GENOTOXIC EVALUATION OF LOCALLY PRODUCED DENTAL PORCELAIN USING THE AMES *SALMONELLA* AND COMET ASSAYS

by

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Dedication

To my beloved wife and parents for their love, patience and sacrifice and to all those who dream of an education but do not have the means…

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LIST OF ABBREVIATIONS

2AA	2-Aminoanthracene
ALS	Alkali-labile sites
ATCC	American Type Culture Collection
Bis-GMA	Bisphenol A-glycidyl methacrylate
CAD/CAM	Computer Aided Design/Computer Aided Manufacturing
CEREC	CEramic REConstruction
CHO	Chinese hamster ovary
DNA	Deoxyribonucleic acid
EMEM	Eagle's minimal essential medium
FDA	Food and Drug Administration
FPDP	Fixed partial denture prostheses
GMA	Glycidyl methacrylate
HEMA	Hydroxyethyl methacrylate
HGPRT	Hypoxanthine-guanine phosphoribosyl transferase
ISO	International Standard Organization
LMA	Low melting agarose
LPDP	Locally produced dental porcelain
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]
NADH	Nicotinamide adenine dinucleotide hydrogenase
NADP	Nicotinamide adenine dinucleotide phosphate
NMA	Normal melting agarose
PBS	Phosphate buffered saline
PFM	Porcelain-fused to metal

PHB	Polyhydroxybutyrate
RNA	Ribonucleic acid
SCG	Single cell gel
SSB	Single strand breaks
TEGDMA	Triethylenglycol dimethacrylate
UDMA	Urethane dimethacrylate
VB	Vogel-Bonner
WBC	World Biomaterials Congress
UDMA	Urethane dimethacrylate
ZTA	Zirconia-toughened alumina
Y-TZP	Yttria–tetragonal zirconia polycrystal

PENILAIAN GENOTOKSIK TERHADAP PORSELIN KELUARAN TEMPATAN MENGGUNAKAN ASSAI AMES *SALMONELLA* DAN ASSAI COMET

ABSTRAK

Porselin merupakan bahan sintetik gantian yang kelihatan paling asli yang digunakan untuk pemulihan gigi, dan ia mempunyai tempat yang istimewa dalam bidang pergigian kerana ia memberikan hasil yang cantik dari sudut estetik. Walaupun porselin gigi secara umumnya dianggap sebagai lengai, keserasian bio bahan ini tidak boleh diabaikan kerana pemulihan ini akan kekal berada pada kaviti gigi untuk bertahun-tahun malah berdekad lamanya. Tujuan kajian ini adalah untuk menentukan ketoksikan geno pada porselin keluaran tempatan (Universiti Sains Malaysia, Malaysia) dengan menggunakan assai kemutagenan *Salmonella*/mikrosom-mamalia (assai Ames) dan satu sel assai gel elektroforesis (assai Comet). Pada assai Ames, empat varian genotip strain *Salmonella* (TA98, TA100, TA1535 dan TA 1537) yang membawa mutasi dalam beberapa gen telah digunakan. Porselin pergigian telah dieramkan dengan keempat-empat strain ini pada lima kepekatan yang berbeza (0.3125, 0.625, 1.25, 2.5 dan 5 mg/piring), bersama dengan kawalan positif dan negatif yang sesuai secara serentak, pada ketidakhadiran dan kehadiran pengaktifan metabolik (S9). Keputusan telah ditafsir berdasarkan kepada bilangan koloni revertan per piring dan jika ia melebihi satu kali ganda bilangan kawalan negatifnya, maka ia dianggap sebagai mutagenik. Pada assai Comet, L929 (CCL-1 ATCC, USA) sel-sel fibroblas tikus telah dirawat dengan menggunakan porselin gigi keluaran tempatan pada tiga kepekatan yang berbeza (50, 100 dan 200mg/ml) bersama dengan kawalan negatif dan positif secara serentak.

Keputusan assai Comet telah dinilai berdasarkan pada ‘tail moment’, yang telah digunakan sebagai parameter untuk menentukan kerosakan DNA dan membandingkannya dengan kawalan negatif. Pada assai Ames, bilangan purata koloni revertan per piring yang dirawat dengan porselin gigi keluaran tempatan adalah kurang daripada sekali ganda jika dibandingkan dengan kawalan negatif, manakala dalam kes assai Comet, ‘tail moment’ adalah hampir sama dengan yang mempunyai kawalan negatif. Kesimpulan kajian terkini ialah porselin keluaran tempatan adalah tidak genotoksik apabila diuji menggunakan kaedah yang digunakan dalam kajian ini.

GENOTOXIC EVALUATION OF LOCALLY PRODUCED DENTAL PORCELAIN USING THE AMES *SALMONELLA* AND COMET ASSAYS

ABSTRACT

Porcelain is the most natural-appearing synthetic replacement dental restorative material, holding a special place in dentistry because of its most aesthetically pleasing result. Even though dental porcelains are generally considered to be inert, their biocompatibility cannot be overlooked as these restorations stay in the oral cavity for years or even decades. The aim of this study was to determine the genotoxicity of locally produced dental porcelain (Universiti Sains Malaysia, Malaysia) using the *Salmonella*/mammalian-microsome mutagenicity assay (Ames assay) and the single cell gel electrophoresis assay (Comet assay). In the Ames assay, four genotypic variants of the *Salmonella* strains (TA98, TA100, TA1535 and TA1537) carrying mutations in several genes were used. The dental porcelain was incubated with these four strains at five different concentrations (0.3125, 0.625, 1.25, 2.5 and 5 mg/plate) along with concurrent appropriate positive and negative controls both in the absence and presence of metabolic activation (S9). The results were assessed based on the number of revertant colonies/plate and if it was more than double the number than that of the negative control, the results are considered mutagenic. In the Comet assay, L929 (CCL-1 ATCC, USA) mouse fibroblast cells were treated with the locally produced dental porcelain at three different concentrations (50, 100 and 200 mg/ml) along with concurrent negative and positive controls. The results of the Comet assay were assessed based on the tail moment, which was used as the parameter to determine the DNA damage and compared

to that of the negative control. In the Ames assay, the average number of revertant colonies per plate treated with locally produced dental porcelain was less than double as compared to that of the negative control, whereas in the case of Comet assay, the tail moment was almost equal to that of the negative control. From the results of the current study, it is inferred that the locally produced dental porcelain is non-genotoxic under the present test conditions.

CHAPTER ONE

INTRODUCTION

1.1 History

The history of the use of porcelain in dentistry dates back to the 18th Century, when a Parisian apothecary, Alexis Duchateau, with the assistance of Nicholas Dubois de Chemant, a Parisian dentist, made the first successful porcelain dentures, replacing the stained and malodorous ivory prostheses of Duchateau. Later, Dubois de Chemant further improved porcelain formulations and fabricated porcelain dentures as part of his practice. In 1808, individually formed porcelain teeth that contained embedded platinum pins were introduced in Paris by Giuseppangelo Fonzi, who called these teeth "terrametallic incorruptibles" and their esthetic and mechanical versatility provided a major advance in prosthetic dentistry (Kelly *et al.*, 1996). Since then, the use of porcelain as a denture material has expanded due to the attainment of better properties. During the past few decades, the advancement in the development of newer and better porcelains has been so tremendous, that, at present, porcelain holds a very promising position in dentistry both in terms of function as well as esthetics.

1.2 Background of the study

The ceramic material known as porcelain holds a special place in dentistry because, not withstanding the many advances made in composites and glass-ionomers, porcelain is still considered to produce the most aesthetically pleasing result. As yet, its color, translucency and vitality cannot be matched by any material, except other ceramics (Noort, 2002).

All porcelains are ceramics, but not all ceramics are porcelains (Anusavice, 1996). Ceramic is defined as any product made essentially from a non-metallic material by firing at a high temperature to achieve desirable properties. Porcelain refers to a family of ceramic materials composed essentially of Kaolin, Quartz and Feldspar. Dental ceramics belong to this family and are commonly referred to as dental porcelains (Craig and Powers, 2002).

The optical properties of different types of porcelain make it aesthetically pleasing as a dental restorative material. Opaque porcelains have very low translucency, allowing them to mask metal substructure surfaces, whereas, enamel porcelains have the highest values of translucency making the restoration look natural (Craig and Powers, 2002). A major advantage of porcelain is that it is chemically very stable and hence, it provides excellent aesthetics that do not deteriorate with time. The thermal conductivity and coefficient of thermal expansion of porcelain is similar to those of enamel and dentine. Porcelain has a high compressive strength and is also a good electrical insulator (Noort, 2002 and Anusavice, 1996).

With all the advantages though, porcelains have a few disadvantages. Porcelains are not ductile with an elastic modulus of approximately 70 Gpa. Even though porcelains have a high compressive strength, they are relatively weak to tensile stresses. Thus, they are brittle and subject to fracture during cementation or chewing (Ferracane, 2001). Another disadvantage is the cost of porcelain restorations. Dental porcelain is comparatively more expensive than other materials. Therefore, ways of reducing the cost of dental porcelain should be explored, which will make it affordable to the poorer patients.

The demand for esthetic restorations has been increasing tremendously in the recent past due to several direct and indirect factors. Increased awareness of the treatment options available is one factor. In an increasingly competitive world, the general population is becoming more aware of their looks, which makes them willing to pay the high price involved with these esthetic restorations. People retain their teeth for much longer than in the past. The demand for porcelain crowns has been increasing at a rate of 50% for every 4 years. Hence, porcelain will be an important restorative material in the years to come (Noort, 2002).

There is a growing demand for porcelain restorations in Malaysia due to the increasing awareness of the masses. One main barrier for these tooth colored restorations is the cost to the patient. At present there is no production of dental porcelain in Malaysia and it has to depend on imported dental porcelains to meet the needs of the population. Since imported dental porcelains are expensive, a good percentage of the local population is unable to afford porcelain restorations. The School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, Penang, Malaysia has set out in collaboration with the School of Dental Sciences, Universiti Sains Malaysia, Kelantan, Malaysia, in the production of a local dental porcelain, which is at present designated as locally produced dental porcelain. The development of this locally produced dental porcelain is carried out with the hope that porcelain restorations will be accessible to more and more people, especially the poorer sections of the society.

The fact that porcelain stays in contact with the oral tissues for prolonged period of time paves the way for research to be conducted on the genotoxicity of porcelain and this has in fact become a routine procedure to be carried out before commercialization of

the product. Considering these facts, this study aims at evaluating the genotoxic potential of locally produced dental porcelain using two different tests.

1.3 Problem Statement

The commercialization of a material is preceded by a series of tests that paves the way for its commercialization. The fate of the biomaterial depends on how it fares in these tests which are set up by international regulatory bodies. The tests are mainly categorized, in order, into *in vitro tests*, animal tests and usage tests which are further divided into other categories. Genotoxicity tests are one of the mandatory tests that a biomaterial has to undergo before it can be commercialized.

The porcelain in the present study was developed by the School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia (USM), Malaysia. The School of Dental Sciences, USM, Malaysia has carried out preliminary *in vitro* and *in vivo* studies on this porcelain. Cytotoxicity of the porcelain material was evaluated by testing on extracts according to ISO 10993-5 (1992) using HOS cell line. Cytotoxicity of porcelain tested was also evaluated by direct contact method according to ISO 10993-5 (1992) using MRC-5 cell line. Cellular response was assessed using MTT assay for measuring the mitochondrial succinate dehydrogenase (SDH) activity. It was concluded that this locally produced dental porcelain is not cytotoxic in terms of *in vitro* cellular response to human osteoblast (HOS) and fibroblast (MRC-5) cell lines and satisfactorily biocompatible, *in vivo,* following a short-term subcutaneous implantation in a rat model.

Dentists quite often place porcelain restorations, which generally last several years or even decades. Hence, it becomes imperative to assess that these restorations do

not cause any genetic damage to the patients as porcelain restorations stay in the oral cavity for prolonged periods of time due to their high survival rate.

1.4 Justification of the study

A number of materials that have previously been thought to be safe are being identified as genotoxic or carcinogenic. The general population is giving more importance to the health and safety aspects of the materials they use or come in contact with, than they used to in the past. International regulatory bodies are also focussing more stringently on the toxic effects of materials that will be put to human use. Before commercialization of any biomaterial or medical device, it has to pass through a series of tests set out by regulatory bodies, of which genotoxicity testing is one. Hence, in this study, the genotoxic potential of locally produced dental porcelain will be evaluated as a step towards commercializing it.

The development of a locally produced dental porcelain which has passed all the tests to reach the stage of commercialization will help to reduce the cost of the material by reducing the cost involved in the import of international porcelains, which will help the poorer sections of the society to a certain extent by reducing the cost of treatment involving these materials. It will also indirectly help in improving the economy of the country and also ensure more jobs, if production can be started in a larger scale.

1.5 Objectives of the study

1.5.1 General objective

To evaluate the genotoxicity of locally produced dental porcelain as one of the initial steps towards the development of a biocompatible restorative dental material

1.5.2 Specific objectives

1. To evaluate the mutagenic effect of locally produced dental porcelain using the Ames *Salmonella* mutagenicity assay

2. To detect the extent of DNA damage caused by locally produced dental porcelain using the single cell gel electrophoresis assay (Comet assay)

1.6 Research hypothesis

Locally produced dental porcelain is non-genotoxic and does not cause mutations or DNA damage.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biomaterials

Artificial biomaterials for the treatment of diseased tissues have been used for more than 2000 years. Wooden teeth and glass eyes are examples of early biomaterials used. Heavy metals such as gold were extensively used in dentistry. In the 1960s, an entirely new field of research was initiated which focussed on the design of new biomaterials with improved biological performance (Leeuwenburgh *et al*., 2008). The single most important factor that distinguishes a biomaterial from any other material is its ability to exist in contact with tissues of the human body without causing an unacceptable degree of harm to that body (Williams, 2008).

Of the several definitions of biomaterials that are used, one of the most commonly accepted is “any substance (other than a drug) or combination of substances synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system which treats, augments, or replaces tissue, organ, or function of the body” (Williams, 1987).

2.2 Ceramics and Porcelain

Until the year 2020, the development of biomaterials that can be used to substitute metals in dental restorations represents the main challenge of future research activities (Holand *et al.*, 2008). Ceramics are usually defined in terms of what they are not: nonmetallic (not metals) and inorganic (not resins). Ceramics are additionally defined as man-made solid objects formed by baking raw materials (minerals) at high

temperatures. The term "ceramics" is derived from the Greek word "keramos" which means "burnt stuff" (Rosenblum and Schulman, 1997).

Porcelain is a specific type of ceramic widely used for nearly 3,000 years and is the most natural-appearing synthetic replacement material for missing tooth substance (Rosenblum and Schulman, 1997). In dentistry, the terms “Porcelain” and “Ceramics” are used interchangeably and dental ceramics are commonly referred to as dental porcelains (Craig and Powers, 2002).

2.2.1 Advantages and disadvantages of dental porcelain

Porcelain is chemically very stable and provides excellent aesthetics that do not deteriorate with time. The thermal conductivity and coefficient of thermal expansion are similar to those of enamel and dentine. Porcelain also has a high compressive strength. It is also a good electrical insulator and has good optical properties (Noort, 2002 and Craig and Powers, 2002).

Even though dental porcelains have acquired a state of near perfection, they still have a number of disadvantages. Their tendency to abrade all structures against which they occlude is the first and most serious disadvantage, especially when the surface of porcelain is unglazed. Glazing of porcelain can minimize such hazardous results but retention of the glazed surface is not guaranteed and once an interruption of the glaze occurs, abrasion will begin. Another problem is that the underlying supporting structures deteriorate more quickly under porcelain-based dentures than under acrylic resin–based dentures. In the case of porcelain, the energy of mastication is readily transferred through the porcelain and into the tissue substrate. In the case of acrylic, a considerable

amount of the energy is absorbed by the polymer rather than being transferred away. Moreover, corrections in contour and finishing must be done in the laboratory, which is an additional problem and repairs of fractures or additions of material must be accomplished extra orally (Leinfelder, 2001). Dental porcelains are brittle and subject to fracture during cementation or chewing because of their weakness to tensile stresses (Ferracane, 2001). Another major disadvantage of dental porcelain restorations is that they are relatively more expensive.

2.2.2 Composition of dental porcelain

Earliest dental porcelains were mixtures of kaolin, feldspar and quartz. In the newer dental porcelains, kaolin has been omitted or very little kaolin is used. The feldspars are mixtures of potassium alumino-silicate ($K_2O.Al_2O_3.6SiO_2$) and sodium alumino-silicate ($Na_2O.Al_2O_3.6SiO_2$). Since feldspars are naturally occurring, the ratio between the potash (K_2O) and the soda (Na_2O) may vary somewhat. The typical oxide composition of a dental porcelain is presented in table 2.1 as given by Noort (2002).

Table 2.1 Typical oxide Composition of a dental porcelain

Material	Weight (%)
Silica	63
Alumina	17
Boric Oxide	7
Potash (K_2O)	7
Soda (Na_2O)	4
Other Oxides	2

The porcelain used by the dental technician is not a simple mixture of the ingredients as shown in table 2.1. These powders are fired once and then, the manufacturer mixes the components, adds additional metal oxides, fuses them and quenches the molten mass in water. This is called 'fritting' and the product is called a 'frit'. This material can be ground easily to produce a fine powder for use by the dental technician (Noort, 2002). Other ingredients of the dental porcelain powders include metal oxides that provide different shades to the porcelain. Metal oxides include titanium oxide for yellowish-brown, manganese oxide for lavender, iron oxide for brown, cobalt oxide for blue, copper or chromium oxides for green and nickel oxide for brown. Tin, titanium and zirconium are used as opacifiers, which block the transmission

of light and reduces transparency of the formulation (Craig and Powers, 2002). Fluorescing agents such as cerium oxide are added to cause porcelain to fluoresce like natural teeth under ultraviolet light (e.g., fluorescent bulbs and sunlight) (Ferracane, 2001).

2.2.3 Classification of dental porcelain

Depending on their application in dentistry, three different types of porcelain compositions are used. One is for denture teeth, one is for ceramo-metal applications and the third for all porcelain restorations (Rosenblum and Schulman, 1997). Porcelain is also classified according to their temperatures of fusion in the dental laboratory (Craig and Powers, 2002).

High-fusing - 1315°C -1370°C

Medium-fusing - 1090°C -1260°C

Low-fusing - 870°C -1065°C

Porcelain can also be classified according to its application (Combe *et al*., 1999).

Core porcelain - Characterized by good mechanical properties

Dentine or body porcelain – It governs the shape and color of the restoration and is more translucent than core porcelain

Enamel porcelain – Used in areas requiring maximum translucency, e.g., at the incisal edge.

2.2.4 Dental applications of porcelain

The dental applications of porcelain are wide and include denture teeth, metal ceramics, veneers, inlays, crowns and anterior bridges (Anusavice, 1996). Other applications include orthodontic brackets and implant materials, including bioactive ceramics (Combe *et al*., 1999).

2.2.5 Evolution of ceramics

Although dental technology existed in Eturia as early as 700 BC and during the Roman first century BC, it remained virtually undeveloped until the eighteenth century. It is obvious that ceramics has acquired a special place in dentistry due to its excellent properties, especially when it comes to esthetics. Although the earliest porcelains are known to date back to thousands of years ago, the history of porcelain as a dental material only goes back just over 200 years (Ferracane, 2001). During the 18th century, several materials were used for artificial teeth which include, human teeth, animal teeth carved to the size and shape of human teeth, ivory and “mineral” or porcelain teeth. Feldspathic dental porcelains were adapted from European triaxial Whiteware formulations (clay-quartz-feldspar). By the 1720s, Europeans had mastered the manufacturing of fine translucent porcelains, comparable to porcelains of the Chinese. In the early 1770s, the first successful porcelain dentures were made at the Guerhard porcelain factory by a Parisian apothecary Alexis Duchateau, with the assistance of a Parisian dentist, Nicholas Dubois de Chemant. In 1808, Giuseppangelo Fonzi introduced individually formed porcelain teeth that contained embedded platinum pins and he called these teeth “terro-metallic incorruptibles” (Kelly *et al*., 1996).

From the time of fabrication of the first successful porcelain dentures in the early 1770s till the middle of the 20th century, the evolution of dental porcelain was quite slow. But since the early 1960s, dental ceramics has developed over the years at a fairly fast pace to reach a current status where no other dental restorative material can outmatch it in terms of biocompatibility and esthetics. Metal-based restorative materials had biocompatibility issues and environmental concerns associated with metals waste and disposal and development of non-metallic restorative materials became a high priority. Ceramics are an ideal candidate for replacing metal-based restorative materials. They provide excellent chemical durability, wear resistance, biocompatibility, environmental friendliness and esthetics (Jeffrey *et al*., 2007).

The introduction of the first successful porcelain-fused-to-metal system was in the early 1960s. Since then, there has been increasing demand for ceramic restorative materials. Till 1990, of the estimated 35 million crowns placed by private practice dentists, more than 71 percent had porcelain as one of the components. Because of its relatively low tensile strength and brittleness, porcelain had been generally fused to a metal substrate to increase resistance to fracture which affected the aesthetics of the porcelain. In addition, some patients have allergic reactions or sensitivity to various metals. These drawbacks, together with the material and labor costs associated with metal substrate fabrication have prompted the development of new all-ceramic systems (Rosenblum and Schulman, 1997).

Of all the abstracts accepted at the World Biomaterials Congress (WBC), 2008, held in Amsterdam, the term “ceramics” ranked 13th among the list of master keywords as shown in Fig. 2.1 (Leeuwenburgh *et al*., 2008).

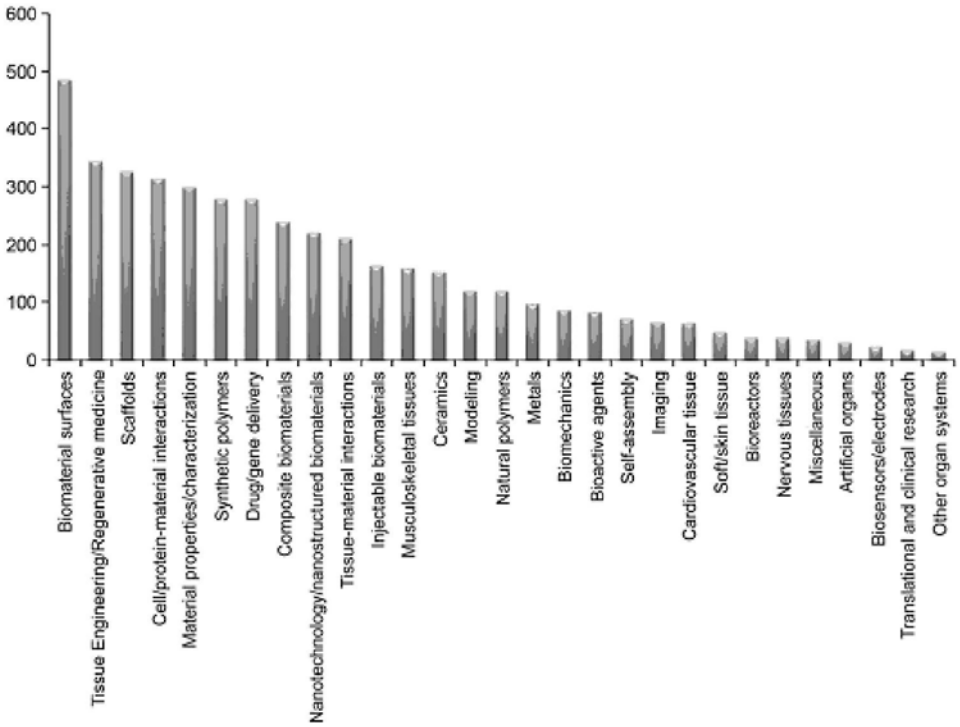

Fig. 2.1 Occurrence of master keywords for abstracts at the World Biomaterials Congress (WBC) in 2008

For more than a decade, all-ceramic crowns have been increasingly used and during the recent years, this use has been extended to include posterior regions. External loading that led to the propagation of cracks starting at flaws and other defects made dental ceramics brittle and weak to tensile stresses (Seghi *et al.*, 1995). Therefore, various types of ceramic materials have been introduced to improve these mechanical properties. IPS Empress 2 (Ivoclar AG, Schaan, Lichtenstein), the In-Ceram alumina and zirconia systems (Vita Zahnfabrik, Bad Sackingen, Germany), Procera AllCeram (Nobelpharma, Goteborg, Sweden) and Denzir (Decim AB, Skelleftea, Sweden) are examples of such ceramics (Sundh and Sjogren, 2004).

2.2.5.1 The In-Ceram system

Of late, there has been tremendous development with regards to dental ceramics. The combination of esthetic veneering porcelains with strong ceramic cores led to the popularity of all-ceramic restorations. Table 2.2 lists the all-ceramic restorations combining esthetic veneering porcelains with strong ceramic cores. Introduced in 1989, the In-Ceram Alumina was the first all-ceramic system which was available for single-unit restorations and 3-unit anterior fixed partial denture prostheses (FPDPs) (Conrad *et al*., 2007). The In-Ceram system uses a reinforcing aluminium oxide core to provide enhanced mechanical properties (Probster, 1992 and Giordano *et al*., 1995). In the in-Ceram system, since very densely stacked alumina particles lead to dispersion strengthening of the ceramic, the resulting bending strengths were the highest reported for dental ceramics (Giordano *et al*., 1995 and Seghi and Sorensen, 1995). The aluminous core provides an enhanced structural support while retaining some translucency and offering good marginal integrity (Pera *et al*., 1994). By using other core materials instead of aluminium oxide core, the aesthetics and strengths of In-Ceram have increased. The In-Ceram Spinell ceramic (Spinell) (Vita Zahnfabrik, Bad Sackingen, Germany), introduced in 1994 has been designated as an inlay/onlay ceramic core material in which aluminium oxide has been substituted with magnesium aluminate ($MgAl_2O_4$), which resulted in improved translucency (Hwang and Yang, 2001). The In-Ceram Zirconia system was developed by adding 34 wt% ZrO_2 partially stabilized zirconia to In-Ceram Alumina (Kou *et al*., 2006) which helped to strengthen the ceramic (Sundh and Sjogren, 2004).

Table 2.2 Ceramic materials and systems and manufacturer-recommended clinical indications (Conrad *et al.*, 2007)

Core Material	System	Manufacturing Techniques	Clinical Indications
Glass Ceramic			
Lithium-disilicate	IPS Empress 2 (Ivoclar Vivadent, Schaan, Liechtenstein)	Heat pressed	Crowns, anterior FPDP
	IPS e.max Press (Ivoclar Vivadent)	Heat pressed	Onlays, 3/4 crowns, crowns, FPDP
(SiO_2-Li_2O)			
Leucite	IPS Empress (Ivoclar Vivadent)	Heat pressed	Onlays, 3/4 crowns, crowns
(SiO_2-Al_2O_3-K_2O)	Optimal Pressable Ceramic (Jeneric Pentron, Wallingford, Conn)	Heat pressed	Onlays, 3/4 crowns, crowns
	IPS ProCAD (Ivoclar Vivadent)	Milled	Onlays, 3/4 crowns, crowns
Feldspathic	VITABLOCS Mark II (VITA Zahnfabrik, Bad Sackingen, Germany)	Milled	Onlays, 3/4 crowns, crowns, veneers
(SiO_2-Al_2O_3-Na_2O-K_2O)	VITA TriLuxe Bloc (VITA Zahnfabrik)	Milled	Onlays, 3/4 crowns, crowns, veneers
	VITABLOCS Esthetic Line (VITA Zahnfabrik)	Milled	Anterior crowns, veneers
Alumina			
Aluminum-oxide	In-Ceram Alumina (VITA Zahnfabrik)	Slip-cast, milled	Crowns, FPDP
(Al_2O_3)	In-Ceram Spinell (VITA Zahnfabrik)	Milled	Crowns
	Synthoceram (CICERO Dental Systems, Hoorn, The Netherlands)	Milled	Onlays, 3/4 crowns, crowns
	In-Ceram Zirconia (VITA Zahnfabrik)	Slip-cast, millled	Crowns, posterior FPDP
	Procera (Nobel Biocare AB, Goteborg, Sweden)	Densely sintered	Veneers, crowns, anterior FPDP
Zirconia			
Yttrium tetragonal	Lava (3M ESPE, St. Paul, Minn)	Green milled, sintered	Crowns, FPDP
zirconia polycrystals	Cercon (Dentsply Ceramco, York Pa)	Green milled, sintered	Crowns, FPDP
(ZrO_2 stabilized by Y_2O_3)	DC-Zirkon (DCS Dental AG, Allschwil, Switzerland)	Milled	Crowns, FPDP
		Milled	Onlays, 3/4 crowns, crowns
	Denzir (Decim AB, Skelleftea, Sweden)	Densely sintered, milled	Crowns, FPDP, implant abutments
	Procera (Nobel Biocare AB)		

2.2.5.2 The Procera system

The concept of the Procera System, developed by Andersson and Oden in 1993, is a computer-assisted design and computer-assisted manufacturing to fabricate an all-ceramic crown composed of a densely sintered, 99.9% high-purity aluminium oxide coping combined with a compatible veneering ceramic (Andersson *et al.*, 1998, Sundh and Sjogren, 2004 and Conrad *et al.*, 2007). Of the alumina-based materials, Procera has

the highest strength and its strength is lower only than zirconia. Due to their increased mechanical properties, zirconia implant abutments (Procera Zirconia Abutment) are now recommended instead of alumina (Conrad *et al*., 2007). The ability to be cemented with standard cements contributed to the rapid acceptance of Procera Zirconia by the profession (Christensen, 2003).

2.2.5.3 The IPS system

The IPS Empress system was introduced in 1991. This leucite-reinforced glass-ceramic material was first described by Wohlwend and Scharer (Qualtrough and Piddock, 1997). In the IPS Empress (Ivoclar-Vivadent, Schaan, Liechtenstein), an injection mold heat-pressed pre-cerammed dentin core reinforced with 40–50% leucite crystals is employed (McLean, 2001). Later on, IPS Empress 2 dentin core reinforced with 60–70% lithium disilicate crystals was developed, with better mechanical properties (McLean and Sced, 1987, Sced and McLean, 1987 and Chen *et al*., 2008).

Numerous clinical studies have confirmed that IPS Empress of the leucite-type fulfills the high standards demanded from aesthetic dental restorations such as inlays, onlays, crowns and veneers. Its translucency, color, fluorescence, and opalescence, in particular, correspond to that of natural teeth and the properties of wear and abrasion resistance match those of natural teeth. One disadvantage of IPS Empress was that, its mechanical strength did not allow the material to be used for dental bridges. The IPS Empress 2 framework material consists of a new microstructure of lithium disilicate crystals embedded in a glassy matrix. The mechanical properties of IPS Empress 2 are improved because the degree of crystallinity of IPS Empress 2 is higher than that of IPS

Empress (Holand *et al*., 2000). The flexural strength of IPS Empress 2 is improved by a factor of 3 over IPS Empress (Conrad *et al*., 2007).

In 1998, the IPS ProCAD (Ivoclar Vivadent) was introduced which is a leucite-reinforced ceramic similar to IPS Empress and has a finer particle size (Fasbinder, 2002). It is designed to be used with the CEREC inLab system (Sirona Dental Systems, Bensheim, Germany) and is available in numerous shades (Fasbinder, 2002, Bindl *et al*., 2003, Attia and Kern, 2004 and Reich *et al*., 2004). In 2005, IPS e.max Press (Ivoclar Vivadent) was introduced as an improved press-ceramic material compared to IPS Empress 2 (Conrad *et al*., 2007).

2.2.5.4 The Zirconia ceramics

Compared to feldspathic ceramics, alumina and zirconia ceramics have better mechanical properties due to their increased crystalline content, chemical composition and microstructure (Tinschert *et al*., 2000, Guazzato *et al*., 2004a and Guazzato *et al*., 2004b). Pure zirconia has three polymorphic forms at atmospheric pressure: monoclinic from room temperature until 1170ºC, tetragonal (1170–2370°C) and cubic (2370–2680°C) (Lazar *et al*., 2008).

Zircon has been known as a gem from ancient times. The name zirconium, comes from the Arabic *Zargon* (golden in colour), which in turn comes from the two Persian words *Zar* (Gold) and *Gun* (Colour). The research on the use of zirconia ceramics as biomaterials started about twenty years ago (Piconi and Maccauro, 1999). Zirconia, which has been recently introduced in prosthetic dentistry for the fabrication of crowns and fixed partial dentures, in combination with CAD/CAM (Computer Aided Design/computer Aided Manufacturing) techniques, holds a unique place amongst oxide

ceramics due to its excellent mechanical properties. The three zirconia-containing ceramic systems used to date in dentistry are yttrium cation-doped tetragonal zirconia polycrystals (3Y-TZP), magnesium cation-doped partially stabilized zirconia (Mg-PSZ) and zirconia-toughened alumina (ZTA) (Denry and Kelly, 2008).

One of the most remarkable innovations in the ceramic field is the concept of stress-induced phase transformation in zirconia ceramics. Zirconia exhibits a transformation toughening mechanism which increases its crack propagation resistance. Yttria-stabilized zirconia ceramics, usually called Y-TZP can exhibit a strength of more than 1 GPa with a toughness of about 6–10MPam$^{1/2}$. One of the most successful applications of Y-TZP ceramics is found in orthopedics, with femoral heads for total hip replacement (Chevalier *et al*., 2004).

Y-TZP has attractive mechanical properties; namely, its chemical and dimensional stability, high mechanical strength and fracture-toughness (Aboushelib *et al*., 2005). Lava (3M ESPE, St. Paul, Minn) which uses a Y-TZP framework with high flexural strength, high fracture toughness and low elastic modulus compared to alumina, exhibits transformation toughening when subjected to tensile stress (Luthardt *et al*., 1999 and Piconi and Maccauro, 1999). A die is scanned by a contact-free optical process for 5 minutes for a crown and 12 minutes for a 3- unit fixed partial denture prosthesis. The CAD software designs an enlarged framework that is milled from softer presintered blanks. After 35 minutes of milling for a crown and 75 minutes for a 3-unit FPDP, the framework can be colored in 1 of 7 shades, followed by sintering in a special automated oven for 8 hours (Piwowarczyk *et al*., 2005). Other CAD/CAM systems available for designing and milling zirconia restorations are Cercon (Dentsply Ceramco, York, Pa),

DCS Precident (DCS Dental AG, Allschwil, Switzerland) and Denzir (Decim AB, Skelleftea, Sweden) (Conrad *et al*., 2007).

Traditional crowns fail after about 6–10 years due to adhesion or fracture failure originating at the interior surface (Hojjatie and Anusavice, 1990, Kelly *et al*., 1990 and Esquivel-Upshaw and Anusavice, 2000). Advances in strengthening bioceramics have been made over decades using several techniques. However, further improvements are needed because mechanical failure is still the limiting factor of their lifetime (Kelly *et al*., 1995 and Denry and Kelly, 2008). Laser interference direct structuring has been proven to scale and improve mechanical properties. It has been shown that this technology can periodically treat and control grain sizes and pore structures on the surface of zirconia without chemically changing the material or introducing phase transformations. The morphology of the ceramic surface is well controllable and the appearance of the material does not change and flexural strength of the dental restorative material is improved significantly (Daniel *et al*., 2008).

Even though the use of porcelain-fused to metal (PFM) is declining slightly as many new all-ceramic and resinbased composite crowns and fixed-prosthesis products flood the market, the venerable PFM crown or fixed prosthesis still dominates the tooth-coloured restoration market (Christensen, 2003).

2.3 Biocompatibility

During the Consensus Conference in Liverpool in 1991 (II Consensus, 1991), biocompatibility was defined as “the ability of a material to perform with an appropriate host response in a specific application” (Gatti and Knowles, 2002). It is necessary to

carry out a variety of different screening methods in order to determine the biocompatibility of a material.

Previously, materials were selected, or occasionally developed, on the basis that they would be non-toxic, non-immunogenic, non-thrombogenic, non-carcinogenic, non-irritant and so on, such a list of negatives becoming, the definition of biocompatibility, by default. A re-evaluation of this position was initiated by three factors. The first was that it became obvious that the response to specific individual materials could be different from one application site to another. Secondly, a number of applications required that the material should specifically react with the tissues rather than be ignored by them, as required in the case of an inert material. Thirdly, some applications required that the material should degrade over time rather than remain indefinitely in the body (Williams, 2008).

Most scientists agree that no material is truly inert in the body (Lemons, 1990). Biocompatibility is an ongoing process and not a static one. It is possible that a dental implant that is osseointegrated today may or may not be osseointegrated in the future. Corrosion or fatigue may cause changes in the material, or the loads placed on the material may change through changes in the occlusion or diet. When a material is placed into living tissue, interactions occur with the complex biologic systems around the material, which depend on the material, the host and the forces and conditions placed on the material (its function). Regardless, the material affects the host and the host affects the material. An absence of such interactions implies the inertness of the material (Wataha, 2001).

Williams (2008) redefined biocompatibility as “the 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 in that specific situation and optimising the clinically relevant performance of that therapy".

Although, in general, dental ceramic materials are generally regarded as being more or less inert, their possible effects of degradation products on biological systems must not be overlooked. The composition, microstructure and physical properties of newly launched ceramic materials are different from those of traditional ones, which may affect the inertness. Safety cannot be inferred from measurements of one ceramic formulation to other compositions or conditions (Anusavice, 1992 and Milleding *et al*., 1999).

2.4 Types of biocompatibility tests

Biomaterials are developed in order to evaluate, treat, augment or replace human tissue, organ or function. Biocompatibility is the main prerequisite for their safe use as medical devices (Kejlova *et al*., 2005). *In vitro* biocompatibility tests are less expensive ways to survey newly developed materials. They simulate biological reactions to materials when they are placed on or into tissues of the body and reduce the probability of surprises when animal usage tests or clinical trials are performed (Hanks *et al*., 1996). In order to assess the biocompatibility of a material, it is necessary to do a battery of tests which includes tests for genotoxicity, depending on the intended use, location and duration the material is to come in contact with the tissues. Biocompatibility is measured with 3 types of biologic tests: *in vitro* tests, animal tests and usage tests (Wataha, 2001).

2.4.1 *In vitro* tests

These tests are performed in a test tube, cell-culture dish, or otherwise outside of a living organism in which cells or bacteria are generally placed in contact with a material. For example, a strain of bacteria may be used to assess the ability of a material to cause mutations (the Ames test). The advantages of *in vitro* biocompatibility tests are, being experimentally controllable, repeatable, fast, relatively inexpensive and relatively simple. Another major advantage is that these tests generally avoid the ethical and legal issues that surround the use of animals and humans for testing. The primary disadvantage of *in vitro* biocompatibility tests is their questionable relevance to the use of a material in the mouth (Wataha, 2001).

2.4.2 Animal tests

In animal tests, the material is placed into an animal, usually a mammal. For example, the material may be implanted into a mouse or placed into the tooth of a rat, dog, cat, sheep, goat or monkey (Wataha, 2001). Animal models allow the evaluation of materials over long time durations and in different tissue qualities (e.g. normal healthy or osteopenic bone) and ages. Not only can the tissues in the immediate vicinity be assessed, but, tissues in remote locations of the implant can also be studied, which is particularly relevant to the study of wear particle debris. In human patients, such debris has been reported to travel into different distant organs such as liver and spleen (Urban *et al*., 2000 and Pearce *et al*., 2007). The disadvantages are that it is difficult to control variables in these tests, questions about the appropriateness of an animal species to represent the human response and that they are time-consuming and expensive. In

animal tests, ethical concerns and animal welfare issues are very important (Wataha, 2001).

2.4.3 Usage tests

The usage test is, by definition, the most relevant biocompatibility test. These tests are essentially clinical trials of a material in which the material is placed into a human volunteer in its final intended use. These tests are expensive, time-consuming, extraordinarily difficult to control, difficult to interpret and may be legally and ethically complex (Wataha, 2001). Usage tests are done only if satisfactory results are obtained in the *in vitro* and animal tests.

2.5 Genotoxicity testing in biomaterials

Historically, biomaterials have always been viewed as inert. However, this view is false, as even the most chemically stable materials undergo some degradation, albeit at very low levels. A number of well-known tests are available, such as the Ames test for *in vitro* gene mutation or the micronucleus test for *in vivo* chromosomal damage (Gatti and Knowles, 2002). The International Standard Organization (ISO) ISO 10993-3 (1992) maintains that certain genotoxicity tests be performed in the biological evaluation of medical devices which consists of a battery of tests, of which, two tests were selected in this study.

The ISO states that, when the genetic toxicity of a medical device has to be experimentally assessed, a series of *in vitro* tests should be used. This series should include at least three assays and at least two of these should preferably use mammalian cells as a target. The tests should preferably cover the three levels of genotoxic effects: