (DK)), and one feldspathic ceramic block (Vitabloc Mark II (VM)). Microhardness was measured using a Vickers indenter tester (FM-700, Future Tech Corp., Japan), under a load of 300 g, and a dwell time of 20 s, at day 0 and after 30 and 90 days storage in distilled water, artificial saliva and 75% E/W at 37°C. The data were analysed by three-way ANOVA, followed by one-way ANOVA and Tukey’s post hoc (α=0.05 for all tests).

Results: The storage time, storage media and material type had a significant effect on the microhardness. The specimens stored in the water had a hardness reduction ranging from 0.9% for VM to 24.4% for BC. In artificial saliva, the specimens had a hardness reduction ranging from 2.8% for VM to 23.2% for DK. The hardness reduction percentage in 75% E/W ranged between 3.8% for VM and 35.3% for CS.

Conclusions: The hardness of CAD/CAM composite blocks was affected by different storage media, and they were not as stable as ceramic, with PICN exhibited superior hardness stability to all of the resin-composite blocks in all the storage media and was comparable to VM ceramic. DK also exhibited comparable hardness stability to both EN and VM in 75% E/W. Water and artificial saliva had similar effects as storage media, while 75% E/W had a more pronounced effect on the Vickers hardness of the investigated materials. The hardness reduction percentage of the CAD/CAM composite blocks was influenced by the filler loading and resin-matrix composition.

Keywords: CAD/CAM composite blocks, Resin-composite blocks, Polymer-infiltrated ceramic network, Vickers hardness
5.2 Introduction

Ceramics have many advantages, including biocompatibility, aesthetics and strength (Barizon et al., 2014). However, material stiffness and brittleness are drawbacks, which affect the clinical durability and their milling process (Giordano, 2006; Lebon et al., 2015). Ceramics are highly abrasive, and hence might cause wear and roughness to opposing enamel (Fasbinder et al., 2010; Lee et al., 2014). The ceramic brittleness influences the restoration longevity as it usually fails through crack propagation that ends in catastrophic failure (Gonzaga et al., 2011). Also, ceramic might chip or crack during processing/machining (Tsitrou et al., 2007).

Resin-composite materials have been undergoing improvements in many aspects related to their mechanical properties by means of innovative composition including monomer resins, initiation systems, and higher filler loading and polymerisation modes (high temperature and/or high pressure polymerisation) (Awada & Nathanson, 2015; Nguyen et al., 2012; 2014). Consequently, resin-composites exhibit improvements in tensile and compressive strength, hardness, elastic modulus and wear resistance (Cetin & Unlu, 2012; Nguyen et al., 2013).

Aesthetic CAD/CAM processed restorative materials can be either ceramics (glass ceramics, polycrystalline alumina and zirconia) or resin-based composites (Fasbinder, 2010; Giordano, 2006; Ruse & Sadoun, 2014). Compared to ceramics, CAD/CAM composite materials exhibit less hardness and stiffness, therefore causing less enamel wear clinically (Lawson et al., 2016), and they are easily fabricated and repaired. Further, they are less brittle than ceramics (Ruse & Sadoun, 2014) and consequently catastrophic failure in clinical conditions and chipping during manufacturing are less likely (Tsitrou et al., 2007). Based on their structure, composite blocks can be either polymer-infiltrated ceramic network (PICN), which is a porous ceramic network infiltrated with a polymer network, or a resin-composite block (RCB) that is similar to conventional resin-composite but manufactured under high pressure and high temperature (Lambert et al., 2017; Mainjot et al., 2016; Nguyen et al., 2012). In addition, PEEK material designed for CAD/CAM systems is gaining more attention in prosthodontic applications (Mehta et al., 2019; Schmidlin et al., 2010).
For simplicity, the term CAD/CAM composite blocks will describe both PICN and resin-composite blocks (resin-composite designed for CAD/CAM systems). In cases where distinction is required, they will be referred to as PICN or RCB.

CAD/CAM composite mechanical properties, such as flexural strength, modulus of resilience and hardness, are essential in terms of predicting material clinical success and performance (Charlton et al., 2008; Zhang & Kelly, 2017). Microhardness is greatly influenced by the material composition, ageing and water sorption (Martos et al., 2003; Sonmez et al., 2018). Some studies have investigated the hardness of CAD/CAM composite materials (Alamoush et al., 2018; Lawson et al., 2016) at dry condition, and others with long-term water storage (Al-Harbi et al., 2017) or short-term storage in food simulating agents (Ilie, 2019) and some drinks like coffee (Saba et al., 2017) and acidic drinks (Colombo et al., 2019). However, no study has evaluated the effect of different storage media, such as 75% ethanol/water and artificial saliva, on microhardness over relatively long-term storage times. Therefore, this study aimed to assess the long-term effect of different storage media on the microhardness of the investigated materials. The null hypotheses were 1) there is no significant difference in the hardness reduction percentage between the investigated materials, 2) there is no significant difference in the surface microhardness provoked by different storage media and storage time, and 3) there is no effect of filler loading on the hardness reduction percentage of the investigated materials.

5.3 Materials and methods

5.3.1 Sample preparation

Eight CAD/CAM blocks were investigated: Five resin-composite blocks (RCB) (Grandio blocs (GR), Lava™ Ultimate (LU), Cerasmart (CS), BRILLIANT Crios (BC), Block HC (HC)), one polymer-infiltrated ceramic network (PICN) block (Enamic (EN)), one ceramic-filled poly ether ether ketone (PEEK) block (Dentokeep (DK)), and one feldspathic ceramic block (Vitabloc Mark II (VM)), as shown in Table 5-1.
Table 5-1: The manufacturers’ compositional information and experimentally determined filler weight percentage of the materials investigated.

<table>
<thead>
<tr>
<th>Material (Code)</th>
<th>Manufacturer</th>
<th>Composition by weight represented by the manufacturers</th>
<th>Filler weight % (Alamoush et al., 2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feldspathic ceramic block</td>
<td>Vitabloc Mark II (VM)</td>
<td>0</td>
<td>Fine-particle feldspar ceramic</td>
</tr>
<tr>
<td>Polymer-infiltrated ceramic network (PICN)</td>
<td>Vita Enamic (EN)</td>
<td>14%</td>
<td>86% fine structure feldspar ceramic</td>
</tr>
<tr>
<td>Resin-composite blocks (RCB)</td>
<td>Grandio Blocs (GR)</td>
<td>14%</td>
<td>86% nanohybrid fillers</td>
</tr>
<tr>
<td></td>
<td>Lava™ Ultimate (LU)</td>
<td>20%</td>
<td>80% silica and zirconia nano particles</td>
</tr>
<tr>
<td></td>
<td>Cerasmart (CS)</td>
<td>71% silica and barium glass nanoparticles</td>
<td>66.1(0.2)</td>
</tr>
<tr>
<td></td>
<td>BRILLIANT Crios (BC)</td>
<td>70% glass and amorphous silica</td>
<td>70.1(0.05)</td>
</tr>
<tr>
<td></td>
<td>Block HC (HC)</td>
<td>61% silica powder, microfumed silica, and zirconium silicate</td>
<td>63(0.02)</td>
</tr>
<tr>
<td>Ceramic-filled PEEK</td>
<td>Dentokeep (DK)</td>
<td>80% PEEK</td>
<td>20% TiO₂</td>
</tr>
</tbody>
</table>
Each CAD/CAM block was sectioned by a diamond blade (MK 303, MK Diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Saw, Buehler Co, IL, USA) under constant water irrigation into rectangular bars of 16×4×2 mm dimensions. Each specimen was wet ground and polished using a lapping machine (MetaServ 250, Buehler Co, IL, USA) with a series of silicon carbide papers of P320, P500, P1200, P2400, and P4000 grit (Buehler Co, IL, USA) under water cooling and then polished with 0.25 μm diamond suspension (Meta Di Supreme, Buehler Co, IL, USA). All specimens were then cleaned in an ultrasonic bath (Ultrasonic Cleaning System, L&R Co, NJ, USA) with distilled water for 5 min.

Forty-eight specimens were prepared, comprising 6 of each of the 8 materials. Microhardness of each material (n=6, 5 readings per specimen) was undertaken under dry conditions at day 0, and then 2 samples (n=2) of each material were immersed in 10 ml of either water, artificial saliva or 75% E/W, and stored at 37°C in individual glass vials. Microhardness was measured after 30 and 90 days. The Macknight-Hane and Whitford formula was used to prepare artificial saliva (AS) (McKnight-Hanes & Whitford, 1992). All the materials used and their concentrations are shown in Table 5-2.

**Table 5-2: The compounds used for artificial saliva preparation.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound amount g/l (distilled water)</th>
<th>Manufacturer</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 4-hydroxybenzoate</td>
<td>2.00</td>
<td>Sigma-Aldrich</td>
<td>99-76-3</td>
</tr>
<tr>
<td>Sodium carboxymethyl cellulose (CMC)</td>
<td>10.00</td>
<td>Sigma-Aldrich</td>
<td>9004-32-4</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.625</td>
<td>BHD Chemicals Ltd, Poole, England</td>
<td>7447-40-7</td>
</tr>
<tr>
<td>Calcium chloride (CaCl₂)</td>
<td>0.166</td>
<td>Sigma-Aldrich</td>
<td>10043-52-4</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH₂PO₄)</td>
<td>0.326</td>
<td>May &amp; Baker LTD, Dagenham, England</td>
<td>7778-77-0</td>
</tr>
<tr>
<td>Magnesium chloride (MgCl₂)</td>
<td>0.059</td>
<td>Sigma-Aldrich</td>
<td>7786-30-3</td>
</tr>
<tr>
<td>Potassium hydroxide (KOH)</td>
<td>To adjust pH 6.75</td>
<td>May &amp; Baker Ltd, Dagenham, England</td>
<td>1310-58-3</td>
</tr>
</tbody>
</table>
5.3.2 Microhardness test

Microhardness was measured using a Vickers indenter tester (FM-700, Future Tech Corp., Japan) under a load of 300 g, and a dwell time of 20 s. Vickers hardness number (VHN) was automatically calculated by the machine and it can also be calculated using the following equation (ISO 6507-1, 2006):

\[
VHN = 1.854 \times \frac{P}{D^2}
\]  

(1)

Where \( P \) is the applied load in kg and \( D \) is the indentation diagonal length in mm.

The hardness reduction (as a percentage) after 90 days storage was calculated as follows:

\[
HR\% = \frac{VHN(d_0) - VHN(d_{90})}{VHN(d_0)} \times 100\%
\]  

(2)

Where \( VHN(d_0) \) and \( VHN(d_{90}) \) are the Vickers hardness numbers at day 0 and day 90, respectively.

5.3.3 Statistical analysis

The data were analysed using statistical software (SPSS ver. 23, IBM, IL, USA) and found to exhibit normal distribution (Shapiro–Wilk test). The homogeneity of variance was calculated by Levene’s statistics. Three-way ANOVA was performed to investigate the interaction between the materials, storage medium and storage times, and Vickers hardness. One-way ANOVA was followed by Tukey’s post hoc analysis for multiple comparisons. The Pearson correlation was conducted for correlation of the hardness reduction and filler weight percentages in different media (\( \alpha=0.05 \) for all tests).
5.4 Results

Three-way ANOVA showed a significant effect of time, material and storage media on microhardness. The interaction between the material and storage media was statistically significant ($p<0.0001$). Simple main effects analysis showed that the material effect was significantly the most in 75% E/W, followed by water and finally AS ($p<0.03$).

The hardness reduction after 90 days storage was the most in 75% E/W for all the investigated materials except in DK; this was less than the hardness reduction in water and AS. Water and AS were comparable as storage media in terms of hardness reduction for all the investigated materials except for DK; hardness reduction was 10.8 (3.1) in water compared to 23.2 (1.7) in AS. Specimens stored in the water had a hardness reduction ranging from 0.9% for VM to 24.4% for BC. In AS, the specimens had a hardness reduction ranging from 2.8% for VM to 23.2% for DK. The hardness reduction in 75% E/W ranged between 3.8% for VM and 35.3% for CS (Table 5-3, 5-4, Figures 5-1, 5-2).

Pearson correlation of the hardness reduction (in different media) and filler weight percentage, measured in a previous study (Alamoush et al., 2018), showed a negative correlation that was only significant in AS ($r=-0.74$, $p=0.037$). Pearson correlation of the hardness reduction (in different media) and filler weight percentage, excluding DK as a non-methacrylate-based composite, showed a negative correlation that was significant in all media ($p<0.04$, $r=-0.77$; $p=0.025$, $r=-0.81$; and $p=0.004$, $r=-0.91$) with linear regression ($R^2= 0.60$, 0.67, and 0.82) for water, AS and 75% E/W, respectively (Figure 5-3).
Table 5-3: The mean and standard deviation values of Vickers hardness (VHN) at day 0, and after 30 and 90 days storage in distilled water, artificial saliva and 75% E/W. Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)) in each column (higher-case letters) and in each row (lower-case letters).

<table>
<thead>
<tr>
<th>Media</th>
<th>Dry</th>
<th>Distilled water</th>
<th>Artificial saliva</th>
<th>75% E/W</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 d</td>
<td>30 d</td>
<td>90 d</td>
<td>30 d</td>
</tr>
<tr>
<td>VM</td>
<td>502.4 (2.28)</td>
<td>498.6 (3.7)</td>
<td>497.8 (4.7)</td>
<td>494.4 (9.3)</td>
</tr>
<tr>
<td>EN</td>
<td>203.1 (0.43)</td>
<td>199.8 (1.6)</td>
<td>198.1 (3.2)</td>
<td>193.9 (5.9)</td>
</tr>
<tr>
<td>GR</td>
<td>121.8 (2.1)</td>
<td>115.9 (3.3)</td>
<td>112.3 (3.2)</td>
<td>114 (3.3)</td>
</tr>
<tr>
<td>LU</td>
<td>112.6 (0.44)</td>
<td>90.3 (2.8)</td>
<td>86.3 (1.6)</td>
<td>90.2 (2.7)</td>
</tr>
<tr>
<td>BC</td>
<td>82.6 (0.49)</td>
<td>67.1 (1.2)</td>
<td>62.4 (0.8)</td>
<td>66.2 (1.1)</td>
</tr>
<tr>
<td>CS</td>
<td>80.1 (0.76)</td>
<td>67.2 (3.3)</td>
<td>63.6 (1.8)</td>
<td>65.5 (2.4)</td>
</tr>
<tr>
<td>HC</td>
<td>73.1 (1.04)</td>
<td>64.2 (1.6)</td>
<td>63.4 (1.0)</td>
<td>62.5 (2.0)</td>
</tr>
<tr>
<td>DK</td>
<td>27.7 (0.19)</td>
<td>25.9 (0.2)</td>
<td>24.8 (0.8)</td>
<td>22.3 (0.5)</td>
</tr>
</tbody>
</table>

Table 5-4: The mean and standard deviation values of hardness reduction percentage (HR %) after 90 days storage in distilled water, artificial saliva and 75% E/W. Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)) in each column.

<table>
<thead>
<tr>
<th>Media</th>
<th>Distilled water</th>
<th>Artificial saliva</th>
<th>75% E/W</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR %</td>
<td>HR %</td>
<td>HR %</td>
</tr>
<tr>
<td>VM</td>
<td>0.9 (0.8)</td>
<td>2.8 (2)</td>
<td>3.8 (1)</td>
</tr>
<tr>
<td>EN</td>
<td>2.5 (2.0)</td>
<td>5.6 (2.3)</td>
<td>6.2 (2.4)</td>
</tr>
<tr>
<td>GR</td>
<td>7.8 (2.5)</td>
<td>10.3 (3.2)</td>
<td>12.7 (2.4)</td>
</tr>
<tr>
<td>LU</td>
<td>23.4 (1.5)</td>
<td>22.2 (1.5)</td>
<td>30.1 (0.89)</td>
</tr>
<tr>
<td>BC</td>
<td>24.5 (2.2)</td>
<td>22.7 (2.4)</td>
<td>34.5 (2.2)</td>
</tr>
<tr>
<td>CS</td>
<td>20.5 (2.6)</td>
<td>21.7 (2)</td>
<td>35.3 (1.6)</td>
</tr>
<tr>
<td>HC</td>
<td>13.3 (1.9)</td>
<td>14.8 (1.7)</td>
<td>29.2 (3.2)</td>
</tr>
<tr>
<td>DK</td>
<td>10.8 (3.1)</td>
<td>23.2 (1.7)</td>
<td>5.5 (1.8)</td>
</tr>
</tbody>
</table>
Figure 5-1: Mean VHN values over 90 days storage in distilled water, artificial saliva, and 75% E/W.
Figure 5-2: A bar chart illustrating the mean values of hardness reduction after 90 days storage in distilled water, artificial saliva, and 75% E/W. Error bars represent the standard deviation.

Figure 5-3: A scatter plot showing a negative correlation and linear regression between the filler weight percentage (measured experimentally) and the hardness reduction percentage in different storage media (p<0.04, r=-0.77; p=0.025, r=-0.81; and p=0.004, r=-0.91) with linear regression (R²=0.60, 0.67, and 0.82) for distilled water, artificial saliva and 75% E/W, respectively.
5.5 Discussion

In the present study, significant differences in hardness reduction percentage between the investigated materials were found, and the first null hypothesis was consequently rejected. The storage medium and time showed a significant effect on the surface microhardness, and thus the second null hypothesis was rejected. Materials with different filler loading exhibited significant differences in hardness reduction, and the third null hypothesis was therefore rejected.

VM showed greater hardness stability than all other tested materials; this is a ceramic material feature, as it is particularly difficult for solvents to penetrate, in contrast to polymer-based materials where solutions can diffuse through the polymeric matrix of the material (Colombo et al., 2019; Ferracane et al., 1998).

EN exhibited softening resistance higher than all resin-composite blocks and comparable to that of VM, attributable to the ceramic-polymer network and resistance to breakdown (Alamouchi et al., 2018; Coldea et al., 2013; Lambert et al., 2017). GR showed the least hardness reduction all the resin-composite blocks due to the high filler percentage (86 wt%). LU, BC and CS had the same level of hardness reduction as they have similar filler weight percentage (75 wt%, 70% and 66%). HC, despite having the least filler weight percentage (63%), showed higher softening resistance than LU, BC and CS; this might be due to the matrix composition of UDMA and TEGDMA without Bis-GMA, which usually absorbs more water than UDMA, leading to more softening of the material (Ertas et al., 2006; Sideridou et al., 2003; Sideridou & Karabela, 2011). Additionally, LU may have more zirconium silicate in its filler composition than HC, and thus is more prone to hydrolysis of the silane-coupling agent attributable to the inefficient salinisation of high crystalline content zirconium silicate (Druck et al., 2015).

Linear regression has been used (figure 5-3), and similar patterns can be noticed. All materials were affected more by 75% E/A followed by AS and water. However, a linear relationship can be noticed in materials with UDMA and no Bis-GMA (EN, GR and HC) in their composition while Bis-GMA containing materials (LU and BC) and (CS) have more like a ‘horizontal’ relationship in both AS and water. This emphasises the fact that not only filler loading but also resin-matrix composition influences the hardness reduction levels.
An interesting finding was that DK was the least affected by 75% E/W, reflecting the material resistance and stability to such a strong solvent. This could be due to DK having a different solubility parameter to that of ethanol, unlike the rest of the investigated methacrylate-based composites with solubility parameters close to that of ethanol (Manojlovic et al., 2013). DK (PEEK with 20% ceramic filler) exhibited the highest hardness reduction in artificial saliva of all the storage media; this might be due to the interaction between AS and DK, which is in line with a study that showed that filled PEEK mechanical properties were more affected by artificial saliva ageing than unfilled PEEK (Gao et al., 2015). PEEK has very stable chemical and physical properties due to the presence of an aryl ring containing ketone and other groups (Williams, 2008a). Consequently, it has resistance to surface modification by different chemical treatments (Noiset et al., 2000) except concentrated sulfuric acid (Kurtz & Devine, 2007), it is stable at high temperatures (Eschbach, 2000), and has shown low solubility and water sorption compared to other polymer-based CAD/CAM materials (Liebermann et al., 2016).

Hardness reduction from day 0 to 30 was higher than from day 30 to 90, as can be noted in Figure 5-1 for all materials and all media; this is consistent with other studies, as most material changes usually occur in the first few days, after which the material may reach an equilibrium (Alshali et al., 2015a; Sunbul et al., 2016; Van Landuyt et al., 2011; Łagocka et al., 2018).

Three solvents were used in this study to simulate different oral conditions. Storage in water causes hydrolytic degradation of the interfacial silane-coupling agent between fillers and resin-matrix, consequently affecting the mechanical and physical properties of resin-composite (Druck et al., 2015; Drummond et al., 1991; Mair & Padipatvuthikul, 2010; Musanje & Darvell, 2003; Tuna et al., 2008). In this study, the artificial saliva formula used had a comparable viscosity and acidity to human saliva (Darvell, 1978; McKnight-Hanes & Whitford, 1992). The 75% E/W solvent is regarded as food and oral simulating liquid, according to the Food and Drug Administration (FDA) Guidelines of the United States (Moon et al., 2000; Sideridou et al., 2007; United States Food and Drug Administration, 1988).
As 75% E/W has a similar solubility factor to the resin-composite matrix, it can penetrate the resin-composite matrix causing the accelerated ageing of resin-composite materials (Manojlovic et al., 2013) and material softening (Marghalani & Watts, 2013). As found in this study and previous studies, ethanol is a more aggressive solvent than water-based solvents (Ferracane, 1994; Tabatabaee et al., 2009).

Some studies have reported the effect of different ageing media and ageing times on the mechanical properties of CAD/CAM composite blocks. A decrease in hardness of LU after water storage up to 9 months has been found, although the decrease mostly occurs in the first 3 months of water storage (Al-Harbi et al., 2017). Other immersion or storage media have been used such as coffee, which has shown a negative impact on both the colour and microhardness of EN and VM (Saba et al., 2017). Acidic drinks storage has also provoked more microhardness reduction of CAD/CAM composite blocks than that of CAD/CAM ceramics (Colombo et al., 2019).

In this study, the hardness reduction values were negatively correlated to the amount of filler loading for all CAD/CAM composite materials, excluding DK; this is in agreement with similar study (Ilie, 2019). The morphology and constituents of filler particles are reported to improve mechanical properties such as compressive strength, fracture toughness, wear resistance (Curtis et al., 2008; Kim et al., 2007), tensile and compressive strength, hardness, and elasticity modulus (Chung, 1990; Lin-Gibson et al., 2009).

The Vickers microhardness test is a well-documented method to measure hardness for a wide range of materials. Nevertheless, there is a subjective operator variation as the indentation surface area is determined based on the average length of both diagonals (D) (used to calculate the indentation surface area), which is determined microscopically by the naked eye (Ilie et al., 2017).
5.6 Conclusions

Within the limitations of the study, the following can be concluded:

1- The hardness of CAD/CAM composite blocks was affected by different storage media and they were not as stable as ceramic with PICN exhibited superior hardness stability to all of the resin-composite blocks in all storage media, and was comparable to VM ceramic. PEEK also exhibited comparable hardness stability to both PICN and VM in 75% E/W.

2- Water and artificial saliva had comparable effects as storage media, while 75% E/W had a more pronounced effect on the Vickers hardness of the investigated materials.

3- The hardness reduction of CAD/CAM composite blocks was influenced by the filler loading and resin-matrix composition.
Chapter Six:
Long-Term Hydrolytic Stability of CAD/CAM Composite Blocks

R. Alamoush, J. D. Satterthwaite, & N. Silikas.
6.1 Abstract

Objectives: This study aimed to assess water sorption and solubility of CAD/CAM composite blocks compared to CAD/CAM ceramic after 8 months storage in water and artificial saliva.

Materials and methods: Eight CAD/CAM blocks were investigated: Five resin-composite blocks (RCB) (Grandio blocs (GR), Lava™ Ultimate (LU), Cerasmart (CS), BRILLIANT Crios (BC), Block HC (HC)), one polymer-infiltrated ceramic network (PICN) block (Enamic (EN)), one ceramic-filled polyetheretherketone (PEEK) block (Dentokeep (DK)), and one feldspathic ceramic block (Vitabloc Mark II (VM)). 112 specimens were prepared comprising 14 of each of the eight materials, and divided into two groups (n=7) that were randomly immersed in 10 ml of either water or artificial saliva, stored at 37°C and weighed at various time intervals. The data were analysed via repeated measures ANOVA, one-way ANOVA, and Tukey’s post hoc test (α=0.05 for all tests).

Results: All materials attained a stable mass within six to eight months in each storage media. Percentage mass change in water was between -0.04% and 2.2% and in artificial saliva between -0.02% and 2.24%. Sorption values in water lay within the range -1.21 (0.4) to 39.3 (2.1) µg/mm³ and in artificial saliva between -0.7 (0.2) and 41.6 (1.3) µg/mm³. Percentage sorption of the polymer matrix in water was between 0.46 (0.02)% and 6.71 (0.7)% and in artificial saliva it ranged between 0.42 (0.09)% and 6.90 (0.4)%. Solubility values in water were between -0.43 (0.08) and 0.34 (0.18) µg/mm³ and in artificial saliva between -0.53 (0.07) µg/mm³ and 0.33 (0.2) µg/mm³.

Conclusions: CAD/CAM composite blocks were reasonably hydrolytically stable in long-term storage, although not as stable as ceramic, with DK exhibited superior hydrolytic stability to CAD/CAM composite blocks. The water sorption of CAD/CAM composite blocks was dependent on the resin-matrix and was influenced by the filler weight %. Water and artificial saliva showed a comparable effect on water sorption.

Keywords: CAD/CAM composite blocks; Resin-composite blocks, Polymer-infiltrated network; Hydrolytic Stability; Sorption; Solubility.
6.2 Introduction

Compared to ceramics, resin-composites exhibit lower strength and worse colour stability, a result mainly of their solubility and water sorption properties. Storage in water induces hydrolytic degradation of the interfacial silane coupling agent, which maintains the chemical bonding between the resin-matrix and fillers, causing molecular instability (Druck et al., 2015; Musanje & Darvell, 2003). Storage in water, therefore, affects the mechanical and physical properties of resin-composite and it induces hydrolytic degradation of bonding (Drummond et al., 1991; Mair & Padipatvuthikul, 2010; Tuna et al., 2008) with hydrophilic materials being more vulnerable to colour change and staining, depending on the degree of water uptake (Um & Ruyter, 1991).

Resin-composite designed for CAD/CAM are polymerised under high temperature and high pressure (Nguyen et al., 2012; 2013). Compared to conventional resin-composite this polymerisation mode results in higher composite homogeneity and reliability with fewer flaws and pores (Nguyen et al., 2012; Stawarczyk et al., 2012), and increased degree of conversion (Phan et al., 2014); which reduces water sorption (Gajewski et al., 2012; Gupta et al., 2012; Lin-Gibson et al., 2009) and improves mechanical properties (Alt et al., 2011; Hussain et al., 2017), wear resistance (Mormann et al., 2013; Stawarczyk et al., 2013d), flexural strength (Nguyen et al., 2014), fracture toughness and fracture strength (Coldea et al., 2013a; Della Bona et al., 2014). The polymer matrix also influences water sorption and colour stability (Fonseca et al., 2017), for instance, UDMA, which is currently used in most CAD/CAM composites, has lower water sorption and solubility properties compared to Bis-GMA (Ertas et al., 2006; Sideridou & Karabela, 2011).

CAD/CAM composite blocks constitute two main categories: resin-composite block (RCB), which is produced by the incorporation of filler particles in a monomer mixture (Gracis et al., 2015; Lambert et al., 2017), and polymer-infiltrated ceramic network (PICN) (for example, Enamic), comprising a pre-sintered glass-ceramic network infiltrated with a monomer, which is then polymerised (Coldea et al., 2013a; Lambert et al., 2017). PEEK (Polyetheretherketone) has also recently gained attention in CAD/CAM technology for both fixed and removable prostheses (Mehta et al., 2019; Schwitalla et al., 2015).
For simplicity, the term CAD/CAM composite blocks describe both PICN and RCB (resin-composite designed for CAD/CAM systems), otherwise each will be referred to by PICN or RCB.

Given the limited previous research on CAD/CAM composite blocks and the need to evaluate their simulated clinical performance, the present study was intended to assess the long-term water sorption and solubility of CAD/CAM composite blocks in comparison to ceramic after eight months storage in water and artificial saliva. The null hypotheses were (1) that there would be no significant difference in mass change between the materials investigated after eight months storage period in either storage medium and over different time intervals; (2) that there was no difference in the sorption and solubility of the materials investigated; (3) that the sorption and solubility of the materials investigated were not affected by their composition (filler weight percentage); and (4) that the storage medium would have no significant effect on sorption and solubility.

6.3 Materials and methods

6.3.1 Materials and sample preparation

Eight CAD/CAM blocks were investigated: Five resin-composite blocks (RCB) (Grandio blocs (GR), Lava™ Ultimate (LU), Cerasmart (CS), BRILLIANT Crios (BC), Block HC (HC)); one polymer-infiltrated ceramic network block (Enamic, EN); one ceramic-filled polyether etherketone (Dentokeep, DK); and one feldspathic ceramic block (Vitabloc Mark II, VM), as shown in Table 6-1.

Each CAD/CAM block was sectioned using a diamond blade (MK 303, MK Diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Saw; Buehler Co, IL, USA) under constant water irrigation. All specimens were polished using a lapping machine (MetaServ 250, Buehler Co, IL, USA) with a series of silicon carbide (SiC) paper disks P320, P500, P1200, P2400, and P4000 grit (Buehler Co, IL, USA) under water cooling and then polished with 0.25 μm diamond suspension (Meta Di Supreme, Buehler Co, IL, USA). They were then cleaned in an ultrasonic bath (Ultrasonic Cleaning System, L&R Co, NJ, USA) with distilled water for 5 min. 112 specimens were prepared with dimensions 14 × 12 × 3 mm, comprising 14 of each of the eight materials, and divided into two groups (n=7).
Table 6-1: The manufacturers’ compositional information and experimentally determined filler weight percentage of the materials investigated.

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Composition by weight represented by the manufacturers</th>
<th>Filler weight % (Alamoush et al., 2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feldspathic ceramic block</strong></td>
<td>Vitabloc Mark II (VM)</td>
<td>0 Fine-particle feldspar ceramic</td>
<td>0.00(0)</td>
</tr>
<tr>
<td><strong>Polymer-infiltrated ceramic network (PICN)</strong></td>
<td>Vita Enamic (EN)</td>
<td>14% UDMA, TEGDMA 86% fine structure feldspar ceramic</td>
<td>85.1(0.1)</td>
</tr>
<tr>
<td><strong>Resin-composite blocks (RCB)</strong></td>
<td>Grandio Blocs (GR)</td>
<td>14% UDMA, DMA 86% nanohybrid fillers</td>
<td>84.6(0.01)</td>
</tr>
<tr>
<td><strong>Ceramic-filled PEEK</strong></td>
<td>Dentokeep (DK)</td>
<td>80% PEEK 20% TiO$_2$</td>
<td>27.5(0.06)</td>
</tr>
</tbody>
</table>
The drying procedure followed was as described by ISO, FDIS 4049:2009 (ISO/FDIS 4049, 2009). Specimens were placed in separate glass vials and stored in a lightproof desiccator with anhydrous self-indicating silica gel at (37 ± 1) °C. After 22 h, they were stored at room temperature (23 ± 1) °C for 2 h in another desiccator and then weighed to an accuracy of 0.01 mg using a calibrated electronic analytical balance (Ohaus Analytical Plus, Ohaus Corporation, USA). This procedure was repeated every 24 h until a constant mass was obtained (defined as the loss of mass of each specimen is below 0.1 mg in successive 24 h periods). This constant mass was taken to be the initial mass of the specimen (m1). Using the average mean sample dimensions, the volume \( V, \text{mm}^3 \) of each specimen was calculated.

### 6.3.2 Sorption and solubility

Samples of each material (n=7) were randomly immersed in 10 ml of either water or artificial saliva and stored at 37 ± 1°C in individual glass vials. Artificial saliva (AS) was prepared according to the Macknight-Hane and Whitford formula (McKnight-Hanes & Whitford, 1992). All materials used and their concentrations are shown in Table 6-2. The specimens were weighed periodically over eight months at various immersion intervals (0, 7, 14, 28, 90, 180 and 240 days) and the mass at each point recorded (m2) \((t)\). At each point, the specimens were dried on filter paper, agitated in the air for 15 s, and after 1 min weighed and returned to the individual glass vials. The pH of the storage media was checked using pH meter at the time of each measurement, and if it had changed the storage medium was replaced. At the final time point (8 months), the specimens were dried according to ISO, FDIS 4049:2009 to constant mass (m3) (ISO/FDIS 4049, 2009).
Table 6-2: The compounds used for artificial saliva preparation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound amount g/l (distilled water)</th>
<th>Manufacturer</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 4-hydroxybenzoate</td>
<td>2.00</td>
<td>Sigma-Aldrich</td>
<td>99-76-3</td>
</tr>
<tr>
<td>Sodium carboxymethyl cellulose (CMC)</td>
<td>10.00</td>
<td>Sigma-Aldrich</td>
<td>9004-32-4</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.625</td>
<td>BHD Chemicals Ltd, Poole, England</td>
<td>7447-40-7</td>
</tr>
<tr>
<td>Calcium chloride (CaCl₂)</td>
<td>0.166</td>
<td>Sigma-Aldrich</td>
<td>10043-52-4</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH₂PO₄)</td>
<td>0.326</td>
<td>May &amp; Baker LTD, Dagenham, England</td>
<td>7778-77-0</td>
</tr>
<tr>
<td>Magnesium chloride (MgCl₂)</td>
<td>0.059</td>
<td>Sigma-Aldrich</td>
<td>7786-30-3</td>
</tr>
<tr>
<td>Potassium hydroxide (KOH)</td>
<td>To adjust pH 6.75</td>
<td>May &amp; Baker Ltd, Dagenham, England</td>
<td>1310-58-3</td>
</tr>
</tbody>
</table>

6.3.3 Calculations

The percentage mass change (weight loss) of each material during storage was calculated using the following equation:

\[
Mg\% = \frac{m_2 (t) - m_1}{m_1} \times 100\%
\]  

(1)

The sorption of each material in micrograms per cubic millimetre (μg/mm³) at the end of the eight-month storage (8m) period was calculated as follows:

\[
SP = \frac{m_2 (8m) - m_3}{V}
\]  

(2)

The percentage quantity of water or artificial saliva absorbed by each material was calculated as follows:

\[
SP\% = \frac{m2 (8m) - m3}{m1} \times 100\%
\]  

(3)

The percentage amount of water or artificial saliva absorbed by the polymer matrix (where absorption mainly occurred) was calculated using the following equation (Sideridou et al., 2011):

\[
SP_{pm} (%) = \frac{SP\%}{a}
\]  

(4)
The solubility of each material was calculated using the following equation:

\[ SL = \frac{m_1 - m_3}{V} \]  

(5)

The percentage solubility, defined as the total weight of components extracted by the storage medium, was calculated as follows:

\[ SL\% = \frac{m_1 - m_3}{m_1} \times 100\% \]  

(6)

Where \( m_1 \) is the initial-recorded mass, \( m_2 \) the mass recorded after each time interval, \( m_3 \) the final-recorded mass, \( V \), the volume in mm\(^3\), and \( a \) is the proportional weight of the polymer matrix of CAD/CAM blocks as measured in a previous study (Alamoush et al., 2018).

6.3.4 Statistical analysis

The data were analysed using SPSS (SPSS ver. 23, IBM, IL, USA) and found to exhibit normal distribution (Shapiro-Wilk test). The percentage of mass change was analysed to assess the effect of storage time using repeated-measures ANOVA. The mean sorption and solubility of different materials were compared using one-way ANOVA, followed by Tukey post hoc analysis for multiple comparisons. An independent \( t \)-test was used to compare the sorption and solubility values obtained for each material in water and artificial saliva. Pearson correlation was used to assess the relationship between the variables. All tests were conducted at a significance level of \( \alpha = 0.05 \).
6.4 Results

Mass percentage change (Mg%) in the materials investigated ranged from -0.04% to 2.20% in water and from -0.02% to 2.24% in artificial saliva, with a significant difference between all materials tested (Table 6-3). The highest increase in mass change was observed during the first week, and it then began to decrease towards the second week, increasing at a slower rate up to three months, before gradually decreasing until equilibrium was reached around the eighth month (Figures 6-1 and 6-2). VM was virtually stable throughout the test period. Repeated-measures ANOVA showed a significant time effect (p<0.01). The percentage mass change values in artificial saliva were significantly higher than water for VM, CS and HC.

A significant difference in water sorption was observed for all the materials investigated in the two groups. Sorption (SP) in water ranged from -1.2 (0.4) to 39.3 (0.9) μg/mm³ and in artificial saliva from -0.7 (0.2) to 41.6 (1.3) μg/mm³. VM, LU, CS, and HC showed significantly higher sorption (SP) in artificial saliva than in water. Conversely, GR showed significantly higher SP in water than in artificial saliva. The remainder of the materials showed no significant difference in sorption between the two media (Tables 6-4, 6-5 and Figure 6-3).

HC showed significantly higher SP in artificial saliva (41.61 (1.33) μg/mm³) followed by LU (36.03 (1.40) μg/mm³), whereas VM had significantly the lowest value (-0.70 (0.20) μg/mm³) in artificial saliva. In water, HC gave significantly higher SP values (39.31 (0.90) μg/mm³) than other materials, followed by LU (34.79 (0.42) μg/mm³), whereas VM showed significantly the lowest value (-1.21 (0.40) μg/mm³). A similar order was observed in the case of SP pm values of these materials in both water and artificial saliva. However, LU exhibit higher, but not significantly different, SP pm than that of HC in artificial saliva.

In water, EN and GR showed comparable SP 15.77 (0.60), and 14.4 (0.58) μg/mm³, respectively, while all the other materials had significantly different SP values. In artificial saliva, the SP values of the materials investigated were significantly different, with the same order noticed in water as follows: VM < DK < GR < EN < BC < CS < LU < HC.
SP in water and artificial saliva was significantly negatively correlated to the measured percentage filler weight, excluding DK as a non-methacrylate based composite, (Pearson correlation coefficient, r=-0.89, -0.88) for water and AS respectively, with linear regression for both, R²=0.78, (p=0.008) (Figure 6-4).

In water, HC gave a significantly higher solubility (SL) value of 0.34 (0.18) μg/mm³ compared to the other materials. Solubility in water ranged from -0.43 (0.08) μg/mm³ to 0.34 (0.18) μg/mm³, with negative solubility values in the case of EN, GR, LU, BC, and CS (Table 6-4, Figure 6-5). In artificial saliva, LU and HC showed significantly higher solubility values compared to the other materials, 0.33 (0.2), and 0.30 (0.06) μg/mm³, respectively, (Table 6-5, Figure 6-5). Solubility in artificial saliva ranged from -0.53 (0.07) to 0.33 (0.2) μg/mm³ and was significantly lower than that in water in the case of VM, EN, and HC, but higher than water for LU, with negative solubility values for some materials (EN, GR, BC and CS).

Sorption (SP) values in water and artificial saliva were positively correlated (Pearson correlation coefficient, r=0.998, p=0.0001). SL values in water and artificial saliva were also positively correlated (Pearson correlation coefficient, r=0.80, p=0.018).

Table 6-3: The mean and standard deviation values of mass percentage change after eight months storage in distilled water and artificial saliva. Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)). Values with the same superscript numbers indicate a non-significant difference for each individual material between the two storage media (Independent t-test (α=0.05)).

<table>
<thead>
<tr>
<th>Material</th>
<th>Mass percentage change in water</th>
<th>Mass percentage change in artificial saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM</td>
<td>-0.048(0.01)</td>
<td>-0.028(0.009)</td>
</tr>
<tr>
<td>EN</td>
<td>0.72(0.03)</td>
<td>0.71(0.05)</td>
</tr>
<tr>
<td>GR</td>
<td>0.55(0.01)</td>
<td>0.54(0.02)</td>
</tr>
<tr>
<td>LU</td>
<td>1.50(0.05)</td>
<td>1.54(0.07)</td>
</tr>
<tr>
<td>BC</td>
<td>1.04(0.03)</td>
<td>1.05(0.04)</td>
</tr>
<tr>
<td>CS</td>
<td>1.22(0.03)</td>
<td>1.29(0.04)</td>
</tr>
<tr>
<td>HC</td>
<td>2.20(0.03)</td>
<td>2.24(0.02)</td>
</tr>
<tr>
<td>DK</td>
<td>0.30(0.03)</td>
<td>0.31(0.03)</td>
</tr>
</tbody>
</table>
Figure 6-1: Mean percentage mass change (Mg %) over eight months storage in distilled water.

Figure 6-2: Mean percentage mass change (Mg %) over eight months storage in artificial saliva.
Table 6-4: The mean and standard deviation values of sorption and solubility (μg/mm³) after eight months storage in distilled water. Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)).

<table>
<thead>
<tr>
<th>Material</th>
<th>SP (μg/mm³)</th>
<th>SP%</th>
<th>SPpm%</th>
<th>SL (μg/mm³)</th>
<th>SL%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM</td>
<td>-1.21 (0.40)</td>
<td>-0.01(0.02)</td>
<td>0</td>
<td>0.09(0.02)</td>
<td>0.04(0.01)</td>
</tr>
<tr>
<td>EN</td>
<td>15.77 (0.60)</td>
<td>0.52(0.04)</td>
<td>3.23(0.4)</td>
<td>-0.43(0.08)</td>
<td>-0.2(0.03)</td>
</tr>
<tr>
<td>GR</td>
<td>14.4 (0.58)</td>
<td>0.48(0.01)</td>
<td>2.95(0.12)</td>
<td>-0.2(0.04)</td>
<td>-0.08(0.01)</td>
</tr>
<tr>
<td>LU</td>
<td>34.79 (0.42)</td>
<td>1.49(0.07)</td>
<td>5.89(0.05)</td>
<td>-0.05(0.19)</td>
<td>-0.02(0.09)</td>
</tr>
<tr>
<td>BC</td>
<td>21.75 (0.42)</td>
<td>0.99(0.03)</td>
<td>3.37(0.15)</td>
<td>-0.09(0.1)</td>
<td>-0.05(0.04)</td>
</tr>
<tr>
<td>CS</td>
<td>24.54 (1.28)</td>
<td>1.12(0.06)</td>
<td>3.42(0.49)</td>
<td>-0.2(0.19)</td>
<td>-0.1(0.09)</td>
</tr>
<tr>
<td>HC</td>
<td>39.31 (0.90)</td>
<td>2.40(0.1)</td>
<td>6.71(0.7)</td>
<td>0.34(0.18)</td>
<td>0.19(0.1)</td>
</tr>
<tr>
<td>DK</td>
<td>5.09 (0.60)</td>
<td>0.32(0.02)</td>
<td>0.46(0.02)</td>
<td>0.03(0.04)</td>
<td>0.02(0.02)</td>
</tr>
</tbody>
</table>

Table 6-5: The mean and standard deviation values of sorption and solubility (μg/mm³) after eight months storage in artificial saliva. Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)).

<table>
<thead>
<tr>
<th>Material</th>
<th>SP (μg/mm³)</th>
<th>SP%</th>
<th>SPpm%</th>
<th>SL (μg/mm³)</th>
<th>SL%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM</td>
<td>-0.70 (0.20)</td>
<td>0.01(0.008)</td>
<td>0</td>
<td>0.04(0.02)</td>
<td>0.02(0.01)</td>
</tr>
<tr>
<td>EN</td>
<td>15.49 (0.70)</td>
<td>0.47(0.03)</td>
<td>3.09(0.4)</td>
<td>-0.53(0.07)</td>
<td>-0.25(0.03)</td>
</tr>
<tr>
<td>GR</td>
<td>13.8(1.03)</td>
<td>0.48(0.02)</td>
<td>3.01(0.22)</td>
<td>-0.18(0.05)</td>
<td>-0.07(0.01)</td>
</tr>
<tr>
<td>LU</td>
<td>36.03 (1.40)</td>
<td>1.69 (0.1)</td>
<td>6.90(0.4)</td>
<td>0.33(0.2)</td>
<td>0.14(0.09)</td>
</tr>
<tr>
<td>BC</td>
<td>21.58 (0.46)</td>
<td>1.00(0.07)</td>
<td>3.65(0.2)</td>
<td>-0.1(0.18)</td>
<td>-0.05(0.08)</td>
</tr>
<tr>
<td>CS</td>
<td>26.47 (0.91)</td>
<td>1.23(0.07)</td>
<td>3.89(0.39)</td>
<td>-0.12(0.1)</td>
<td>-0.06(0.05)</td>
</tr>
<tr>
<td>HC</td>
<td>41.61 (1.33)</td>
<td>2.41(0.04)</td>
<td>6.64(0.2)</td>
<td>0.30(0.06)</td>
<td>0.16(0.03)</td>
</tr>
<tr>
<td>DK</td>
<td>5.19 (0.44)</td>
<td>0.32(0.04)</td>
<td>0.42(0.09)</td>
<td>0.01(0.06)</td>
<td>0.008(0.04)</td>
</tr>
</tbody>
</table>
**Figure 6-3:** A bar chart illustrating the mean values of sorption (μg/mm$^3$) after eight-month storage in water and artificial saliva of the investigated materials; Error bars represent the standard deviation. Stars represent a significant difference in sorption between water and artificial saliva of each investigated material.

**Figure 6-4:** A scatter plot showing a negative linear regression between filler weight percentage (measured experimentally) and sorption in water and artificial saliva, $R^2=0.78$, $p=0.008$. Excluding DK, as all other CAD/CAM composite materials had methacrylate-based polymer matrix.
Figure 6-5: A bar chart showing the mean values of solubility (μg/mm³) after eight months storage in water and artificial saliva of the investigated materials; Error bars represent the standard deviation. Stars represent a significant difference in solubility between water and artificial saliva of each investigated material. The negative solubility values of (EN, GR, BC, and CS) do not necessarily mean that these materials are insoluble, as this could be caused by the incomplete dehydration of these materials.

6.5 Discussion

Water sorption and solubility of eight different CAD/CAM blocks were assessed over eight months in water and artificial saliva. All materials had reached equilibrium toward eight months, but the mass change percentage after eight months was significantly different between all the tested materials with significant time effect, and the first null hypothesis was therefore rejected. The results showed significant differences in water sorption and solubility between different materials in the two media, and the second null hypothesis was consequently rejected. There were significant effects of filler loading on water sorption between the materials tested, and the third null hypothesis was therefore rejected. The storage medium showed a significant effect for some materials, and small or non-significant differences for others; the fourth null hypothesis was thus partially accepted.
Most mass changes take place within the first week as most water absorption usually occurs within the first few days (Alshali et al., 2015b; Huang et al., 2002; Martin et al., 2003; Zankuli et al., 2014; Łagocka et al., 2018). VM exhibited a negligible degree of sorption and solubility, which is a feature of the material. In the case of ceramics, it is more difficult for the water to penetrate in comparison to the polymer-based materials under test, which is the area of the material where the highest water diffusion takes place (Ferracane et al., 1998).

GR showed the lowest sorption value among CAD/CAM composite blocks, followed by EN. Although EN is considered stiffer and has a robust microstructure (PICN), (Alamoush et al., 2018; Coldea et al., 2013a; Lambert et al., 2017), making it harder to penetrate by storage media, it exhibited a comparable sorption value to that of GR (resin-composite block). This showed that the filler weight and composition have a more considerable influence on water sorption than did the manufacturing technique.

HC exhibited the highest sorption values, which could be attributable to filler content, as this is the lowest among the CAD/CAM composites, as well as having zirconium silicate in the filler composition that makes it more prone to hydrolysis of the silane coupling agent due to the inefficient salinization of high crystalline content zirconium silicate (Druck et al., 2015). Sorption of HC was significantly higher in artificial saliva (41.61 (1.33) µg/mm³). This slightly exceeds the maximum recommended value of 40 µg/mm³ according to ISO standards for dental resin restorations, ISO FDIS 4049:2009 (ISO FDIS 4049, 2009).

DK exhibited the lowest sorption value of the CAD/CAM composite materials under test (5.09 (0.60) mg/mm³); which is in line with similar study findings (Liebermann et al., 2016). DK was different from the other CAD/CAM composite tested, mainly due to the polymer matrix, polyetheretherketone (PEEK), whereas the others were based on methacrylate polymer matrix. PEEK is regarded as a very stable material; it has stable chemical and physical properties due to its chemical structure comprising an aryl ring containing ketone and other groups (Williams, 2008a).
The sorption (SP) values were significantly different between the materials tested in both media, in the following order: VM < DK < GR < EN < BC < CS < LU < HC. However, in the case of LU, SP$_{pm}$ was higher, but not significantly different, from that for HC. The LU polymer wt% was 25.7%, which is lower than that of HC (36.2%). However, it had slightly higher sorption of the polymer matrix (SP$_{pm}$) than that of HC, which might be due to the effect of artificial saliva on the LU matrix being more prominent than on HC, or due to the different resin-matrix composition as LU contains Bis-GMA, which has greater water sorption and solubility properties than UDMA, whereas both LU and HC contain TEGDMA (Ertas et al., 2006; Sideridou & Karabela, 2011).

CS and BC exhibited lower sorption values compared to LU, which has a higher filler content than either, which might again be attributed to the difference in resin-matrix composition. The polymer matrix composition and structure influences the resin-composite degree of conversion, water sorption and solubility, and colour stability (Fonseca et al., 2017). TEGDMA has the highest water absorption, followed by Bis-GMA, and finally by UDMA and Bis-EMA (Bagheri et al., 2005; Ertas et al., 2006; Sideridou et al., 2003). Most of the resin-composite CAD/CAM materials investigated had UDMA and TEGDMA as the main components of the polymeric matrix. LU had all these monomers, which might explain the higher sorption SP than CS and BC, although both CS and BC had lower filler content. In addition, nanocluster filler particles in LU normally have defects and voids leading to sorption and surface degradation (Zhang & Kelly, 2017).

Water sorption and solubility after 8 months storage in water were much higher than those previously reported for some CAD/CAM composite materials when immersed for one week, EN for example have SP of 15.77 µg/mm$^3$ and SL of -0.43 µg/mm$^3$ after 8 months compared to 7.0 µg/mm$^3$ and -2.8 µg/mm$^3$ after one week, respectively. This showed an increase in sorption with an increasing period of storage allowing more water diffusion to take place (Lauvahutanon et al., 2017). Solubility values of EN (-0.45 µg/mm$^3$ in water and -0.47 µg/mm$^3$ in artificial saliva) were comparable to those given in a previous study (around -1.43 µg/mm$^3$ in water and -1.7 µg/mm$^3$ in artificial saliva, respectively) measured after six months storage (Liebermann et al., 2016).
The sorption values were negatively correlated to the amount of filler loading, except for DK, which has a very low filler weight combined with very low water sorption. As the filler percentage increased, the polymer matrix percentage decreased, which as the polymer is the main factor in water absorption means less water diffusion into the material (Alshali et al., 2015b; Sideridou et al., 2003). Other factors, such as the storage medium and storage period, can also influence the degree of sorption and solubility. Storage in ethanol, for example, has a significant influence on the degree of sorption and solubility compared to water-based storage (Ferracane, 1994; Zhang & Xu, 2008).

The negative solubility values for EN, GR, BC, and CS do not necessarily imply that these materials are insoluble, as it could be the result of incomplete dehydration. Another explanation might be the interaction between the glass fillers, metal oxides and the storage media (Ortengren et al., 2001).

In the present study, sorption (SP) and solubility (SL) values in water and artificial saliva were positively correlated (Pearson correlation coefficient for SP were; \( r=0.998, p=0.0001 \) and for SL were; \( r=0.80, p=0.018 \)); indicating that the two storage media had a similar effect, in line with previous studies (Alshali et al., 2015b; Liebermann et al., 2016; Sideridou et al., 2011). However, material differences exist, for example, LU exhibits a high solubility value in AS but much lower in water which might be due to the interaction between the components of AS with the resin-matrix, which does not take place in water.

Water and water-based artificial saliva were used at 37 °C, simulating intraoral fluids and temperature. The artificial saliva formula used in the study had acidity (pH) and viscosity comparable to that of human saliva, which may influence the level of water sorption into the materials under test relative to distilled water (Darvell, 1978; McKnight-Hanes & Whitford, 1992). In addition, the pH of the storage medium was checked at each measurement (time point) and adjusted in order to avoid the possible effect of pH on the study results (Örtengren et al., 2004). The study was limited to eight months, as this was the period during which most of the materials reached equilibrium, with no further mass change. Due to clinical indications and the shape and size of the CAD/CAM blocks, the specimen dimensions (14 × 12 × 3 mm) employed in the study for resin-composite differed from those specified in ISO FDIS 4049:2009 (ISO FDIS 4049, 2009).
6.6 Conclusions

Within the limitations of the study, the following can be concluded:

1. CAD/CAM composite blocks were reasonably hydrolytically stable in long-term storage, although not as stable as ceramic, with DK exhibited superior hydrolytic stability to CAD/CAM composite blocks.

2. The water sorption of CAD/CAM composite blocks was dependent on the resin-matrix composition and influenced by the filler weight percentage.

3. Water and artificial saliva showed a comparable effect on water sorption.
Chapter Seven:
Viscoelastic Stability of Pre-cured Resin-composite CAD/CAM Structures

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Viscoelastic stability of pre-cured resin-composite CAD/CAM structures

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ABSTRACT

Objectives. To study the effect of water storage (3 months) on the creep deformation and recovery of CAD/CAM composite materials to determine their viscoelastic stability.

Materials and methods. Five CAD/CAM composite blocks, with increasing filler loading, and one polymer-infiltrated ceramic network (PICN) were studied. Six specimens of each material were separated into two groups (n=3) according to their storage conditions (24 h dry storage at 23°C versus 3 months storage in 37°C distilled water). A constant static compressive stress of 20 MPa was applied on each specimen via a loading pin for 2 h followed by unloading and monitoring strain recovery for a further period of 2 h. The maximum creep strain (%) and permanent set (%) were recorded. Data were analysed via two-way ANOVA followed by one-way ANOVA and Bonferroni post hoc tests (p<0.05) for comparisons between the materials. Homogeneity of variance was calculated via Levene’s statistics.

Results. The maximum creep strain after 24 h dry ranged from 0.45% to 1.06% and increased after 3-month storage in distilled water to between 0.71% and 1.85%. The permanent set after 24 h dry storage ranged from 0.033% to 0.15% and increased after 3-month water storage to between 0.087% and 0.18%. The maximum creep strain also reduced with increasing filler loading.

Significance. The PICN material exhibited superior dimensional stability to all the pre-cured resin composite blocks in both storage conditions with deformation being predominantly elastic rather than viscoelastic. Notwithstanding, two of the resin-matrix composite blocks approached the PICN performance, when dry, but less so after water storage.

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1. Introduction

Resin-based materials designed for use with computer aided design/computer aided manufacturing (CAD/CAM) systems were first introduced as filled or unfilled polymethylmethacrylate (PMMA) with modified polymer networks [1]. New and improved resin materials have been subsequently developed utilising various matrix and filler constituents [2]. New formulations of CAD/CAM materials aim to provide an enhanced combination of resin-based materials and ceramics utilising their advantageous properties such as durability, strength and colour stability of ceramics, and improved flexural and fatigue properties and low abrasiveness of resins [3–7].

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CAD/CAM resin composites are typically composed of polymer matrices with a high volume fraction of different ceramic fillers such as porcelain, glass, ceramics, and glass-ceramics. CAD/CAM resin composites are considered as ceramic-like materials in a new classification proposed by Graci, et al. [8]. However, CAD/CAM composites can be classified based on their micro-structural geometry into two main types: resins with dispersed fillers and polymer infiltrated ceramic networks [PICN] [9].

The main difference between the two is the incorporation method. Simple mixing is used to formulate a composite with dispersed inorganic filler particles, where the matrix resin is formed from photocurable monomers [10]. However, for PICN, a network between ceramic and polymer is created in two steps: first, a porous pre-sintered ceramic network is fabricated and conditioned by a coupling agent. Then this ceramic network is infiltrated with a polymeric resin [11,12]. This manufacturing technique results in a three-dimensional or a double network hybrid, which enhances the stress distribution and promotes resistance to the breakdown of the material [13,14].

Crep can be defined as the strain generated within a material in response to a load application [15]. Depending on the form of the load, the creep can be static (constant load application) or dynamic (cyclic load application) [16]. Static and dynamic creep can be correlated, as reported by some studies [15-18]. The viscoelastic behavior (creep) of resin composites is influenced by the filler microstructure (volume percent, size and distribution) and the resin matrix composition [15,19]. As a property, it has been studied extensively for dental restorative materials, including amalgam and resin-composites. However, this is the first study of the creep behavior of CAD/CAM composite blocks.

This study aimed to assess the viscoelastic creep and recovery of different CAD/CAM blocks under compressive loading conditions. The null hypotheses for the investigated materials were that: (1) there is no difference in the creep behavior between the materials; (2) there is no effect of water storage (3 months) on the creep deformation of CAD/CAM composite materials and (3) the creep behavior will not be affected by their composition (filler weight percentage).

2. Materials and methods

Five CAD/CAM composite blocks and one polymer infiltrated ceramic network (PICN) were investigated. The details of manufacturers’ compositional information and experimentally determined filler weight percentages are given in Table 1. In total 36 cylindrical specimens were prepared: CAD/CAM blocks were sectioned and shaped into cylinders of 4 mm diameter and 6 mm height using a diamond blade (MK 303, MK diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Cutter, Buehler Co., IL, USA) under constant water irrigation. All specimens were wet-ground and polished with silicon carbide papers (SiC) P300, P1200 (Buehler Co, Illinois, USA). For each material, the specimens were divided into two groups (three specimens in each group, n = 3) for storage, as follows: Group one: 24 h dry at 23 °C, Group two: 3 months in 37 ± 1 °C distilled water.

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Flexure strength (MPa)</th>
<th>Creep 24 h (μm)</th>
<th>Creep 3 mo (μm)</th>
</tr>
</thead>
</table>
The static creep deformation of the CAD/CAM composite blocks was measured using a creep apparatus as described previously [20]. A constant compressive stress of 20 MPa was applied on each specimen via a loading pin for 2 h followed by load removal for a further measurement period of 2 h (total: 4 h). The creep strain and recovery were monitored continuously in real time using a LVDT transducer system [16,20]. The maximum creep strain (%), and permanent set (%) were obtained after loading and recovery respectively. Maximum creep strain, maximum creep recovery, percentage creep recovery and permanent set were obtained from the creep/time plots.

Data were analysed using statistical software (SPSS ver. 23, SPSS Inc., Illinois, USA). Homogeneity of variance was calculated by Levene’s statistics. Two-way ANOVA (2 factors; material and storage) followed by one-way ANOVA and the Bonferroni post hoc tests were used for comparisons between the materials for each group. Independent sample t-test was used for the difference between the two storage groups for each individual material. All tests were conducted at a significance level of $\alpha = 0.05$.

3. Results

The results are presented in Figs. 1–11 and Table 2. Figs. 1–6 show the creep and recovery in real time after 24 h of dry storage and after three months of water storage for the six CAD/CAM blocks.

There was a statistically significant difference in creep behaviour between the investigated materials. The maximum creep strain after 24 h storage ranged from 0.45% to 1.09% and increased after 3 m storage in distilled water to between 0.71% and 1.85%. The permanent set after 24 h storage ranged from 0.03% to 0.15% and increased after 3 m storage in distilled water to between 0.087% and 0.18%. The percentage creep recovery after 24 h storage ranged from 85.01% to 96.0% and slightly reduced after 3 m storage in distilled water to between 83.5% and 94.05%. Figs. 7–10 show maximum creep strain, permanent set, maximum creep recovery, and percentage creep recovery of the two groups (24 h dry and 3 m water storage) of the CAD/CAM composite blocks. Increased filler loading
**Table 2 - Maximum creep strain (%), permanent set (%), maximum creep recovery (%), and maximum recovery percentage after 24 h dry and three months water storage.**

<table>
<thead>
<tr>
<th>Storage</th>
<th>Max. creep strain (%)</th>
<th>Permanent set (%)</th>
<th>Max. creep recovery (%)</th>
<th>Percentage of creep recovery</th>
<th>Max. creep strain (%)</th>
<th>Permanent set (%)</th>
<th>Max. creep recovery (%)</th>
<th>Percentage of creep recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h dry storage at 23°C</td>
<td>FCN (EN)</td>
<td>0.44% (0.07)</td>
<td>0.96% (0.11)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>GR (GR)</td>
<td>0.50% (0.04)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>LU (LU)</td>
<td>0.72% (0.08)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>CAD CAM blocks</td>
<td>0.55% (0.08)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>BC (BC)</td>
<td>0.50% (0.06)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>CI (CI)</td>
<td>0.90% (0.04)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>HC (HC)</td>
<td>1.09% (0.09)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td>Three month water storage at 37°C</td>
<td>FCN (EN)</td>
<td>0.44% (0.07)</td>
<td>0.96% (0.11)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
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<tr>
<td></td>
<td>GR (GR)</td>
<td>0.50% (0.04)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>LU (LU)</td>
<td>0.72% (0.08)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>CAD CAM blocks</td>
<td>0.55% (0.08)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
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<tr>
<td></td>
<td>BC (BC)</td>
<td>0.50% (0.06)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
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<tr>
<td></td>
<td>CI (CI)</td>
<td>0.90% (0.04)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
<tr>
<td></td>
<td>HC (HC)</td>
<td>1.09% (0.09)</td>
<td>0.96% (0.03)</td>
<td>0.16% (0.06)</td>
<td>0.36% (0.06)</td>
<td>0.71% (0.15)</td>
<td>0.71% (0.07)</td>
<td>0.82% (0.04)</td>
</tr>
</tbody>
</table>

Vitalumatic (EN), Gradio Bloos (GR), Lava™ Ultimate (LU), Brilliant Crux (BC), Cerasmart (CI), Block HC (HC).

Values with the same small superscript letters represent non-significant difference among different materials (Kruskall-Wallis post hoc tests (p < 0.05)). Values with the same capital superscript letters represent non-significant difference among different behaviour for each material, independent sample t test.

ONE WAY significance: max. creep strain (%) at 24 h: p < 0.05; permanent set (%): p = 0.1; max. creep recovery (%): p < 0.05; percent creep recovery: p = 0.1. *Max. creep strain (%) at 3 m: p < 0.05; permanent set (%): p = 0.7; max. creep recovery (%): p < 0.05; percent creep recovery: p = 0.7.
Fig. 8 – Permanent set (%) of the two groups (24 h dry and 3 m water storage) of CAD/CAM composite blocks.

Fig. 9 – Maximum creep recovery (%) of the two groups (24 h dry and 3 m water storage) of CAD/CAM composite blocks.

Fig. 10 – Percentage of creep recovery of the two groups (24 h dry and 3 m water storage) of CAD/CAM composite blocks.

Fig. 11 – A scatter plot showing a negative correlation and linear regression between filler weight percentage (the filler weight percentage were as determined experimentally, rather than the manufacturer’s figure) and maximum strain percentage after 24 h dry storage $R^2 = 0.74$, $p = 0.02$ and 3 m water storage $R^2 = 0.96$, $p = 0.0007$.

$F (5,24) = 6.178$, $p = 0.0008$. A significant effect of storage and material type ($p < 0.0001$) was found. Simple main effects analysis showed that material effect was significantly more in 3 m water storage than the material effect in 24 h dry storage ($p < 0.03$).

4. Discussion

CAD/CAM resin composites have some improved mechanical properties [21] compared to conventional indirect composites. They have better wear resistance [22,23], flexural strength [24], fracture toughness, and fracture strength [3,11]. This can be attributed to the higher degree of conversion of CAD/CAM composites [25,26] as they are polymerised under high pressure and high temperature [27,28]. This results in higher composite homogeneity and reliability with fewer flaws and poros compared to conventional indirect composites [27,29] that enables incorporation of higher filler content [30]. Further, when compared to all-ceramic materials, CAD/CAM composites are less hard and less stiff, so the opposing enamel exhibits less wear clinically. Also, they are easily fabricated and repaired [31].

This study investigated five CAD/CAM composite blocks and one polymer infiltrated ceramic network (PICN) with different filler loadings and resin matrices. There was a statistically significant difference in the creep/recovery behaviour between the investigated materials. Thus the first null hypothesis was rejected.

In this study, the specimens of Group two; stored for 3 months in 37 ± 1°C distilled water in the incubator, exhibited higher creep strain and recovery than those stored dry for 24 h at 23°C. A significant effect of water storage and material type ($p < 0.0001$) was found. Therefore the second null hypothesis was rejected. Storage in water can lead to a reduction in material stiffness due to plasticization of the polymer matrix [32,33]. Water sorption increased creep and reduced
creep recovery of the composite materials. This corresponds to some previous studies [17,34–36]. For the purpose of correlation, plots with filler loading (w/w), from previously published data on filler content were utilized [36]. There was a negative correlation between maximum creep percentage and filler weight percentage. However, this correlation was more statistically significant for Group two: water storage for 3 m (R² = 0.96, p = 0.0007) compared to Group one: dry storage for 24 h (R² = 0.74, p = 0.02). Therefore, the third null hypothesis was rejected.

The PICN material (EN) showed higher creep resistance, attributable to the robust ceramic-matrix microstructural geometry of PICN as compared to other CAD/CAM composite blocks with resin incorporating dispersed ceramic fillers. However, both EN, and GR had comparable creep resistance and permanent set under both dry and wet conditions. HC with the lowest filler content (63% w/w) showed the highest creep strain followed by CS (66% w/w) and BC (70% w/w). LU (75% w/w) had a middle-ranking creep strain.

These findings are in agreement with a Hertzian indentation study of two experimental PICN materials with different filler loadings compared to ceramics. The PICN with lower filler incorporation had greater indentations. Both PICN materials had greater indentations than that of investigated ceramics [37].

All investigated materials exhibited minimal permanent set and high recovery. Both characteristics were not significantly different between the investigated materials, reflecting the material resistance to any permanent change. The maximum permanent set obtained after 3 months of water storage was 0.18 % by BC, which has (70% w/w) but it was not significantly different from all other investigated materials.

The resistance to creep and the recovery were generally higher than that of conventional composites. They also exhibited a lower permanent set. A recent study on bulk fill composites with immediate measurement after immersion in different media using 3-point bending showed higher permanent set especially in water up to 1.14%, and lower recovery ranging from 45 to 64% [38]. Many studies showed that conventional composites, particularly some bulk fill types with as high a filler loading as our investigated materials, exhibit higher creep strain even in shorter-term wet storage [18,35,38].

In this study, the deformation was predominantly elastic rather than viscoelastic as expected in conventional composites attributable to the higher degree of conversion and improved mechanical properties of CAD/CAM composite blocks.

Maximum creep recovery depends upon the level of maximum creep strain; hence both properties have a similar trend in terms of highest to lowest values. Creep can range from 1-6% depending on the filler content [20,39]. All investigated materials showed less than 1% creep under dry conditions and less than 2% after wet storage for 3 months. Compressive creep resistance reflects the materials viscoelastic stability and its resistance to catastrophic failure under loading [16,40]. The applied stress in this study (20 MPa) was similar to the average in-vivo bite force [41] and corresponds with the maximum occlusal force intraorally during occlusion [42].

5. Conclusions

- The PICN material exhibited superior dimensional stability to all of the precured resin composite blocks with deformation being predominantly elastic rather than viscoelastic.
- Creep deformation and maximum recovery demonstrated lower viscoelastic stability of pre-cured CAD/CAM composite blocks upon water storage.
- Pre-cured CAD/CAM composite showed better viscoelastic stability compared to conventional direct or indirect resin composites.

Data availability

The data used to support the findings of this study are included within the article.

REFERENCES

Chapter Eight:
Analysis of Long-Term Monomer Elution from CAD/CAM Composite Blocks Using HPLC

R. Alamoush, R. Sung, J. D. Satterthwaite, & N. Silikas
8.1 Abstract

Objectives: This study aimed to assess monomer elution from CAD/CAM composite blocks compared to conventional resin-composite using high performance liquid chromatography (HPLC) after storage in different media for 3 months.

Materials and methods: Eleven materials with different filler loadings were investigated: 7 CAD/CAM blocks and 4 conventional resin-composites. One hundred and sixty-five specimens were prepared comprising 15 of each of the materials, and divided into three groups (n=5) immersed in 3 ml of either water, artificial saliva or 75% ethanol: water (E/W) solution and stored at 37°C for 1 and 3 months. Monomer release in the storage media was quantified by HPLC. The data were analysed with two-way ANOVA, one-way ANOVA, Tukey’s post hoc test and the independent t-test (α=0.05 for all tests).

Results: All materials, except GR (resin-composite block) and DK (Polyetheretherketone (PEEK)), showed a variable extent of monomer elution into 75% E/W with significantly higher amounts eluted from conventional composites. GRA and GND (conventional resin-composites) eluted TEGDMA in artificial saliva and GRA eluted TEGDMA in water.

Conclusions: Minimal or no monomer elution from CAD/CAM blocks was detected. Both artificial saliva and water were similar in terms of a non-influencing effect on monomer elution, except for GRA and GND. DK exhibited high compatibility with 75% E/W despite its high polymer content.

Keywords: CAD/CAM composite blocks, Polymer-infiltrated ceramic network, Monomer elution, HPLC, Storage media.
8.2 Introduction

Alternative materials for aesthetic dental restorative materials have been introduced along with the increased use of CAD/CAM technology; the two main materials used for CAD/CAM processed aesthetic restorations are ceramic and resin-composite (Fasbinder, 2010; Giordano, 2006; Ruse & Sadoun, 2014). Ceramic is more biocompatible, stronger and better aesthetically than resin-composite (Barizon et al., 2014). However, it is a very stiff, hard, and brittle material, which affects its clinical performance, durability, and machinability (Lee et al., 2014). Resin-composite shows less hardness and stiffness; however, it is more susceptible to the degradation of components over time (Ferracane, 1994; Gupta et al., 2012; Van Landuyt et al., 2011). Composite blocks are classified as polymer-infiltrated ceramic network (PICN), which is a ceramic network that is infiltrated with a monomer, or resin-composite blocks (RCB), which is resin-composite polymerised under high pressure and/or high temperature (Lambert et al., 2017; Phan et al., 2014; Tang et al., 2014). PEEK is considered a promising material for many dental applications for use with CAD/CAM systems (Mehta et al., 2019; Schwitalla et al., 2015; Stawarczyk et al., 2013c; 2015).

Resin-composites might release low molecular weight monomers such as HEMA and TEGDMA, high molecular weight monomers such as Bis-GMA and UDMA, free radicals, and photoinitiator molecules (Kingman et al., 2012; Leprince et al., 2013; Van Landuyt et al., 2011). Monomer elution can compromise the material biocompatibility, as well as mechanical properties (Bakopoulou et al., 2009; Krifka et al., 2013; Leprince et al., 2013). Many factors influence monomer elution from resin-composite, including the degree of conversion, the solvent type, the chemical structure of the eluted molecules (Ferracane, 1994), the filler composition and the microstructure (Polydorou et al., 2009).
CAD/CAM composite blocks have a higher degree of conversion and less residual monomer, than conventional resin-composites, due to high temperature and high pressure polymerisation (Bagis & Rueggeberg, 2000; Phan et al., 2014). UDMA, is the main monomer used in CAD/CAM composite blocks, and less monomer release has been reported in UDMA-containing resin-composite (Goncalves et al., 2010; Phan et al., 2014). Moreover, CAD/CAM composite blocks are more resistant to breakdown and components leaching out due to their increased hardness compared to conventional composites (Alamoush et al., 2018; Coldea et al., 2015; Swain et al., 2016; Van Landuyt et al., 2011).

There is a paucity of research regarding monomer elution of the newly introduced CAD/CAM composite materials, particularly in simulated oral conditions (ageing). One study evaluated the monomer elution of some CAD/CAM composite blocks for up to 2 months in ethanol and distilled water (Mourouzis et al., 2019).

This study aimed to assess monomer elution from CAD/CAM composite blocks compared to conventional indirect and direct resin-composites using high performance liquid chromatography (HPLC) after storage in different media for 1 and 3 months. The null hypotheses are 1) there will be no difference in the monomer elution quantity between the CAD/CAM composite blocks and conventional resin-composites, 2) there will be no difference in the monomer elution quantity between two time points (1 month and 3 months), and 3) there will be no difference in eluted monomers between the different storage media.

For simplicity, the term CAD/CAM composite block describes PICN and RCB (resin-composite designed for CAD/CAM systems); otherwise, each will be referred to as PICN or RCB.
8.3 Materials and Methods

8.3.1 Materials

Seven CAD/CAM blocks and 4 conventional resin-composite materials, 2 indirect and 2 direct, were investigated. A list of materials investigated, with the manufacturers’ compositional information, is given in Table 8-1. All solvents were HPLC grade. Water, methanol, ethanol, acetonitrile, caffeine, and ethoxylated Bis-phenol A dimethacrylate (Bis-EMA) were from Sigma-Aldrich (UK). Bis-phenol A glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and urethane dimethacrylate (UDMA) were supplied by Röhm GmbH (Germany).

8.3.2 Specimen preparation

Each CAD/CAM block was sectioned into 10×10×3 mm dimensions using a diamond blade (MK 303, MK Diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Saw, Buehler Co, IL, USA) under constant water irrigation. Conventional resin-composite materials were prepared of 10×10×3 mm dimensions using a polytetrafluoroethylene (PTFE) mould, and they were cured according to their manufacturers recommendations using a LED light-curing unit with an output irradiance of 1200 mW/cm² (Elipar™, 3M ESPE, USA). All specimens were wet ground and polished with a series of silicon carbide papers (SiC) (P320, P500, P1200, P2400, and P4000 grit (Buehler Co, IL, USA)) under water cooling and then polished with 0.25 µm diamond suspension (Meta Di Supreme, Buehler Co, IL, USA) using a lapping machine (MetaServ 250, Buehler Co, IL, USA). Finally, the specimens were cleaned in an ultrasonic bath (Ultrasonic Cleaning System, L&R Co, NJ, USA) with distilled water for 5 min. Each specimen had a surface area of 320 mm² and a volume of 300 mm³. One hundred and sixty-five specimens were prepared, comprising 15 of each material, and divided into three groups (n=5) immersed in 3 ml of either water, artificial saliva or 75% E/W solution and stored at 37°C for 1 and 3 months.
Table 8-1: A list of materials investigated with manufacturers’ compositional information.

<table>
<thead>
<tr>
<th>Materials (Code)</th>
<th>Composition by weight</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polymer- infiltrated ceramic network (PICN)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VitaEnamic (EN)</td>
<td>Filler: 86% fine structure feldspar ceramic, Polymer: 14% UDMA+TEGDMA</td>
<td>Vita Zahnfabrik, Germany</td>
</tr>
<tr>
<td>Grandio Blocs (GR)</td>
<td>Filler: 86% nanohybrid fillers, Polymer: 14% UDMA+DMA</td>
<td>VOCO GmbH, Germany</td>
</tr>
<tr>
<td><strong>Resin-composite blocks (RCB)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lava™-Ultimate (LU)</td>
<td>Filler: 80% silica and zirconia nanoparticles, Polymer: 20% Bis-GMA, UDMA, Bis-EMA, TEGDMA</td>
<td>3M™ESPE™, USA</td>
</tr>
<tr>
<td>Cerasmart (CS)</td>
<td>Filler: 71% silica and barium glass nanoparticles, Polymer: Bis-HEPP, UDMA, DMA</td>
<td>GC dental products, Europe</td>
</tr>
<tr>
<td><strong>Ceramic-filled PEEK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dentokeep (DK)</td>
<td>Filler: 20% TiO₂, Polymer: 80% PEEK</td>
<td>NT-Trading, Germany</td>
</tr>
<tr>
<td><strong>Conventional resin-composites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Indirect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceramage (CMG)</td>
<td>Filler: 73% zirconium silicate micro fine ceramic particles, Polymer: UDMA, UDA</td>
<td>Shofu, Japan</td>
</tr>
<tr>
<td>Gradia Plus (GRA)</td>
<td>Filler: nanohybrid Silica-based, Polymer: UDMA and other DMA</td>
<td>GC dental products, Europe</td>
</tr>
<tr>
<td><strong>Direct</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GrandioSO (GND)</td>
<td>Filler: 89% glass ceramic and silica-nanoparticles, Polymer: Bis-GMA, Bis-EMA, TEGDMA</td>
<td>VOCO GmbH, Germany</td>
</tr>
<tr>
<td>Tetric EvoCeram (TET)</td>
<td>Filler: 79-81% nanohybrid fillers, Polymer: 20-21% UDMA+TEGDMA + Bis-GMA</td>
<td>Ivoclar Vivadent</td>
</tr>
</tbody>
</table>
Caffeine (CF) was used as an internal standard, and 0.1 mg/ml was added to all storage media. Artificial saliva was prepared using the Macknight-Hane and Whitford formula (McKnight-Hanes & Whitford, 1992). The monomers of interest (Bis-GMA, UDMA, TEGDMA, Bis-EMA) were dissolved in methanol with CF as an internal standard at different concentrations and were assessed by HPLC to identify each eluted monomer retention time and to obtain a calibration curve to quantify each monomer. In addition, control samples of only storage media were assessed by HPLC. The specimens were stored in the dark at 37°C, and after 1 month, all storage solutions were collected for analysis and replaced with fresh solutions. After 3 months, the storage solutions were collected again for analysis and the total eluted monomer quantity in 3 months was calculated by adding the values obtained at the 2 time points.

8.3.3 Analysis of eluted monomers

The collected solutions at each time point were placed in HPLC vials and assessed by HPLC (Agilent 1100 series, Agilent Technology, Germany) to identify and quantify the eluted monomers using the calibration curves and retention times of the monomers of interest. Chromatography was performed using a reverse-phase column with isocratic separation. HPLC samples of 1 µL were injected into Phenomenex SphereClone 5 µm ODS (2) column of dimensions 4.6x250 mm (Phenomenex, USA). Chromatographic separation was achieved using a mixture of acetonitrile and water (65%:35%) at a flow rate of 0.5 ml/min. The column temperature was set at 22°C with a run time of 70 min for each sample, and the UV detector was set at 205 and 210 nm. Figure 8-1 illustrates an HPLC chromatogram of the detected monomers (Bis-GMA, UDMA, TEGDMA) and their retention times.
Figure 8-1: HPLC chromatogram of the internal standard (CF), the main detected monomers peaks (TEGDMA, UDMA, Bis-GMA) and their retention times, with CF at 4.9 min, TEGDMA at 8.99 min, UDMA at 12 min, and Bis-GMA at 14.3 min.

The calibration curve was obtained by plotting the HPLC peak area of monomer to peak area of CF against the concentration of monomer to the concentration of CF, of the calibration solutions as follows:

\[
\left( \frac{\text{Monomer peak area}}{\text{CF peak area}} \right) \text{ versus } \left( \frac{\text{Monomer concentration}}{\text{CF concentration}} \right)
\]  

(1)

Then, a linear regression analysis of the plotted ratio was carried out. The linearity, slope (b), intercept (a), calibration range and the detection range are shown in Table 8-2. The following equation was used to calculate the eluted monomer concentration (µg/ml):

Eluted monomer concentration (µg/ml)

\[
= \left\{ \left( \frac{\text{Monomer peak area}}{\text{CF peak area}} + \frac{\text{CF peak area}}{\text{CF concentration}} \right) - a \right\} \times \text{CF concentration}
\]  

(2)

Where the intercept (a), the slope (b) resulting from calibration curves, monomer and CF peak areas were obtained from HPLC, and the CF concentration (µg/ml) is known as an internal standard (100 µg/ml).

The eluted monomer amount (nmol/mm²) was calculated using the following equation (Van Landuyt et al., 2011):

Eluted monomer amounts (nmol/mm²)

\[
= \text{conc} \times \text{solvent volume} \times \left( \frac{1}{Mm} \right) \times \left( \frac{1}{\text{sample surface area}} \right)
\]  

(3)

Where conc is the eluted monomer concentration in µg/ml units, solvent volume is in ml, Mm is the molecular mass in nmol/µg, and the sample surface area is in mm².
Table 8-2: The calibration curves data and the monomers detection range (nmol/mm²).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity (R²)</th>
<th>Slope (b)</th>
<th>Intercept (a)</th>
<th>Calibration range (μg/ml)</th>
<th>Detection range (nmol/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDMA</td>
<td>0.999</td>
<td>0.536</td>
<td>0.099</td>
<td>10-1200</td>
<td>0.29-34.82</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>0.995</td>
<td>0.355</td>
<td>0.059</td>
<td>10-1200</td>
<td>0.477-57.22</td>
</tr>
<tr>
<td>Bis-GMA</td>
<td>0.986</td>
<td>1.17</td>
<td>0.45</td>
<td>10-1200</td>
<td>0.266-31.96</td>
</tr>
<tr>
<td>Bis-EMA</td>
<td>0.996</td>
<td>0.67</td>
<td>0.19</td>
<td>10-1200</td>
<td>0.302-36.2</td>
</tr>
</tbody>
</table>

8.3.4 Statistical analysis

The data were analysed using statistical software (SPSS ver. 23, IBM, IL, USA). A Shapiro–Wilk test was performed to test normality, while the homogeneity of variance was calculated by Levene's statistics. Two-way ANOVA (2 factors: material and storage time) followed by one-way ANOVA and Tukey’s post hoc tests were used for comparisons between materials for each monomer at each time point. An independent t-test was performed for differences in-between the two time points (1 month, 3 months) of the monomer elution for each material. All tests were carried out at α=0.05.

8.4 Results

The eluted monomers were TEGDMA, UDMA, and Bis-GMA. Two-way ANOVA of the eluted monomer amounts for all investigated materials was performed to assess the material and storage time effect, and their interaction. There was statistically significant time and material effect on the elution amounts of both TEGDMA and UDMA, with a significant interaction between the investigated materials and the storage time (p<0.005). There was a significant material effect with a significant interaction between the investigated material and the storage time (p<0.005), and a non-significant time effect on the elution of Bis-GMA (p=0.09).

Most of the investigated materials eluted monomers in 75% E/W with conventional resin-composites showed a significantly higher amounts of eluted monomers. Table 8-3, figures (8-2, 8-3, and 8-4) show the eluted monomer amounts by the investigated materials in 75% E/W. Only TEGDMA was eluted in artificial saliva by GRA, where the total after 3 months was 2.72 (0.38) nmol/mm², while for GND the total was 1.85 (0.5) nmol/mm². Only GRA eluted TEGDMA in water after 1 month (0.47 (0.3) nmol/mm²),
Table 8-4. CMG eluted unknown monomer (possibly UDMA and HEMA; see appendix-2) in 75% E/W (17.95 (0.65) nmol/mm²) (see Table 8-4).

The monomer elution amounts in 75% E/W was in the following order from highest to lowest: UDMA>Bis-GMA>TEGDMA. Only TEGDMA was eluted in water and artificial saliva. Although three of the investigated materials contained Bis-EMA, it was not detected. The highest eluted UDMA amount was found in CMG, while the highest eluted TEGDMA amount was found in GRA, with both being indirect resin-composite materials. The highest eluted Bis-GMA amount was released from TET, a direct bulk-fill resin-composite material.

For the CAD/CAM composite blocks, GR showed no detectable monomer elution. The least detected monomer amounts were found in BC, which only eluted Bis-GMA (0.06 (0.02) nmol/mm²) at 1 month and none at 3 months, followed by EN, which only eluted UDMA (0.37 (0.17) nmol/mm²). CS also only eluted UDMA (0.73 (0.13) nmol/mm²) and LU only eluted Bis-GMA (0.84 (0.12) nmol/mm²). HC released both UDMA (1.17 (0.04) nmol/mm²) and TEGDMA (1.5 (0.07) nmol/mm²), which were the highest amounts released from the CAD/CAM composite blocks. However, there was no statistically significant difference of eluted monomers between all the investigated CAD/CAM composite blocks. DK (Ceramic-filled PEEK) also showed no detectable monomer elution.

The independent t-test showed a significant difference between the eluted monomer amounts at the two time points (1 month and 3 months) for most of the investigated materials with different eluted monomers. The monomer detected in the first time point (1 month) was significantly higher than the monomer detected at the second time point (3 months). LU eluted more TEGDMA in 3 months than in 1 month, and HC eluted more UDMA in 3 months than in 1 month, while GND eluted more Bis-GMA in 3 months than in 1 month. TET was the only material that did not show a significant difference in the eluted monomer amounts between the two time points. In addition, the unknown molecule eluted by CMG showed a non-significant difference between the two time points.
Table 8-3: The mean and standard deviation values of eluted monomers (nmol/mm²) at 1 month and 3 months, and the total amounts after 3 months storage in 75% E/W.

<table>
<thead>
<tr>
<th>Material</th>
<th>UDMA</th>
<th>TEGDMA</th>
<th>Bis-GMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1 month</td>
<td>1-3 months</td>
<td>Total</td>
</tr>
<tr>
<td>EN</td>
<td>0.25 (0.15) a,1</td>
<td>0.11 (0.02) a,1</td>
<td>0.37 (0.17) a</td>
</tr>
<tr>
<td>GR</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LU</td>
<td>ND</td>
<td>0.33 (0.007) a,2</td>
<td>0.51 (0.12) a,2</td>
</tr>
<tr>
<td>BC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CS</td>
<td>0.62 (0.1) a,1</td>
<td>0.11 (0.02) a,2</td>
<td>0.73 (0.13) a</td>
</tr>
<tr>
<td>HC</td>
<td>0.47 (0.018) a,1</td>
<td>0.7 (0.02) a,2</td>
<td>1.17 (0.04) a</td>
</tr>
<tr>
<td>DK</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CMG</td>
<td>7.7 (0.27) b,1</td>
<td>5.68 (0.25) b,2</td>
<td>13.4 (0.5) b</td>
</tr>
<tr>
<td>GRA</td>
<td>4.96 (0.5) c,1</td>
<td>2.07 (0.06) c,2</td>
<td>7.02 (0.58) c</td>
</tr>
<tr>
<td>GND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TET</td>
<td>7.13 (0.72) b,1</td>
<td>5 (1.5) h,1</td>
<td>12.13 (2.2) h</td>
</tr>
<tr>
<td></td>
<td>15.8 (1.49) c,1</td>
<td>13.1 (3.9) h,1</td>
<td>28.9 (5.4) c</td>
</tr>
</tbody>
</table>

Values with the same superscript letters/numbers represent a non-significant difference for each monomer elution between different materials (Tukey's post hoc test (α=0.05)) and a non-significant difference for each eluted monomer for each material between the 1 month and 3 month time points (independent t-test (α=0.05)). ND stands for not detected.

Table 8-4: The mean and standard deviation values of eluted monomers (nmol/mm²) at 1 month and 3 months, and the total amounts after 3 months storage in water, artificial saliva and 75% E/W.

<table>
<thead>
<tr>
<th>Storage media</th>
<th>Material</th>
<th>Monomer</th>
<th>Monomer elution amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 month</td>
</tr>
<tr>
<td>Water</td>
<td>GRA</td>
<td>TEGDMA</td>
<td>0.47 (0.3)</td>
</tr>
<tr>
<td>Artificial saliva</td>
<td>GND</td>
<td>TEGDMA</td>
<td>2.27 (0.3) b,1</td>
</tr>
<tr>
<td>GRA</td>
<td>TEGDMA</td>
<td>1.6 (0.4) b,1</td>
<td>0.25 (0.05) b,2</td>
</tr>
<tr>
<td>CMG</td>
<td>possibly UDMA in HEMA paste</td>
<td>8.93 (0.22) 1</td>
<td>9.02 (0.4) 1</td>
</tr>
</tbody>
</table>

Values with the same superscript letters/numbers represent a non-significant difference for each monomer elution between different materials (Tukey’s post hoc test (α=0.05)) and a non-significant difference for each eluted monomer for each material between the 1 month and 3 month time points (independent t-test (α=0.05)). ND stands for not detected.
Figure 8-2: A bar chart illustrating the mean values of eluted UDMA from the investigated materials after 3 months storage in 75% E/W. Error bars represent the standard deviation.

Figure 8-3: A bar chart illustrating the mean values of eluted TEGDMA from the investigated materials after 3 months storage in 75% E/W. Error bars represent the standard deviation.
Figure 8-4: A bar chart illustrating the mean values of eluted Bis-GMA from the investigated materials after 3 months storage in 75% E/W. Error bars represent the standard deviation.
8.5 Discussion

There was a significant difference in the eluted monomer amounts between CAD/CAM and the conventional resin-composite materials; therefore, the first null hypothesis was rejected. There was a significant difference between the eluted quantities of each monomer in the two points (1 month and 3 months), so the second null hypothesis was rejected. There was a significant difference in monomer elution in the three storage media used in this study; therefore, the third null hypothesis was rejected.

All the investigated materials expressed a variable extent of different monomer elution into 75% E/W, except for GR and DK. Only two of the investigated materials eluted monomer in artificial saliva and water, and this showed the significant effect of different solvents on monomer elution (Rathbun et al., 1991). Composite elutes more monomer in organic solvents than in water or water-based solutions (Van Landuyt et al., 2011). GR is a resin-composite block with a high filler content of 86% and no TEGDMA in the resin-matrix, and as mentioned earlier UDMA containing composite with no TEGDMA exhibits less monomer release (Phan et al., 2014). DK has an etheretherketone polymer matrix with 20% ceramic filler content, but it showed no monomer elution. DK belongs to the PEEK family, which has been reported to be very biocompatible owing to their uniquely stable chemical structure of an aryl ring connected with ketone and other groups (Williams, 2008a). Also, it is very compatible with many solvents, including ethanol, but one exception is concentrated sulfuric acid (Kurtz & Devine, 2007). All other resin-composite and PICN blocks eluted non-significantly different small monomer amounts. EN (PICN) and LU (RCB) have been investigated for monomer elution in the short term (7 days), and have not shown any detectable amounts of monomer (Hussain et al., 2017).

Of the conventional resin-composites, TET and GND eluted monomers were broadly in agreement with a previous study (Alshali et al., 2015a). However, due to the larger sample dimensions and higher ethanol concentration (75%), higher monomer amounts were detected in our study. TET eluted the highest amount of monomers followed by GRA, then CMG and GND. This could be attributed to the filler weight percentage and polymer matrix composition; GND has the highest filler weight percentage (89%) (Łagocka et al., 2018).
Only TEGDMA was released into the water and artificial saliva, and it was slightly higher in artificial saliva. Similar findings were noticed in other studies, where higher TEGDMA was released in artificial saliva than water (Alshali et al., 2015a; Moharamzadeh et al., 2007). This might be attributed to the small molecular size of TEGDMA, which makes it easily mobilised and eluted (Pelka et al., 1999).

Monomer amounts detected in the first time point (1 month) was significantly higher than the monomer amounts detected at the second time point (3 months). Most monomer elution usually occurs in the first few days, and then the material might reach equilibrium (Alshali et al., 2015a; Van Landuyt et al., 2011; Łagocka et al., 2018). For conventional resin-composite, uncured or unreacted monomers tend to be eluted in the first few days (Ferracane, 1994); however, monomer elution may continue in a small amount up to one year (Alshali et al., 2015a; Polydorou et al., 2007; 2009; 2012). According to ISO standards (ISO 10993-13), devices intended to be used for more than 30 days and tested in simulated conditions should be tested at 1 month, 3 months, 6 months, and 1 year (ISO 10993-13, 2010). Therefore, our study tested the monomer elution at 1 and 3 months, and since CAD/CAM blocks eluted minimal amounts at 3 months, the study did not extend beyond this time point. However, conventional resin-composites are expected to elute more monomer even after 3 months in 75% E/W, as they do not appear to reach equilibrium.

The monomer levels detected in this study in RCB and PICN are considered minimal and below the ED$_{50}$ cytotoxicity levels compared to the ones detected in the conventional composites, in line with a similar study (Mourouzis et al., 2019). The only exception was HC with the lowest filler content of all RCB (61 wt%), which released UDMA slightly above ED$_{50}$ cytotoxicity levels (Geurtsen et al., 1998). However, this does not necessarily exclude the cytotoxic effects of them, where EN and LU have shown a higher cytotoxic effect than conventional composites in a recent study (Hussain et al., 2017). The monomer levels detected from conventional resin-composites exceeded ED$_{50}$ cytotoxicity levels; the only exception was TEGDMA released from GRA in water (Geurtsen et al., 1998). No data on the recommended limits of monomer intake is available up to date (Putzeys et al., 2019), which might be ascribed to the differences in experimental settings of monomer elution studies such as sample size, solvent type and
volume, chemical analytical methods, storage time, and measurement units (Van Landuyt et al., 2011).

The 75% E/W solvent used in this study is considered as food and oral simulating fluid by the Food and Drug Administration (FDA) Guidelines of the United States (United States Food and Drug Administration, 1988), and has been used in many studies (Gul et al., 2014; Mazzaoui et al., 2002; Miletic et al., 2009; Ortengren et al., 2001; Tang et al., 2014; Tokay et al., 2015). Hence, it could simulate clinical conditions (Moon et al., 2000; Sideridou et al., 2007). In addition, 75% E/W has a similar solubility parameter to that of the resin-composite matrix. Hence, it could penetrate the resin-composite matrix, and consequently accelerate the ageing process of resin-composite materials (Manojlovic et al., 2013). In addition, the artificial saliva formula used in the study had similar acidity (pH) and viscosity to the human saliva (Darvell, 1978; McKnight-Hanes & Whitford, 1992).

Each specimen had a surface area of 320 mm$^2$. Previous work has suggested that the bottom surface of the sample that is in contact with the container surface is less exposed to the storage solution and hence contributes less to monomer elution than other surfaces (Van Landuyt et al., 2011). Therefore, 220 mm$^2$ was considered as the actual surface area exposed to the storage solutions, excluding the bottom surface that was in contact with the storage container floor and was less exposed to the solution. This is comparable to the surface area of the anterior or premolar crown restoration, which has been cited as a reference standard in a meta-analysis study on monomer elution of resin-composite (Van Landuyt et al., 2011). The ratio between the specimen mass and the storage medium volume was greater than 1:10 and the specimens were fully immersed in the medium, which is in line with the requirements of ISO 10993-13 (ISO 10993-13, 2010). The eluted monomer amounts were calculated in nmol/mm$^2$ as the mol, defined as the number of molecules, was considered as a better option than the mass (µg) to present the eluted monomer amounts. Moreover, the sample surface area is included, which better reflects clinical conditions in terms of the ability to calculate the eluted monomer amount using the restoration surface area (Van Landuyt et al., 2011). Even though a representative sample surface area was used, intraoral circumstances still differ from the in vitro ones. The continuous salivary flow and
enzymatic degradation and how the restorative material interacts with them are all factors that considerably affect monomer elution (Heil et al., 1996). In addition, equilibrium can be reached between the eluted molecules and the non-eluted ones in the solvent, which is not the case intraorally, where equilibrium is not expected due to the continuous salivary flow (Van Landuyt et al., 2011). Hence, it is expected that there will be more monomer elution in the *in vivo* long-term circumstances.

CAD/CAM composite blocks are likely to exhibit superior biocompatibility compared to conventional resin-composites due to limited monomer elution. This might be attributed to the higher degree of conversion (Gupta et al., 2012; Phan et al., 2014), using less toxic monomers and no photoinitiators in their manufacture (Krifka et al., 2013), and their resistance to breakdown and leach out of their components (Coldea et al., 2015; Swain et al., 2016; Van Landuyt et al., 2011).

HPLC is usually utilised to assess monomer release in dental research (Ortengren et al., 2001) as it is considered to be a highly sensitive and effective analysis method and it is also not very costly (Van Landuyt et al., 2011). However, other methods can be used to assess monomer release, such as gas chromatography, mass spectroscopy, and electrospray ionisation (Mazzaoui et al., 2002; Zhang & Xu, 2008).

**8.6 Conclusions**

Within the limitations of this study, the following can be concluded:

1- Minimal or no monomer elution from RCB, PICN and PEEK blocks was detected.

2- Both artificial saliva and water were similar in terms of having a non-influencing effect on monomer elution, except for GRA and GND.

3- DK (PEEK) exhibited high compatibility with 75% E/W despite its high polymer content.
Chapter Nine: Biocompatibility of CAD/CAM Composite Blocks

R. Alamoush, E. Kushner, J. M. Yates, J. D. Satterthwaite, & N. Silikas
9.1 Abstract

Aims and objectives: This study aimed to investigate the influence of CAD/CAM composite materials on human gingival fibroblasts (HGF) and gingival keratinocytes (HGK).

Materials: Four materials were investigated: two resin-composite blocks (RCB), Grandio Blocs (GR) and Block HC (HC); one polymer-infiltrated ceramic network (PICN) (Enamic, EN); and one conventional resin-composite, Grandioso (GND). HGF and HGK were cultured as per the supplier’s protocol (ATCC, UK). Cell proliferation and cytotoxicity were evaluated at 1, 3, 5 and 10 days using LDH and Alamar Blue assays. Indirect immunostaining was used to assess the Caspase-3 activity. The data were analysed with two-way ANOVA, one-way ANOVA and Tukey’s post hoc test ($\alpha=0.05$ for all tests).

Results: HGK showed the highest cell proliferation when cultured with GR blocks (100%, 3.0), while HGF showed the highest cell proliferation with GND composite (126%, 1.9). HGF cell proliferation was higher than HGK with almost all investigated materials and at all time points. Cytotoxicity was not significantly different between the investigated materials in either cell line. However, it was the highest on day 1, and lowest on day 5 for HGK, while it was the highest on day 10 and lowest on day 1 for HGF. No Caspase-3 activity was detected in either cell line.

Conclusions: All investigated materials influenced HGK proliferation and appeared to cause higher cell cytotoxicity than for HGF. Different manufacturing techniques of resin-composites had no significant effect on their biological properties. EN showed cytotoxic effects in HGK.

Keywords: Biocompatibility, Polymer-infiltrated ceramic network, Resin-composite blocks, Cell viability, Cytotoxicity.
9.2 Introduction

Biocompatibility of dental materials is crucial, especially for materials that are in direct contact with oral tissues such as dentine, the dental pulp and adjacent gingiva (Schmalz & Arenholt-Bindslev, 2009). Ceramic is more biocompatible compared to resin-composite (Barizon et al., 2014) as resin-composite components can be released due to incomplete polymerisation or degradation of components over time (Ferracane, 1994; Gupta et al., 2012; Van Landuyt et al., 2011).

Resin-composites designed for use with CAD/CAM systems are polymerised under high temperature and/or high pressure with high filler content and sometimes with innovative compositions (Awada & Nathanson, 2015; Nguyen et al., 2012; Ruse & Sadoun, 2014). In theory, these formulations of CAD/CAM composites should have superior biocompatibility compared to conventional resin-composites due to their higher degree of conversion (Gupta et al., 2012; Lin-Gibson et al., 2009) and lower levels of residual monomer (Lin-Gibson et al., 2009). Additionally, fewer potentially toxic monomers and no photoinitiators are used in the manufacture of CAD/CAM composite blocks (Gupta et al., 2012; Krifka et al., 2013). In addition, UDMA is the main monomer used in CAD/CAM composite rather than Bis-GMA. UDMA is considered less toxic than Bis-GMA as it is not synthesised from bisphenol A (BPA), and has been found to induce less cytotoxicity on human gingival and pulp fibroblasts in vitro (Gupta et al., 2012). Finally, CAD/CAM blocks exhibit high breakdown-resistance; therefore, the release of toxic components is less likely (Coldea et al., 2015; Swain et al., 2016; Van Landuyt et al., 2011).

CAD/CAM composite blocks can be classified into two main categories based on their microstructural geometry and fabrication method: resin-composite blocks (RCB), which are manufactured by incorporation of filler particles into a monomer mixture; and polymer-infiltrated ceramic networks (PICN) (Lambert et al., 2017; Mainjot et al., 2016). Production of PICNs is achieved in two stages: fabrication of a porous pre-sintered ceramic network followed by coupling agent conditioning, then polymer infiltration into this ceramic network takes place (Coldea et al., 2013a; Della Bona et al., 2014).
Human gingival keratinocytes (HGK) are considered the main cell population of the marginal keratinised gingiva (epithelial layer) of the oral mucosa, which is supported by the subepithelial connective tissues in which human gingival fibroblasts (HGF) reside. Both cell types play an essential role in the maintenance of soft tissue integrity and the oral wound healing and regeneration process (Pabst et al., 2014). CAD/CAM composite blocks used for a coronal restoration, whether on natural teeth or implants, will come into close contact with oral soft tissues including the keratinised marginal gingiva. Hence, adverse side effects such as inflammation, allergic reactions or tissue cytotoxicity could occur (Jerg et al., 2018; Nicholson & Czarnecka, 2008; Schulz et al., 2012; Urcan et al., 2010).

There is little research regarding the biocompatibility of the newly introduced CAD/CAM composite materials. Therefore, this study aimed to evaluate the biocompatibility of three manufacturing techniques (conventional resin-composite, resin-composite blocks and PICN) and their influences on HGF and HGK proliferation and cytotoxicity.

The null hypotheses were –

(1) There is no difference in cell proliferation or cytotoxicity between the investigated materials.

(2) Cell proliferation and cytotoxicity is not affected by exposure time and material type.

9.3 Materials and Methods

9.3.1 Samples preparation

Four materials were tested: two resin-composite blocks (RCB), Grandio Blocs (GR) and Block HC (HC); one polymer-infiltrated ceramic network (PICN), Enamic (EN); and one conventional resin-composite, Grandioso (GND), Table 9-1. Each CAD/CAM block was sectioned into 5×5×5 mm specimens using a diamond blade (MK 303, MK diamond, CA, USA) mounted on a saw (Isomet 1000 Precision Saw; Buehler Co, IL, USA) under constant water irrigation. Conventional resin-composite samples were prepared using a polytetrafluoroethylene (PTFE) mould, and were cured according to manufacturer recommendations using a LED light-curing unit with an output irradiance of 1200 mW/cm² (Elipar™, 3M ESPE, USA). In total, 96 specimens were prepared comprising 24 of each material divided into 12 for each cell line (HGK and HGF) of each material that
were divided into four samples (n=4) for each of three replicas. All experiments were performed using appropriate controls with biological and instrumental triplicates. All specimens were wet ground and polished with a lapping machine (MetaServ 250, Buehler Co, IL, USA) with a series of silicon carbide papers (SiC) P320, P500, P1200, P2400, and P4000 grit (Buehler Co, IL, USA) under water cooling. Specimens were then cleaned in an ultrasonic bath (Ultrasonic cleaning system, L&R Co, NJ, USA) with phosphate-buffered saline solution, rinsed four times with purified cell culture water and then ultrasonically cleaned in 80% ethanol. Finally, they were sterilized by UV exposure for 1 hour per side.

**Table 9-1: Materials investigated and manufacturers’ compositional information.**

<table>
<thead>
<tr>
<th>Materials (Code)</th>
<th>Composition by weight</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polymer- infiltrated ceramic network (PICN)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VitaEnamic (EN)</td>
<td>86% fine structure feldspar ceramic</td>
<td>14% UDMA+TEGDMA</td>
</tr>
<tr>
<td><strong>Resin- composite blocks (RCB)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grandio Blocs (GR)</td>
<td>86% nanohybrid fillers</td>
<td>14% UDMA+ DMA</td>
</tr>
<tr>
<td>Block HC (HC)</td>
<td>61% silica powder, microfumed silica and zirconium silicate</td>
<td>UDMA+TEGDMA</td>
</tr>
<tr>
<td><strong>Conventional resin-composite</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GrandioSO (GND)</td>
<td>89% glass ceramic and silica-nanoparticles</td>
<td>Bis-GMA, Bis-EMA, TEGDMA</td>
</tr>
</tbody>
</table>
9.3.2 Cell culture preparation

Commercially available cells were obtained and cultured according to a standard protocol for cell culture, maintenance, freezing and thawing. Two cell lines, primary human gingival fibroblast (Lot-201018) and primary human gingival keratinocytes (Lot-201014) (ATCC, VA, USA), were grown in their relevant growth media.

Human gingival fibroblasts (HGF) were cultured with fibroblast basal medium supplemented with fibroblast growth kit (Lot-804014). The final concentration for each component was as follows: L-glutamine: 7.5 mM; rh FGF beta: 5 ng/ml; rh insulin: 5 µg/ml; hydrocortisone: 1 µg/ml; ascorbic acid: 50 µg/ml; foetal bovine serum: 2%. Also, 1 % penicillin/streptomycin was added.

Human gingival keratinocytes (HGK) were grown in their relevant growth media: dermal cell basal medium and Keratinocyte growth kit (Lot-80923177). The final concentration for each component in complete keratinocyte growth medium was as follows: 0.4% bovine pituitary extract (BPE); 0.5 ng/ml rh TGA-alpha; 3 mM L-glutamine; 100 ng/ml hydrocortisone; 5 µ/l insulin; 1.0 µg/ml epinephrine; 5 µg/ml apo-transferrin with 1 % penicillin/streptomycin.

The cells were cultured in a T75 flask then kept in an incubator (5 % CO₂ and 95 % air) at 37°C. Then cells were passaged at regular periods based on their growth characteristics using 0.25% trypsin. Once the T75 flask was confluent, cells were detached and seeded into a 24-well culture plate, 5x10⁴ cells in 500 µl of complete growth medium. Once the cells attached, composite specimens were placed in the centre of each well signifying the start of the experiment according to ISO standard 10993-5 (ISO 10993-5, 2009).

9.3.3 Cell viability

Cellular viability of 100% was attributed to control wells, where cells were cultured with no composite blocks (LC or positive growth control). Cellular viability was quantified via a colorimetric assay using AlamarBlue™ cell viability reagent, DAL1100 (Thermo Fisher Scientific, IL, USA). At least one biological replica (24-well plate) was used for each assay at each time point (1, 3, 5 and 10 days).
HGF and HGK at each time point were exposed to AlamarBlue™ (1:10) for 1 hour at 37°C. Then 100 µl of supernatant was transferred into a 96-well plate for analysis at each time point. Cell viability was measured at the four-time points 1, 3, 5 and 10 days of cell growth. The 96-well plate was read with a UVM 340-microplate reader at 570 nm and 600 nm (ASYS, Scientific laboratory supplies). Cell viability was calculated according to equation (1) (Thermo Fisher Scientific, 2018):

\[
\text{Cell viability} \% = \frac{A_{570} - (A_{600} \times R_O) \text{ for test well}}{A_{570} - (A_{600} \times R_O) \text{ positive growth control}} \times 100\%
\]  

(1)

Where \(A_{570}\) and \(A_{600}\) are absorbance at 570 and 600 nm respectively and \(R_O\) is the correction factor calculated from \((A_{570}/A_{600})\) of the positive growth control.

**9.3.4 Cytotoxicity**

The cytotoxic potential of the tested materials was investigated using a Pierce™ LDH cytotoxicity assay kit, 88954 (Thermo Fisher Scientific, IL, USA). At least one biological replica (24-well plate) was used for each assay at each time point.

Cytotoxicity in HGF and HGK at each of the four time points (day 1, 3, 5 and 10) were measured using 50 µl of the supernatant and 50 µl of LDH cell reaction solution incubated for 30 minutes at room temperature in the dark. The reaction was stopped using the LDH “stop” solution. Appropriate controls were used as per the manufacturers’ protocol; maximum LDH release from the cells was set by adding membranolytic-particles, and was considered the positive (high) control, and the spontaneous LDH release control (water-treated) was considered the low control. The 96-well plate was read with a UVM 340-microplate reader at 490 nm subtracted from 680 nm (ASYS, Scientific laboratory supplies) and cytotoxicity was calculated according to equation (2) (Thermo Fisher Scientific, 2014):

\[
\text{Cytotoxicity} \% = \frac{\text{Specimen-treated LDH activity} - \text{Spontaneous LDH activity}}{\text{Maximum LDH activity} - \text{Spontaneous LDH activity}} \times 100\%
\]

(2)

Where Specimen-treated LDH activity is the LDH amount expressed by cells cultured with composite materials; maximum LDH activity, is the LDH amount expressed by cells treated with membranolytic-particles; and the spontaneous LDH activity is the LDH amount expressed by cells treated with water.
9.3.5 Cell morphology and Immunostaining

Cell morphology was evaluated using light microscopy imaging at days 1, 3, 5, 7, and 10, at ×10 magnification (Olympus IX51 inverted fluorescence microscope). Indirect immunostaining was used to assess the Caspase 3 activity. At the last time point, the growth medium was aspirated, and cell cultures in the plate were fixed using 4% paraformaldehyde (PFA) for 20 min and then permeabilization was done using 0.2% Triton-X for 30 min – both at room temperature. Blocking of Fc receptors (to prevent non-specific antibody binding) was done using normal goat serum with 0.1 BSA (Dilution factor 1:20) for 1 h at room temperature. Specimens were incubated with 1:250 primary antibody, mouse monoclonal Caspase 3 (Santa Cruz Biotechnology, Inc) at 4°C for 24 h. Secondary antibody 1:500, Alexafluor Goat anti-mouse (Life Technologies Corporation) was added after 24 h and kept in the dark for 1.5 h at room temperature. Cell nuclei were stained using DAPI (Vector Laboratories, CA, USA). Specimens were then observed using an Olympus IX51 fluorescence microscope connected to a digital camera at ×10 magnification (Olympus IX51 inverted fluorescence microscope).

9.3.6 Statistical analysis

Data were analysed using statistical software (SPSS ver. 23, IBM, IL, USA) and found to be normally distributed (Shapiro-Wilk’s test). Two-way ANOVA was performed for material effect, time effect and their interaction, followed by one-way ANOVA and Tukey’s multiple comparisons performed to compare cell viability and cytotoxicity for different materials at each time point (α=0.05 for all tests).
9.4 Results

HGK showed the highest cell proliferation when cultured with GR blocks at day 1 and day 10 with 100% (3.0) and 82.85% (3.3) proliferation percentage respectively, which was significantly different from HC and EN at day 1, and EN at day 10. At day 3, HGK exhibited high proliferation with all materials, but still lower than day 1, with the following order from lowest to highest: EN<GR<HC<GND. Notably, HGK exhibited the lowest proliferation rates with all materials at day 5, with the following order from lowest to highest: EN<HC<GR<GND. HGK exhibited high proliferation with all materials excluding EN on day 10. HGK (with EN) showed the lowest proliferation percentage at days 3, 5, and 10 with 73.8% (1), 52.7% (0.2), 53.8% (2.6) proliferation percentage respectively (Table 9-2 and Figure 9-1).

HGF showed the significantly highest cell proliferation when cultured with GR blocks on day 1 with a proliferation of 100% (2.0), and it exhibited high proliferation with all materials (>70%) with the following order from lowest to highest: HC<EN<GND<GR. Notably, HGF (with GR) and (with GND) exhibited their lowest proliferation at day 3, but they were still significantly higher than that with EN, with the following order from lowest to highest: EN<HC<GND<GR. HGF exhibited significantly different proliferation percentages between all materials at day 5, with the following order from lowest to highest: EN<HC<GND<GR. HGF (with GND) exhibited the significantly highest proliferation at day 10, 126% (1.9), which was the highest proliferation noted over all the time points of all the investigated materials in both cell lines. HGF with All materials exhibited a high cell proliferation percentage of more than 100% at day 10 (Table 9-2 and Figure 9-2).

HGF cell proliferation was higher than that of HGK in almost all investigated materials and all time points. Two-way ANOVA highlighted significant material and time effects on cell proliferation and significant interaction (p<0.0001) in both cell lines (Table 9-2).
Cytotoxicity was the highest on day 1, but not significantly different between the investigated materials in HGK, with the following order from highest to lowest: GND>GR>EN>HC. The highest cytotoxicity percentage was 23% (3), exhibited by GND. Cytotoxicity decreased on days 3 and 5, and slightly increased again on day 10. In HGF, cytotoxicity was the lowest on day 1 with no significant differences. It increased gradually toward day 10 to reach the highest cytotoxicity, with the highest cytotoxicity percentage being 15% (2) exhibited by GR (Table 9-3, Figure 9-3 and 9-4).

Two-way ANOVA was performed for material and time effect on cytotoxicity and their interaction. There was a significant effect of time and a significant interaction ($p<0.0001$) but an insignificant effect of material ($p=0.057$) for HGK. For HGF, only a significant effect of time ($p<0.0001$) was found.

Both assays are compared for each material over time points in both cell lines (Figure 9-5 and 9-6). In HGK, a positive correlation was found between proliferation and cytotoxicity in all investigated materials that was only significant in EN ($p=0.023$). While in HGF, a non-significant negative correlation was found between proliferation and cytotoxicity in both EN and HC. A non-significant positive correlation was found between proliferation and cytotoxicity in both GND and GR.

Cell imaging provided an idea about cell count and shape on days 1, 3, 5, 7 and 10. HGK (with GR and HC) exhibited more proliferation and were comparable to the control with time. HGK (with GND) exhibited less cell proliferation on day 3, but after that, it was comparable to the control. HGK (with EN) exhibited the least cell proliferation compared to the control and other investigated materials over all the test period. HGF exhibited an increasing proliferation with time in the control and all materials. The cells appeared to maintain their standard shape over the test period (Figure 9-7 and 9-8). No Caspase-3 activity was detected for any of the investigated materials in both cell lines based on immunostaining results. In addition, HGF showed more cell density than HGK at day 10 (Figures 9-9 and 9-10).
Table 9-2: The mean and standard deviation values of cell proliferation percentage at days 1, 3, 5 and 10 in both cell lines (HGK and HGF). Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)) between investigated materials at each time point.

<table>
<thead>
<tr>
<th>Material</th>
<th>EN</th>
<th>GR</th>
<th>HC</th>
<th>GND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (days)</td>
<td>HGK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>93.1(0.5)A,B</td>
<td>100(3)C</td>
<td>89.6(2)A</td>
<td>97(2)B,C</td>
</tr>
<tr>
<td>3</td>
<td>73.8(1)A</td>
<td>79(2)D</td>
<td>84(1)B</td>
<td>98.8(1)C</td>
</tr>
<tr>
<td>5</td>
<td>52.7(0.2)A</td>
<td>65.7(1.9)B</td>
<td>62.8(1.6)B</td>
<td>70.08(0.5)C</td>
</tr>
<tr>
<td>10</td>
<td>53.8(2.6)A</td>
<td>82.85(3.3)B</td>
<td>81.4(2.6)B</td>
<td>77.5(2.9)B</td>
</tr>
<tr>
<td>Time (days)</td>
<td>HGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>92.5(1.0)A</td>
<td>100(2.0)C</td>
<td>90.4(1.0)B</td>
<td>94(2.0)A</td>
</tr>
<tr>
<td>3</td>
<td>77.9(1.5)A</td>
<td>87.8(3.5)C</td>
<td>80.7(2.5)A</td>
<td>84(3.5)B</td>
</tr>
<tr>
<td>5</td>
<td>75.8(0.7)A</td>
<td>100(1.7)D</td>
<td>77.3(1.0)B</td>
<td>86.3(0.9)C</td>
</tr>
<tr>
<td>10</td>
<td>102(3.7)A</td>
<td>100(4.8)A</td>
<td>102(1.4)A</td>
<td>126(1.9)B</td>
</tr>
</tbody>
</table>

Table 9-3: The mean and standard deviation values of cytotoxicity percentage at days: 1, 3, 5, and 10 in both cell lines (HGK and HGF). Values with the same superscript letters represent a non-significant difference (Tukey’s post hoc test (α=0.05)) between investigated materials at each time point.

<table>
<thead>
<tr>
<th>Material</th>
<th>EN</th>
<th>GR</th>
<th>HC</th>
<th>GND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (days)</td>
<td>HGK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21(5)a</td>
<td>22(3.8)a</td>
<td>18(7)a</td>
<td>23(3)a</td>
</tr>
<tr>
<td>3</td>
<td>13(5)a,c</td>
<td>3(6)b,c</td>
<td>1(2)p</td>
<td>2(2)b</td>
</tr>
<tr>
<td>5</td>
<td>-7(4)a</td>
<td>5(2)b</td>
<td>1(3)p</td>
<td>1(2)b</td>
</tr>
<tr>
<td>10</td>
<td>-2(1)a</td>
<td>13(3)b</td>
<td>11(4)b</td>
<td>7(4)b</td>
</tr>
<tr>
<td>Time (days)</td>
<td>HGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6(1)a</td>
<td>9(4)a</td>
<td>7(3)a</td>
<td>6(3)a</td>
</tr>
<tr>
<td>3</td>
<td>7(1)a</td>
<td>7(1)a</td>
<td>6(2)a</td>
<td>8(2)a</td>
</tr>
<tr>
<td>5</td>
<td>12(6)a</td>
<td>13(2)a</td>
<td>11(4)a</td>
<td>12(2)a</td>
</tr>
<tr>
<td>10</td>
<td>10(3)a,c</td>
<td>15(2)c</td>
<td>8(1)a,b</td>
<td>12(3)a,c</td>
</tr>
</tbody>
</table>
**Figure 9-1:** A bar chart illustrating the mean values of cell proliferation (Viability) percentage at days 1, 3, 5 and 10 for HGK. Error bars represent the standard deviation.

**Figure 9-2:** A bar chart illustrating the mean values of cell proliferation (Viability) percentage at days 1, 3, 5 and 10 for HGF. Error bars represent the standard deviation.
Figure 9-3: A bar chart illustrating the mean values of cytotoxicity percentage at days 1, 3, 5 and 10 for HGK. Error bars represent the standard deviation.

Figure 9-4: A bar chart illustrating the mean values of cytotoxicity percentage at days 1, 3, 5, and 10 for HGF. Error bars represent the standard deviation.
Figure 9-5: Mean viability and cytotoxicity percentages over 10 days for HGK.
Figure 9-6: Mean viability and cytotoxicity percentages over 10 days for HGF.
Figure 9-7: Cell morphology of HGK at days 1, 3, 5, 7, and 10 for the control and investigated materials. The scale bar at the right bottom corner is 100 µm; CTR stands for control.
**Figure 9-8:** Cell morphology of HGF at days 1, 3, 5, 7, and 10 for the control and investigated materials. The scale bar at the right bottom corner is 100 µm; CTR stands for control.
Figure 9-9: Immunofluorescence microscopy images, nuclei stained with DAPI (blue), representing the cell density of HGK at the end of the test (day 10). The scale bar is 100 µm.

Figure 9-10: Immunofluorescence microscopy images, nuclei stained with DAPI (blue), representing the cell density of HGF at the end of the test (day 10). The scale bar is 100 µm.
9.5 Discussion

The present study showed that cell proliferation and cytotoxicity were significantly different between some materials in both cell lines; the first null hypothesis was consequently partially accepted. However, there was a difference between the two cell lines in both their proliferation and cytotoxicity level. In a sense, this could be expected due to differences between the two cell lines used in this study. Keratinocytes are considered less resistant and not as strong as fibroblasts. Fibroblasts are the main cell population of the basement membrane, which is the supportive layer for the superficial epithelial cell layer where keratinocytes are considered the main cell population (Pabst et al., 2014), nevertheless, the cells appear to maintain their normal shape through the test period as observed by light microscopy imaging.

There was a significant effect of both material type and time on cell proliferation and only significant effect of time on cytotoxicity of both cell lines, so the second null hypothesis was partially accepted. In addition, no Caspase-3 activity was detected. In other words, the materials investigated did not induce apoptosis in either cell line. In this study, HGK seemed to be more affected by composite materials than HGF; this finding compliments current literature (Jerg et al., 2018; Schulz et al., 2012; 2015), although other studies have shown more material influence on HGF than on HGK (Grenade et al., 2016; 2017).

The materials used in this study were selected according to the results of a previous study on monomer elution (Chapter 8). EN was selected, as it is the only PICN currently available commercially (Lambert et al., 2017) that eluted minimal monomer amounts (less than ED\textsubscript{50}). GR is a resin-composite block with the same filler percentage as EN and it did not elute any detectable monomer amounts. HC has the lowest filler weight of all investigated resin-composite blocks, and it showed a minimum ED\textsubscript{50} cytotoxic level of monomer elution. GND is a conventional resin-composite with the highest filler weight of all investigated materials and showed ED\textsubscript{50} cytotoxic level of monomer elution (Geurtsen et al., 1998).

Both cell lines with GND and GR showed better proliferation than with EN and HC. HGK showed more than 30% reduction in proliferation with EN on day 5 and 10, and with GR
and HC on day 5. This exceeds the maximum value accepted for proliferation reduction (30%) for medical devices, according to ISO-10993:5 (ISO 10993-5, 2009). On day 5, HGK exhibited the lowest proliferation level for all investigated materials; however, the proliferation rate increased (>70%) on day 10 for all investigated materials except for EN.

The results for EN are in line with a recent study that showed a cytotoxic effect of EN (Hussain et al., 2017). However, another in vitro study showed that EN and other experimental PICN materials had no cytotoxic effects on gingival and pulpal stem cells, and were considered very biocompatible (Tassin et al., 2016). Moreover, an experimental PICN (with no TEGDMA and no photoinitiator with different filler particles) showed comparable biocompatibility to both zirconia and titanium (Grenade et al., 2016; 2017). TEGDMA has many cytotoxic and genotoxic effects due to its small molecular size, which enhances diffusion processes (Krifka et al., 2013, Pelka et al., 1999). In this matter, the presence of TEGDMA (a low weight monomer) as part of the polymer matrix in the commercial PICN (EN) can be attributed the cytotoxic effect (Huang, et al., 2015; Krifka et al., 2013; Lin-Gibson et al., 2009).

In the present study, cell proliferation in both cell lines decreased at day 3 and 5 and then increased toward day 10, due to the short time, 2 days interval, which allows less time for the cells to proliferate, but then toward day 10, where cells have 5 days interval, the proliferation level increased again. Decreased proliferation should not necessarily mean that cells are dying, as they might be reacting to the material by differentiation rather than proliferation, and increased proliferation might be a reaction to stimuli (Lloyd, 2013).

All of the investigated materials showed similar cytotoxicity level, but none exceeded the ISO standard level (30%) (ISO 10993-5, 2009) except EN, which showed a proliferation cytotoxic effect in HGK. It is worth mentioning that both HC and GND showed ED_{50} cytotoxic level monomer release in 75% E/W in a previous study (Chapter 8). Nevertheless, ethanol is considered an aggressive medium compared to oral conditions. Moreover, cells cannot withstand such an aggressive medium, and the water-based medium is more comparable to oral conditions (Ferracane, 1994;
Tabatabaee et al., 2009). Although the investigated materials eluted various monomer levels (measured in a previous study, Chapter 8), they had comparable cytotoxicity levels, except for EN, which indicates that factors other than eluted monomer levels, such as nanoparticles, could be involved in material cytotoxicity (Schmalz & Galler, 2017). One study assessing filler elements leached out from CAD/CAM composite blocks in purified water at different temperatures showed a variable amount of leached filler elements that was dependent on the material composition, especially oxides components of the fillers, as well as heating temperatures (Iwata et al., 2019). Research in this particular area is still scarce, and further investigation is needed.

Apoptosis is a programmed cell death that takes place in pathology, in reaction to toxicants or in normal development (Schweikl et al., 2006). This physiological process differs from necrosis in that necrosis sets off a tissue inflammation process associated with clinical symptoms (Majno & Joris, 1995; Zhivotovsky, 2004). Many studies detect apoptosis in various cell lines in reaction to composite material eluates and polymethacrylates (Cimpan et al., 2000a; 2000b; Gough & Downes, 2001; Quinlan et al., 2002); these reactions were more likely associated with unpolymerised resin compounds (Schweikl et al., 2006). Hence, in this study, due to CAD/CAM manufacturing of fully polymerised composite with a high degree of conversion, no Caspase-3 activity was detected. Furthermore, the conventional composite used in this study had a high filler content of 89%, so it was expected to have a smaller percentage of unpolymerised resin and in this case, did not induce any apoptotic activity. EN also showed no apoptosis induction in another study (Tassin et al., 2016).

The specimen surface area of 150 mm² is in between the surface area of a central incisor veneer and crown (Van Landuyt et al., 2011) which is representative of the clinical indications of the investigated materials such as veneers, inlay/onlay, and anterior and posterior single crowns (Lambert et al., 2017). Composite materials were incubated on the cell layer in the centre of each of the well at 37°C to simulate oral conditions (ISO 10993-5, 2009). In addition, the ratio of the surface area of the sample to medium volume is 3 cm²/ml, which is within the ISO standard ratio of 0.5-6 cm²/ml, ISO 10933-12 (ISO 10993-12, 2007).
Primary instead of immortalised cells were used as they are more representative of human cells (Pabst et al., 2014; 2016; Pendegrass et al., 2015) with direct contact with the investigated materials, which is considered the most sensitive method as it can measure low cytotoxicity levels (De Melo et al., 2013). There is a continuous wash-away of leached out components from restorative materials by saliva and consumable fluids in patient mouth, in addition to tooth-brushing, so changing the medium, although not as comparable, could have had a slight washing effect. Finally, finding cytotoxic effects in the cell medium over the short testing time in this study could be an alarming point to further investigate the biocompatibility and biological properties of CAD/CAM composite materials.

9.6 Conclusions

All investigated materials were more influential on HGK proliferation and caused higher cytotoxicity than on HGF. EN showed a proliferation cytotoxic effect in HGK. Different manufacturing techniques of composite materials had no significant effect on their biological properties, as conventional composite showed comparable and even better proliferation and lower cytotoxicity levels than CAD/CAM counterparts.
Chapter Ten: General Discussion, Conclusions and Future Work Recommendations
10.1 General discussion

This research aimed to characterise and compare different mechanical, chemical and biological aspects of CAD/CAM composite materials (RCB and PICN) and PEEK to CAD/CAM ceramic and, where appropriate, to the tooth structure and conventional resin-composites; and to explore any relationships between their composition and their properties. The CAD/CAM blocks investigated were: Five resin-composite blocks: (Grandio blocs (GR), Lava™ Ultimate (LU), Cerasmart (CS), BRILLIANT Crios (BC), Block HC (HC)); one polymer-infiltrated ceramic network ceramic block (Enamic, EN); one ceramic-filled poly ether ether ketone (Dentokeep, DK), one unfilled PEEK (Ceramill PEEK, PE); and one feldspathic ceramic block (Vitabloc Mark II, VM). The conventional resin-composites investigated were two indirect; Ceramage (CMG), and Gradia plus (GRA); and two direct; GrandioSO (GND) and Tetric evoceram (TET) materials.

Dental restorative materials are exposed to a variety of acidity levels, temperatures and stresses. Thus, storage in simulated oral cavity conditions and solvents is more reflective of clinical conditions (Musanje & Darvell, 2003). Water and water-based artificial saliva were used in this research at 37 °C, simulating intraoral fluids and temperature. The artificial saliva formula used in this research had a comparable viscosity and acidity to human saliva. In addition, 75% ethanol/water (E/W) solvent was considered as a food and oral simulating liquid, according to the Food and Drug Administration (FDA) guidelines of the United States (Moon et al., 2000; Sideridou et al., 2007; United States Food and Drug Administration, 1988). 75% E/W has similar solubility parameter to that of resin-composite (Manojlovic et al., 2013) and hence it can penetrate resin-matrix, leading to sorption, material softening and monomer elution (Boaro et al., 2013; Ferracane, 1994; Marghalani & Watts, 2013; Sideridou et al., 2003; Zhang & Xu, 2008). 75% E/W has been used in many studies to assess material softening and monomer elution (Gul et al., 2014; Mazzaoui et al., 2002; Miletic et al., 2009; Ortengren et al., 2001; Sunbul et al., 2016; Tang et al., 2014; Tokay et al., 2015). However, ethanol-based solvents are considered more aggressive than water-based solvents (Ferracane, 1994; Tabatabaei et al., 2009), which was also found in this research. Nevertheless, ethanol-based solvent can be used to allow extraction of all components that can be extracted for better evaluation (Van Landuyt et al., 2011).
Mechanical properties can predict the material clinical performance (Charlton et al., 2008; Zhang & Kelly, 2017). Hence, in chapter 4, mechanical properties of CAD/CAM composite blocks (hardness and elastic modulus) were investigated and compared to those of CAD/CAM ceramic, enamel and dentine. The influence of the filler loading on these properties was also investigated. For the next chapters, unfilled PEEK (Ceramill PEEK, PE) was excluded as deemed very similar to PEEK with 20% ceramic filler and the latter is more relevant to the investigated composite materials.

Mechanical properties tested under simulated oral conditions are more clinically relevant. Thus, in chapter 5, the microhardness of the investigated materials was assessed after short-term (30 days) and long-term (90 days) storage in different solvents (water, artificial saliva, and 75% E/W). Storage in water can cause material softening as found in chapter 5, due to plasticisation of the polymer matrix (Papadogiannis et al., 2008; Sih et al., 1980). Such solvents diffuse and absorb into the resin-matrix, leading to hydrolytic degradation of the interfacial silane coupling agent between the fillers and resin-matrix, causing molecular instability (Druck et al., 2015; Musanje & Darvell, 2003) and degradation of resin-composite components over time (Ferracane, 1994; Ferracane et al., 1998; Martos et al., 2003; Sonmez et al., 2018). Consequently, this negatively influences the colour stability and the mechanical and physical properties of the resin-composite (Um & Ruyter, 1991), and it induces hydrolytic degradation of bonding (Drummond et al., 1991; Mair & Padipatvuthikul, 2010; Tuna et al., 2008). The solubility of resin-composite components is another consequence of hydrolytic degradation, which leads to composite components such as unreacted monomers and fillers leaching out into the oral cavity (Boaro et al., 2013; Ferracane, 1994; Sideridou et al., 2003). Thus, it was important to investigate sorption and solubility of the investigated materials over the long-term (chapter 6).

As viscoelastic stability of a material is an important property and influenced by the material composition and water storage, it seemed pertinent to investigate this property in chapter 7. Findings in chapter 6 elucidate comparable effects of artificial saliva and water (SP: Pearson correlation coefficient, $r=0.998$, $p=0.0001$), (SL: Pearson correlation coefficient, $r=0.80$, $p=0.018$), which was in line with previous studies (Alshali et al., 2015b; Liebermann et al., 2016; Sideridou et al., 2011). Accordingly, in chapter 7,
only distilled water was used to assess viscoelastic stability of PICN compared to RCB. Compressive creep resistance is an indication of material viscoelastic stability and subsequently, its resistance to catastrophic failure under loading (El Hejazi & Watts, 1999; Watts, 1994). The compressive load applied in this study was about 20 MPa, which corresponds to the occlusal force intraorally during occlusion (Hirano & Hirasawa, 1989; de Abreu et al., 2014). Water sorption is associated with increased creep and reduced creep recovery of composite materials, which was confirmed by the findings in chapter 7, and is in line with some previous studies findings (El-Safty et al., 2012; Kildal & Ruyter, 1997; Odén et al., 1988).

The investigated materials exhibited various levels of hardness reduction, water sorption and solubility, and viscoelastic stability (Chapters 5-7). As all these properties were assessed in different storage media, it was necessary to determine what is released into the storage medium following these changes in the material surface and bulk properties (chapter 8). Dental composites can release low molecular weight monomers such as HEMA and TEGDMA, high molecular weight monomers such as Bis-GMA and UDMA, free radicals and photoinitiator molecules (Kingman et al., 2012; Leprince et al., 2013; Van Landuyt et al., 2011). Monomers and some photoinitiator molecules may be related to dermatologic, allergic, cytotoxic, and genotoxic effects (Bakopoulou et al., 2009; Krifka et al., 2013; Leprince et al., 2013).

There was minimal or no monomer elution from the CAD/CAM composite blocks (chapter 8), thus it might be expected that CAD/CAM composite blocks would exhibit superior biocompatibility compared to the conventional resin-composites. Hence, to confirm or reject this assumption, biocompatibility was assessed (chapter 9). Four composite materials with different manufacturing techniques (conventional resin-composite, 2 RCB and PICN) were investigated in terms of their influence on HGF and HGK proliferation, cytotoxicity and Caspase 3 activity (apoptosis). The materials used in this study were selected according to the results of chapter 8 on monomer elution. EN was chosen as the only commercially available PICN (Lambert et al., 2017). GR is a resin-composite block with the same filler percentage (86 wt%) to EN but different manufacturing technique. HC has the lowest filler weight (61 wt%) of all investigated resin-composite blocks, and it showed an ED$_{50}$ cytotoxic level of monomer elution. GND
is a conventional resin-composite with the highest filler weight (89 wt%) of all investigated materials, and also showed an ED$_{50}$ cytotoxic level of monomer elution (Geurtsen et al., 1998). The two gingival cell lines used in this study were human gingival keratinocytes (HGK), which is the primary cell population of the marginal keratinised gingiva (epithelial layer) of the oral mucosa; and human gingival fibroblasts (HGF) that are the supportive connective tissues (subepithelial layer). Both cell types play an essential role in the oral wound healing process and soft tissue integrity and regeneration (Pabst et al., 2014). Primary human cell lines rather than immortalised ones were used for better comparison to human cells (Pabst et al., 2014; 2016; Pendegrass et al., 2015).

This research showed that VM (CAD/CAM ceramic) was very hard and stiff in comparison to both enamel and dentine. The high hardness and stiffness of VM could negatively influence clinical performance and machinability (Tsitrou et al., 2007; Zhang & Kelly, 2017). However, VM showed a superior softening resistance and a negligible amount of sorption and solubility compared to CAD/CAM composite materials. Thus, as long-term restorative material, it is considered stable, especially considering the humidity of the intraoral environment and salivary flow. These factors could influence discolouration and surface degradation, so ceramic is considered the material of choice when a highly aesthetic restoration is demanded (Mainjot et al., 2016). This shows the advantageous stability of ceramics compared to CAD/CAM composites, which show different and higher levels of hardness reduction (Material softening) and sorption due to solvents diffusion through their polymeric matrices (Colombo et al., 2019; Ferracane et al., 1998).

EN, which is currently the only commercially available PICN, showed hardness and stiffness between that of enamel and dentine but closer to enamel, thus EN might be favourable over VM in that it can withstand elastic deformation and is more damage-tolerant (Feng et al., 2003; Mainjot et al., 2016; Swain et al., 2016; Zhang & Kelly, 2017), especially for minimal preparation cases within the enamel (Mainjot, 2018; Peampring, 2014). Additionally, similar hardness and elastic modulus to tooth structure implies lower wear of the opposing tooth structure. In addition, EN showed higher softening resistance than RCBs, and comparable to VM. This may have been due to the interpenetrating ceramic and polymer networks that increase resistance to breakdown
(Coldea et al., 2013a; Lambert et al., 2017; Swain et al., 2016). EN sorption was lower than VM and DK (PEEK); comparable to GR (resin-composite block with similar filler weight %); and higher than other resin-composite blocks, indicating that filler loading had more influence on sorption than the manufacturing technique. The viscoelastic stability of EN was superior to all resin-composite blocks, reflecting the material stability under heavy compressive loads as encountered in cases of bruxism, especially those in which ceramics might fail due to their high brittleness (Ozturk et al., 2008).

Another favourable yet expected finding was the minimal monomer release from EN, which may indicate better biocompatibility of the material, as monomer release is considered the main issue in respect to resin-composite biocompatibility. Nevertheless, EN showed some cytotoxic effects on human gingival keratinocytes.

The hardness and stiffness of resin-composite blocks were more comparable to dentine rather than enamel. They showed variable degrees of hardness reduction, viscoelastic stability and sorption, which were mostly correlated to their filler loading. RCBs with higher hardness and elastic moduli were less likely to degrade in solvent storage. GR, for instance, with highest filler loading (86%), expressed the most favourable properties among RCBs and was most comparable to EN (PICN). In addition, GR released no monomer and found more biocompatible than EN. CS, BC, and LU, with measured filler weight percentages of 66, 70, and 75 wt% respectively, had similar levels of hardness reduction and sorption. HC, despite having the lowest filler weight% (63%), exhibited higher softening resistance than LU, BC and CS. This may be attributed to its resin-matrix composition, as it does not have Bis-GMA, which has more water sorption levels and thus causes more material softening (Ertas et al., 2006; Sideridou et al., 2003; Sideridou & Karabela, 2011). LU had more hardness reduction than HC, and more sorption than CS and BC that might be due to zirconium silicate in the filler composition, thus it is more prone to hydrolysis of the silane coupling agent due to the inefficient silanisation of high-crystalline content zirconium silicate (Druck et al., 2015).

CAD/CAM composite blocks (RCB and PICN) exhibited higher creep resistance, higher creep recovery and lower permanent set than conventional composites investigated in other studies, especially bulk fill composites and in shorter-term wet storage (Alrahlah et al., 2018; El-Safty et al., 2012; Kaleem et al., 2012). Furthermore, an elastic
deformation was exhibited by CAD/CAM composite blocks rather than viscoelastic, which is the case in conventional composites. This may be due to the higher degree of conversion and improved mechanical properties of CAD/CAM composite storage.

RCBs eluted significantly lower and minimal amounts of monomers compared to the conventional resin-composite materials investigated. GR released no monomers, while all other investigated materials released variable amounts of different monomers into 75% E/W and only GRA and GND (conventional resin-composite) eluted monomers in AS and water. This may be ascribed to the solubility factor of 75% E/W being similar to that of resin-composite matrix, allowing it to diffuse into the resin-composite matrix and cause accelerated ageing of resin-composite materials (Manojlovic et al., 2013) and material softening (Ferracane, 1994; Marghalani & Watts, 2013; Tabatabaee et al., 2009; Van Landuyt et al., 2011). All RCBs and PICN released minimal amounts of monomer that were below the ED$_{50}$ cytotoxicity levels, except for HC, which released UDMA slightly above the ED$_{50}$ cytotoxicity level (Geurtsen et al., 1998). Nevertheless, this does not necessarily imply that the materials are inert and not cytotoxic. For instance, EN and LU exhibited higher cytotoxic effects than the tested conventional composite in a study conducted recently (Hussain et al., 2017). However, other studies on experimental PICN, which differs from EN (experimental PICN does not contain TEGDMA), do not show any cytotoxic effects (Grenade et al., 2016; 2017). Regarding conventional resin-composites, all of them except GRA (eluted TEGDMA in water) eluted monomer amounts above the ED$_{50}$ cytotoxicity level (Geurtsen et al., 1998). However, to date, there is no reference data on the recommended limits of monomer intake (Putzeys et al., 2019), which could be due to the absence of experimental standardisation such as sample size, solvent type, volume, chemical analytical methods, storage time and measurement units (Van Landuyt et al., 2011).

DK, which is PEEK, a non-methacrylate based CAD/CAM block, showed the lowest hardness and stiffness, which was expected due to the very low filler content of 20%. However, DK showed hardness softening comparable to both VM and PICN in 75% E/W and a high hydrolytic stability that was superior to PICN and resin-composite blocks. In addition, interestingly, DK did not elute monomers, despite high polymer content (80%). This superior hydrolytic and chemical stability may be attributed to the very stable
chemical and physical properties that are provided by the presence of a unique chemical structure comprising an aryl ring containing ketone and other groups (Williams, 2008a). Accordingly, PEEK have potential favourable properties to allow wide use in prosthodontics applications (Liebermann et al., 2016).

Material changes, i.e. hardness reduction, mass change and monomer elution, mostly occurred in the early time points; relevant to each property. Hardness reduction noticed after 30 days was higher than hardness reduction occurred between 30 and 90 days in all materials and all media, in line with other studies as most material changes occur in the first few days (Al-Harbi et al., 2017; Sunbul et al., 2016; Van Landuyt et al., 2011). All materials exhibited the greatest mass change in the first week of storage, in line with other studies (Alshali et al., 2015b; Huang et al., 2002; Martin et al., 2003; Zankuli et al., 2014). Moreover, monomer detected in the first month was significantly higher than the monomer detected between 1 and 3 months. Most monomers usually leach out in the early few days, and then the material reach equilibrium (Alshali et al., 2015a; Van Landuyt et al., 2011; Łagocka et al., 2018). However, some elution continues in a small amount for up to a year (Alshali et al., 2015a; Polydorou et al., 2007; 2009; 2012).

To a certain extent, increased filler loading is associated with an improvement in mechanical properties. Thus filler loading was positively correlated with hardness, elastic modulus, softening resistance and hydrolytic and viscoelastic stability in this research, in line with other studies (Chung, 1990; Coldea et al., 2014; Curtis et al., 2008; He & Swain, 2011; Ilie, 2019; Kim et al., 2007; Lin-Gibson et al., 2009).
Finally, CAD/CAM composite blocks are advantageous to CAD/CAM ceramics in terms of similar hardness and stiffness to that of tooth structure, and how beneficial that could be in terms of repair, enamel wear, clinical performance and longevity, and machinability. However, when it comes to ageing in different solvents, CAD/CAM composite blocks are not as a stable as ceramics in terms of material softening, sorption and monomer elution, attributable to the resin-matrix features allowing water sorption and surface degradation. Viscoelastic stability is another concern for polymeric-based materials. However, CAD/CAM composite blocks exhibited superior viscoelastic stability to conventional resin-composites measured in other studies. As for biocompatibility, there are some concerns regarding the cytotoxicity of EN, which needs further investigation.

10.2 Limitations

A better representation of the clinical performance will be provided by testing these materials in the oral environment. Thus, in vivo work is warranted to determine the clinical relevance and serviceability of these materials. Nevertheless, in vitro studies are considered a useful tool to provide an initial assessment of different aspects of materials.

As for the different methodologies used in this research, the limitations have been mentioned previously, such as the microhardness test being subjective and dependant on the researcher experience to measure the exact diagonal of the indentation and nanoindentation technique sensitivity. The recommended standards and protocols were followed to reduce variability, increase objectivity and allow comparison with similar studies.

One limitation regarding the sample dimensions of the conventional composites used could be the adequacy of curing of more than 2 mm thickness; however, this has been addressed by adding the composite material in increments and curing of top and bottom sample surfaces.

Using sample shape and geometry that is relevant to the clinical indications of the investigated materials such as onlays/inlays may be more reflective of the material
clinical performance than using flat surface samples. However, this is not always possible, as much mechanical testing, for instance, requires flat surfaces. Besides, the cost of manufacturing such custom restorations, especially using CAD/CAM, presents a barrier.

10.3 Overall Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1) CAD/CAM composite blocks have comparable hardness and elastic moduli to tooth structure, and these are positively correlated to filler weight percentage.

2) The hardness of CAD/CAM composite blocks is affected by different storage media and are not as stable as a ceramic with 75% E/W has a more pronounced effect on Vickers hardness of the investigated materials.

3) CAD/CAM composite blocks are reasonably hydrolytically stable in long-term storage, although not as stable as ceramic, with DK exhibits superior hydrolytic stability.

4) PICN exhibits superior viscoelastic stability compared to all of the resin-composite blocks, with deformation being predominantly elastic rather than viscoelastic.

5) There is minimal or no monomer elution from CAD/CAM composite blocks.

6) The different manufacturing techniques of CAD/CAM composites have no significant effect on their biological properties, as conventional resin-composite shows less impact on cell proliferation and lower cytotoxicity levels than CAD/CAM counterparts. EN shows a proliferation cytotoxic effect in HGK, this finding deserves further investigation.
10.4 Recommendations for future work

In order to complement the findings of the current study and further knowledge of the investigated materials, the following areas are recommended for future investigation:

- Assessment of mechanical performance in a simulated oral environment using different ageing methods such as thermal and mechanical cycling.
- Assessment of other mechanical properties such as fracture strength, toughness, flexural strength and fatigue resistance and explore the relationship between CAD/CAM composite blocks composition and these properties. Also, explore the effect of different ageing mechanisms on these properties.
- Investigation of bacterial accumulation and biofilm formation of different CAD/CAM composite blocks with comparison to both CAD/CAM ceramic blocks and conventional resin-composites.
- Investigation of cell reactions toward different CAD/CAM composite blocks in comparison to both CAD/CAM ceramic blocks and conventional resin-composites using techniques that are more advanced i.e. 3D cell cultures and other relevant cell lines. Also, further investigation of EN (PICN) cytotoxicity.
- Evaluation of the effect of the monomer matrix and filler microstructure, rather than filler loading, on the investigated properties.
- Develop and formulate new composite blocks (RCB and PICN) with optimised properties utilising the best resin-matrices compositions and filler loadings.
- Develop and formulate new PEEK blocks with higher filler loadings and different shades that mimic tooth colour.
References:


European Commission Scientific Committee on Emerging and Newly Identified Health Risks, (2015). 'Final opinion on the guidance on the determination of potential health effects of nanomaterials used in medical devices'.


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Appendices:

Appendix I: HPLC chromatograms of storage media, reference monomers and their calibration curves.

**Figure-Apx I-1:** HPLC chromatogram of a control 75 % ethanol/water solution with caffeine internal standard, CF retention time at 4.76 min.

**Figure-Apx I-2:** HPLC chromatogram of a control water sample with caffeine internal standard, CF retention time at 4.76 min.

**Figure-Apx I-3:** HPLC chromatogram of a control artificial saliva sample with caffeine internal standard, CF retention time at 4.5 min retention time, and strong peak of an artificial saliva component at 5.7 min.
Figure-Apx I-4: HPLC chromatogram of UDMA with caffeine internal standard.

Figure-Apx I-5: HPLC chromatogram of TEGDMA with caffeine internal standard.

Figure-Apx I-6: HPLC chromatogram of Bis-GMA with caffeine internal standard.

Figure-Apx I-7: HPLC chromatogram of Bis-EMA with caffeine internal standard.
Figure-Apx I-8: HPLC calibration curve of UDMA, $R^2=0.995$.

Figure-Apx I-9: HPLC calibration curve of TEGDMA, $R^2=0.999$. 

Figure-Apx I-10: HPLC calibration curve of Bis-GMA, $R^2=0.989$.

Figure-Apx I-11: HPLC calibration curve of Bis-EMA, $R^2=0.996$. 
Appendix II: Fragmentation mass spectrometry

**Figure-Apx II-1**: Fragmentation mass spectrometry of unknown molecule released from CMG in 75% E/W, which is possibly HEMA; MW 130 and UDMA; MW 498.
Appendix III: Immunofluorescence microscopy image (enlarged view).

Figure-Apx III-1: Immunofluorescence microscopy image of EN (enlarged view), nuclei stained with DAPI (blue), representing the cell density of HGK at the end of the test (day 10). The scale bar is 100 µm.