IN VITRO AND IN VIVO EVALUATION OF LOCALLY PRODUCED DENTAL PORCELAIN

By

HAZEM YOUSEF ALI ABU SHARBEH

Thesis submitted in fulfilment of the

requirements for the degree of

Master of Science

July 2007

Dedication

To my Beloved Parents

ACKNOWLEDGEMENTS

I would like to acknowledge with my sincere esteem and gratitude my main supervisor, Dr. Adam Husein, who greatly enriched my knowledge with his exceptional insights of research and clinical aspects of prosthodontic dentistry. I am very fortunate and extremely proud to have been under his supervision.

I am deeply indebted to my co-supervisor, Dr. T.P. Kannan, for his expert supervision, farsighted suggestions and invaluable feedback that guided this work.

I extend my grateful appreciation and thanks to my co-supervisor, Dr. Raja Azman, for his remarkable contribution, persistent support and many fruitful discussions through out my study.

I would like to record my gratitude to Prof. Zainal Arifin Ahamd, for providing the biomaterial, dental porcelain, to carry out this work.

I would like to thank Dr. Mohd Ayub for his great help with statistical analysis. Thanks are also forwarded to Dr. Rajan Saini for his effort and contribution to this work.

My respect and appreciation are given to the staff of Craniofacial Science Laboratory, School of Dental Sciences, USM for their assistance and generous cooperation. These individuals include Asia Abu Bakar, Noor Baizura Ab. Ghani, Siti Fadilah Abdullah and Rodiah Mohd. Radzi.

Finally, I owe a lifetime of gratitude to my parents, whose confidence, support and eternal devotion inspire me and design my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSA	Bovine Serum Albumin
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
ELISA	Enzyme Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
H&E	Haematoxyline and eosin
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]
PBF	Phosphate Buffered Formalin
PBS	Phosphate Buffered Saline
SDH	Succinate dehydrogenase
USM	Universiti Sains Malaysia

IN VITRO AND *IN VIVO* EVALUATION OF LOCALLY PRODUCED DENTAL PORCELAIN

ABSTRACT

Biocompatibility of dental porcelain is of crucial importance to the long-term success of dental prostheses because of its close contact with oral tissues for extended periods. This study was designed to evaluate the biocompatibility of locally produced dental porcelain "test" using *in vitro* and *in vivo* methods. The *in vitro* cytotoxic potential of the test material was evaluated using test on extracts and direct contact test formats as per ISO 10993-5. Cell culture medium was used both as a control and an extractant. Additionally, a commercially available product was included to facilitate comparison of results. HOS cell line (ATCC, USA) was incubated for 72 hours with the extraction solutions of the test and commercial materials powders at various concentrations (50, 100, 150, 200 and 250 mg/ml). Similarly, MRC-5 cell line (ATCC, USA) was incubated for 72 hours with the test and commercial discs (5 mm in diameter and 2 mm thick). Aging process was carried out by submerging the discs into 3% Bovine Serum Albumin (BSA) solution for 96 hours followed by reincubation with the MRC-5 cell line. Cellular response was assessed using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay for measuring the mitochondrial succinate dehydrogenase (SDH) activity of living cells. Optical densities were measured at 570 nm using ELISA (Enzyme Linked Immunosorbent Assay) reader and then converted to a percentage of the control for each cell culture well. Results were compared using one-way ANOVA and Tukey Post-Hoc comparisons at a significance level of $P < 0.05$. For *in vivo* study, materials discs were implanted subcutaneously into 12

Sprague-Dawley male albino rats, which were sacrificed in groups of 3 at 1, 2, 3 and 4 weeks after implantation. A semi-quantitative histological analysis of the tissue surrounding implanted discs was done under an image analyzer. *In vitro* cytotoxicity test on extracts showed that the test material was significantly different from the control at concentrations higher than 150 mg/ml. The mean(SD) percentage of cellular viability was 102.2(12.8) for 50 mg/ml, 98.9(10.3) for 100 mg/ml, 89.4(15.8) for 150 mg/ml, 86.7(14.6) for 200 mg/ml and 82.9(16.5) for 250 mg/ml. However, the commercial product was significantly different from the control at concentrations higher than 50 mg/ml. The mean(SD) percentage of cellular viability was 95.6(14.5) for 50 mg/ml, 85.4(12.4) for 100 mg/ml, 81.5(14.8) for 150 mg/ml, 80.7(14.5) for 200 mg/ml and 79.3(10.9) for 250 mg/ml. Direct test showed that the materials after aging were not significantly different from the control. The mean(SD) percentage of cellular viability was 89.2(13.4) for the test and 89.4(14.6) for the commercial. The materials tested were already significantly different from the control before the conditioning of BSA. The mean(SD) percentage of cellular viability was 88.5(12.1) for the test and 88.5(8.9) for the commercial. However, in both tests, the materials caused mild suppression of SDH activity (<25% of control), which is considered to be accepted clinically. *In vivo* subcutaneous implantation showed that the macrophage was clearly the dominant cell type at the implant surface at the first week after implantation, followed by a gradual decrease as the implantation period increased. On the contrary, fibroblasts and fibrocytes were the dominant cell types in the tissue surrounding test and commercial discs at the third and fourth week after implantation. These findings, from pathological point of view, might be an indicator of biocompatibility.

PENILAIAN PORSELIN PERGIGIAN PENGHASILAN TEMPATAN SECARA *IN VITRO* DAN *IN VIVO*

ABSTRAK

Biokompatibiliti porselin pergigian amat penting untuk kejayaan jangka panjang prostesis pergigian disebabkan persentuhan dengan tisu oral dalam jangka masa yang panjang. Kajian ini direka bentuk untuk menilai ujian biokompatibiliti porselin pergigian keluaran tempatan menggunakan kaedah *in vitro* dan *in vivo.* Bagi menilai potensi sitotoksik kaedah *in vitro,* bahan yang diuji dinilai menggunakan ujian ekstrak dan sentuh langsung mengikut format ISO 10993-5. Medium kultur sel digunakan sebagai kawalan dan ekstraktan. Satu porselin pergigian komersil digunakan untuk tujuan perbandingan. Inkubasi selama 72 jam dilakukan ke atas HOS sel line (ATCC, USA) menggunakan larutan ekstraksi daripada sebuk bahan ujian dan bahan komersil pada tahap kepekatan yang berbeza (50, 100, 150, 200 and 250 mg/ml). MRC-5 sel line juga diinkubasi selama 72 jam untuk bahan ujian dan bahan komersil dalam bentuk ceper (bergaris pusat 5 mm dan ketebalan 2 mm). Proses penuaan dijalankan dengan menenggelamkan ceper tersebut ke dalam larutan 3% Serum Albumin Bovin (BSA) selama 96 jam dan proses inkubasi dilakukan semula dengan MRC-5 sel line. Reaksi sel dianalisa menggunakan MTT [3-(4,5-Dimetilthiazol-2-yl)-2,5-Bromide Difeniltetrazolium] assai bagi tujuan pengukuran aktiviti mitokondrial suksinat dehidrogenase (SDH) sel yang hidup. Ketumpatan beroptik diukur pada 570nm menggunakan bacaan ELISA (Enzyme Linked Immunosoben assay) dan ditukar kepada peratusan kawalan bagi setiap "well" di dalam kultur sel. Hasil-hasil kajian dibanding menggunakan one-way ANOVA dan Tukey Post-Hoc pada tahap makna $P < 0.05$. Untuk kajian *in vivo,*

bahan-bahan dalam bentuk ceper diimplankan di bawah lapisan kulit ke dalam 12 ekor tikus jantan jenis albino Sprague Dawley. Tikus-tikus itu kemudiannnya dikorbankan dalam kumpulan bertiga pada 1, 2, 3 dan 4 minggu selepas implantasi. Analisa histologi secara separa kuantitatif ke atas tisu sekeliling ceper dibuat menggunakan alat penganalisa imej. Bagi kepekatan melebihi 150mg/ml ujian sitotoksisiti secara *in vitro* ke atas ekstrak menunjukkan terdapat bebezaan yang nyata di antara bahan ujian dan bahan kawalan. Peratusan min(SD) sel hidup adalah 102.2(12.8) untuk 50 mg/ml, 98.9(10.3) untuk 100 mg/ml, 89.4(15.8) untuk 150 mg/ml, 86.7(14.6) untuk 200 mg/ml dan 82.9(16.5) untuk 250 mg/ml. Walau bagaimanapun, produk komersil menunjukkan perbezaan yang nyata berbanding kawalan pada tahap kepekatan melebihi 50 mg/ml. Peratusan min(SD) sel hidup adalah 95.6(14.5) untuk 50 mg/ml, 85.4(12.4) untuk 100 mg/ml, 81.5(14.8) untuk 150 mg/ml, 80.7(14.5) untuk 200 mg/ml dan 79.3(10.9) untuk 250 mg/ml. Ujian langsung menunjukkan tiada perbezaan yang nyata di antara bahan yang melalui proses penuaan dan bahan kawalan. Peratusan min(SD) bagi sel hidup adalah 89.2(13.4) untuk bahan ujian dan 89.4(14.6) untuk bahan kawalan. Bahan yang telah diuji sememangnya berbeza dari kawalan sebelum dikondisikan dengan BSA. Peratusan min(SD) sel hidup adalah 88.5(12.1) untuk bahan ujian dan 88.5(8.9) untuk bahan komersil. Namun begitu, dalam kedua-dua ujian, bahan yang digunakan telah menyebabkan penindasan secara ringan kepada aktiviti SDH (<25% bagi kawalan), suatu nilai yang boleh diterima pada tahap klinikal. Ujian *in vivo* menunjukkan dengan jelas bahawa macrophage adalah sel yang dominan pada permukaan ceper implan dalam minggu pertama implantasi, kemudian diikuti dengan penurunan yang sekata dengan peningkatan masa

implantasi. Sebaliknya, fibroblast dan fibrosit adalah sel yang dominan dalam tisu sekeliling ceper implan bahan ujian dan bahan komersil pada minggu ketiga dan keempat implantasi. Dari pandangan patologikal, keputusan ini menunjukkan kemungkinan terdapat biokompatabiliti.

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

A ceramic is a compound formed by the union of metallic and non metallic elements. Most of these materials are oxides, formed by the union of oxygen with metals such as silicon, aluminum, calcium and magnesium (Ferracane, 2001). Porcelain is a specific type of ceramics, which has been widely used for nearly 3,000 years; traditionally it is composed of blends of three naturally occurring minerals: pure white clay, quartz and feldspar. When these three ingredients are pulverized, blended, formed into shapes and baked, they compose of what is known as white-wares, so named because their colour is white after they are baked (Rosenblum and Schulman, 1997). Although many of the materials used in dentistry are ceramics, the term is commonly used to refer to porcelain and its derivatives.

Because of its translucency, porcelain is considered to be the most natural-looking restorative material for esthetic rehabilitation. It can be manufactured with such colours that are indistinguishable from the natural dentition. Furthermore, having an outer surface of metal oxides, porcelain does not permit the absorption of dental plaque byproducts, eliminating therefore the problem of surface colour changes, unlike composite resin (Veronese *et al.*, 2006).

Porcelain differs from metals in that it contains ionic and/or covalent bonds. In contrast to metals, it is usually an electrical insulator because it has few electrons. It is very often transparent or translucent to light and is usually chemically very stable under a wide range of environmental conditions (Combe *et al.*, 1999).

Porcelain now plays a vital role in restorative dentistry. The demands of patients for tooth coloured restorations and the availability of new types of dental porcelain have increased its uses in a variety of restorative situations (Messer *et al.*, 2003).

Common uses of porcelain include full coverage as crowns, inlays and onlays, porcelain bridges, veneering agents, castable ceramics and porcelain fused to metals restorations. Based upon some interesting technology, porcelain can also be cast into molds in the same way as conventional base metals or gold alloys (Leinfelder, 2000).

However, porcelain is a brittle material and has low fracture resistance and relatively low flexural strength (Sundh *et al.*, 2005). The potential for abrading structures against which it occludes and the difficulty in resurfacing and polishing the glazed surface continue to be the biggest problem associated with this commonly used clinical material (Leinfelder, 2000). In addition, porcelain restorations require substantial reduction of the tooth to provide the bulk of material necessary for the translucency and optical properties associated with optimum esthetics. In all-ceramic restorations, the reduction is also necessary to provide the bulk of material required for adequate strength of the restoration. This requirement is also an undesirable side-effect of the material (Mackert, 1992).

Today, in the development of an intraoral material, one must consider not only the strength, esthetics and functional aspects of the material but its biocompatibility as well. Since all materials used in medicine and dentistry interact with tissues, producing changes in both the material and the surrounding tissues (O'Brien, 2002), preclinical assessments of the toxic potential of such materials or

components is needed to minimize the potential hazard to the patient. Thus, biocompatibility is important to manufacturers and materials scientists.

1.2 Problem statement

At no time in recent history has the interest in esthetics, biological safety and the relative cost and efficacy of dental care been greater than at the present time. As people retain their teeth for much longer time than in the past, the need for esthetically acceptable restorations is continuing to increase.

In view of porcelain's desirable esthetic properties and biocompatibility, it is understandable that the demand for porcelain crowns has been increasing at the rate of 50% every 4 years (Noort, 2002). Therefore, porcelain will continue to be an important restorative material for many years to come.

However, porcelain restorations are currently expensive, primarily as a result of the labour-intensive nature of their fabrication. Porcelain crowns, bridges and veneers require a high degree of skills and several steps in their fabrication are critical to the success of these restorations. In addition, porcelain restorations require the exposure of patients to additional materials such as impression and temporary filling materials.

Unfortunately, since there is no production of porcelain in Malaysia; the country has to import this material. Consequently, this leads to an increase in its price and subsequently the treatment cost as well. From this point of view, the School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia (USM), Malaysia, decided to develop locally produced dental porcelain in order to minimize the import expense and therefore reduce the cost of dental treatment.

As for the current status of the locally produced dental porcelain, the School of Materials and Mineral Resources Engineering is working on the evaluation of its physical and mechanical properties, such as X-ray diffraction (XRD), X-ray fluorescence (XRF), scanning electron microscope (SEM), thermal expansion coefficients (TEC) and fire shrinkage.

In fact, testing the biological effect of dental porcelain has lagged far behind the characterization of its mechanical and physical properties (Sjogren *et al.*, 2000 and Messer *et al.*, 2003). Most new porcelain materials have not been tested for biological response with the same scrutiny as has been applied to dental casting alloys (Wataha, 2002 and Schmalz and Garhammer, 2002), amalgams (Wataha *et al.*, 1994), or composites (Wataha *et al.*, 1999). Yet, the biocompatibility of porcelain is critical to the long-term success of dental prosthesis because porcelain restorations are in close contact with oral tissues for extended period. The issue has profound ethical, social, technical and legal effects on prosthodontic practice.

1.3 Justification of the study

The presence of proven biocompatible as well as durable locally produced dental porcelain, which can be provided to the patients in a cost-effective manner, may limit the import of the material, which in turn helps to reduce its cost and ultimately reduce the cost of dental treatment.

1.4 Objectives of study

1.4.1 General objective

To evaluate the biocompatibility of locally produced dental porcelain in order to improve the understanding of material characteristics and increase the ability to develop a more biocompatible product

1.4.2 Specific objectives

1.4.2.1 *In vitro* study

1. To detect the potential ability of locally produced dental porcelain and a commercially available product in inducing toxic effects as observed at the cellular level

2. To detect the potential cellular response changes to the materials tested following the exposure to a biological medium

1.4.2.2 *In vivo* study

To investigate cellular responses to locally produced dental porcelain and a commercially available product after a short-term subcutaneous implantation in a rat model

1.5 Research hypothesis

Locally produced dental porcelain is a satisfactory biocompatible material in terms of toxicity potential at the cellular level and short-term cellular responses to subcutaneous implantation in a rat model.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Biomaterials

Biomaterials can be defined as any substances "other than a drug" or combinations of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system, which treats, augments or replaces any tissue, organ or function of the body (von Recum and LaBerge, 1995).

Dentistry has a wider variety of biomaterials at their disposal than any other profession. Rigid polymers, elastomers, metals, alloys, ceramics, inorganic salts and composite materials are commonly encountered.

2.2 Ceramics

Ceramics have been widely used in biomedical applications for load bearing implants and the dental industry. Most members of this group are characterized by a high Young's modulus, very low elasticity and a hard and brittle surface (Suchanek and Yoshimura, 1998).

2.2.1 Development of dental ceramics

The word ceramic is derived from the Greek word *"keramos"*, which literally means "burnt stuff" but which has come to mean more specifically a material produced by burning or firing. They are usually defined in terms of what they are not: nonmetallic "not metals" and inorganic "not resin". To distinguish them from rocks and minerals, the vast majority of which are also inorganic and nonmetallic, ceramics are additionally defined as man-made objects formed by baking row materials "minerals" at high temperatures (Rosenblum and Schulman, 1997).

Historically, three basic types of ceramic materials were developed. Earthenware, fired at high temperatures up to 900 ^{0}C, is relatively porous as the sintering process only just managed to fuse the particles of clay where they touch. This problem was overcome eventually by fusing a thin layer of a glassy material over the surface of the pot. This technology was used as far back as 5500 BC in various places. Stoneware, which appeared in China in about 100 BC, is fired at a higher temperature than earthenware, which results in both higher strength and also renders the material impervious to water. The third material is porcelain, obtained by fluxing white China clay with "China stone" to produce white translucent stoneware. This material was developed in China in about 1000 AD and it was much stronger than the earthenware and stoneware. Translucent vessels could be produced with very thin walls of only 2 to 3 mm through which light could shine (Jones, 1985 and Noort, 2002).

The early Chinese porcelain was called hard-paste porcelain. This hard paste product is often referred to as "true" porcelain and was highly translucent. The composition of traditional hard-paste porcelain is approximately 50% kaolin, known as china clay, ($Al_2O_3SiO_22H_2O$), 25% feldspar ($K_2OAl_2O_36SiO_2$) and 25% quartz (SiO_2). The first porcelains used in dentistry were originally based upon the triaxial porcelain composition, which falls into the zone of the K_2O-Al_2O_3-SiO_2 phase diagram (Jones, 1985).

Although the earliest examples of porcelain are known to date back a thousands of years ago, the history of porcelain as a dental material only goes back just over 200 years (Ferracane, 2001 and Anusavice, 2003).

The first porcelain tooth material was patented in 1789 by a French dentist "de Chemant" in collaboration with a French pharmacist "Duchateau". The product, an improved version of "mineral paste teeth" that was produced in 1774 by Duchateau, was introduced in England soon thereafter by de Chemant. However, this baked compound was not used to produce individual teeth because there was no effective way at that time to attach the teeth to a denture base material (Noort, 2002 and Anusavice, 2003).

In 1808, individually formed porcelain denture teeth that contained embedded platinum pins were introduced in Paris by Fonzi. Fonzi, an Italian dentist, called these teeth "terro-metalic" and their esthetic and mechanical versatility provided a major advance in prosthetic dentistry (Kelly *et al.*, 1996).

Feldspathic porcelains have been first used to produce porcelain jacket crowns to dentistry ever since Charles Land (1903) described a technique for fabricating ceramic crowns using a platinum foil matrix and high-fusing feldspathic porcelain. Although these crowns are highly esthetic, their inability to withstand tensile and shear forces remains a major limitation (Moffa, 1988).

Alumina ceramic Al_2O_3 has characteristics of high hardness and high abrasion resistance. The reasons for the excellent wear and friction behavior of Al_2O_3 are associated with the surface energy and surface smoothness of this ceramic. Abrasion resistance, strength and chemical inertness of alumina have made it to be recognized as a ceramic for dental and bone implants (Thamaraiselvi and Rajeswari, 2004).

In the early 1950s, the ceramics employed in the conventional porcelain jacket crown were medium to high fusing feldspathic porcelains. Due to the relatively low

strength of this type of porcelain, an alumina-reinforced porcelain core material was developed by McLean for the fabrication of porcelain jacket crowns. The alumina-reinforced crowns were regarded as providing better esthetics for anterior teeth than metal-ceramic crowns (Rizkallah and Jones, 2004).

Aluminous core porcelain is a typical example of strengthening by dispersion of a crystalline phase. Alumina has a high modulus of elasticity and high fracture toughness. Its dispersion in a glassy matrix of similar thermal expansion coefficient leads to a significant strengthening of the core. The first alumina core porcelain provided by McLean contained 40 to 50% alumina by weight. The core was baked on a platinum foil and later veneered with matched-expansion porcelain (Denry, 1996).

However, because of the large sintering shrinkage (approximately 15-25%) of the aluminous porcelain core material at its high firing temperature and the use of a 20-25 μm thick platinum foil, excellent marginal adaptation was difficult to achieve except by highly skilled laboratory technicians. Therefore, the main indication for the use of aluminous porcelain crowns is the restoration of maxillary anterior crowns when aesthetic is of paramount importance and when no other ceramic product is available (Anusavice, 2003).

Zirconia ceramics have several advantages over other ceramic materials. Compared with alumina, stabilized zirconia has the potential advantages of a lower elastic modulus, higher strength, better wear properties and higher fracture toughness (Eliades *et al.*, 2003).

The research on the use of zirconia (ZrO_2) as a biomaterial started about twenty years ago and it is now mainly used in the manufacturing of ball heads for total hip

replacements. However, developments are in progress for applications in other medical devices (Piconi and Maccauro, 1999).

Porcelain fused to metal (metal-ceramic) technology was first described in 1956 and patented in 1962. Alloys were produced with melting points sufficiently high to resist the firing of porcelain. The first alloys had a high noble metal content of around 98% with iron, indium and tin used for hardening and to create a superficial oxide layer to which the ceramic could be bonded (Wassell *et al.*, 2002). During this period, substantial improvements in alloys and veneering porcelains have resulted in widespread acceptance of metal-ceramic restorations. Continued research efforts have led to a more detailed and practical understanding of metal-ceramic systems.

In 1997, adhesive metal-ceramic crown was introduced as an alternative to conventional crowns. The crown requires minimal preparation of the tooth and consists of a metal baking and porcelain labial surface. They are advocated for both anterior and posterior restorations and in the case of posterior restorations; the occlusal surface may be metallic to reduce abrasive damage to opposing teeth. Laboratory procedures involve lost wax casting and porcelain firing on refractory dies. The measurement of strength and durability of such restorations is suggested for further studies (Whitters *et al.*, 1999).

Leucite-based frits, a potassium-aluminum-silicate phase, have been used since the early 1960s in metal-ceramic restorations because of the high coefficient of thermal expansion of leucite, which rises the bulk of porcelain thermal expansion to a level where it is compatible with the metal substrate. However, nowadays,

leucite is also used in all-ceramic restorations, not for thermal compatibility, but as a reinforcing material as it may act as a crack deflector (Cesar *et al.*, 2005).

Although the porcelain fused to metal systems have high strength, the opacity of the metal substructure has encouraged the development of all-ceramic core materials containing crystalline components, which are stronger than the traditional "predominantly glassy amorphous" feldspathic porcelain. This type of core material can then be veneered with a more translucent ceramic material (Jones, 1998). The all-ceramic systems described by Jones (1998) are presented in table 2.1.

2.2.2 Composition of traditional dental porcelain

In the dental laboratory, traditional porcelain for dental restorations is used in fine powder form. Porcelain powder is manufactured from three primary ingredients: feldspar (K_2O-Al_2O_3-$6SiO_2$), silica (SiO_2) and alumina (Al_2O_3). These crystalline ingredients are heated together with fluxes such as sodium carbonate or potassium carbonate. The fluxes cause the other raw ingredients to form a glass that is not crystalline and melts at a relatively low temperature compared with the row materials (Craig *et al.*, 2004).

Feldspar is a range of natural crystalline minerals of aluminum, silicon and oxygen combined with smaller amounts of sodium, potassium and calcium. The presence of the alkalis controls the softening point of feldspar, which is lowered by increased sodium but increases with potassium content (Jones, 1998).

Table 2.1: All-ceramic systems used for producing crowns and inlays

Type	Description	Process	Examples
Aluminous core porcelain	Dispersion strengthening by high modulus crystalline alumina particles in a glass matrix	Condensation of frit sintering, veneering with a more translucent feldspathic frit	Vitadur and Hi - Ceram
Leucite reinforced porcelain	Dispersion strengthening by crystalline leucite particles in a glass matrix	Pressure moulded frit sintered, veneering with translucent feldspathic frit	IPS Empress and Optec
Glass infiltrated alumina	Highly filled crystalline alumina in glass matrix	Slip-cast slurry in porous mould sintered, infiltration by low viscosity glass, veneered with more translucent feldspathic frit	In – Ceram Alumina
Glass infiltrated Spinel	Highly filled crystalline spinel in glass matrix	Slip-cast slurry in porous mould sintered, infiltration by low viscosity glass, veneered with more translucent feldspathic frit	In – Ceram Spinel
Castable glass	A glass which can be crystallized by heat treatment	Cast using lost-wax investment method followed by heat-treatment to precipitate a crystalline phase	Dicor
Glass Ceramic CAD-CAM	A glass ceramic machinable material	Milling (mostly inlays) by computer control	Dicor, MGC and Vitabloc

In dental feldspathic porcelain, feldspar makes up 75 to 85% of the porcelain and serves as the amorphous and glassy phase that holds the silica mineral crystals together. Kaolin clay (aluminum silicate), added at only 3 to 5%, serves as an opaquing agent and enhances the workability of porcelain. In contrast to dental porcelains, decorative porcelain contains high concentrations of kaolin and low concentrations of feldspar (Ferracane, 2001).

Some contemporary feldspathic materials are reinforced by the presence of up to 45% of leucite, which has the formula $KAlSi_2O_6$. In terms of coefficient of the thermal expansion, there is a considerable mismatch between that of leucite (22 parts per million per ^0C) and the glassy phase (8 parts per million per ^0C). On cooling, there are resultant compressive stresses in the glass around the leucite particles. Such stresses act as crack deflectors, resulting in a material with greater modulus of rupture (Combe *et al.*, 1999).

However, only a lower leucite contents "from zero to 20%" has a strengthen effect on the material, as the residual stresses arise from the addition of high quantities of leucite "from 30 to 50%" may cause spontaneous microcracking within the material during cooling, which leads to a significant reduction in the strength values of the porcelain (Cesar *et al.*, 2005).

The recent introduction of the pressed leucite reinforced ceramic system, IPS Empress, has leucite in a different role. This material relies on an increased volume of fine leucite particles to increase flexural strength. Similar versions using finely dispersed leucite grains to increase toughness, strength and modify wear patterns and rates to make them similar to enamel wear rates are now available for metal-ceramic restorations (Ironside and Swain, 1998).

To allow the fabrication of porcelain restorations in tooth colours, small quantities of colouring agents are added to porcelain powders. These pigments "also called colour frits" are derived from metallic oxides that are ground and mixed with feldspar powder; this mixture is then fired and fused to a glass and the pigmented glass is then reground to powder. Commonly used oxides include tin oxide for opaquing, iron oxide for brown shading, copper oxide for blue, nickel oxide for brown and manganese oxide for purple (Nathanson *et al.*, 1999).

Some feldspathic porcelains are supplied as "opalescent porcelains". Opalescence is a light scattering effect achieved by the addition of very small amounts of metallic oxides having a higher refractive index and a particle size near to that of the wave-length of light. Since natural teeth can display some opalescence, the availability of opalescent porcelains adds further to the ability to match natural tooth appearance in every way (McCabe and Walls, 1998).

2.2.3 Classifications of dental porcelain

The porcelain powder is mixed with water to form slurry, which is adapted as a plastic mass to the requisite shape prior to firing in an oven. According to a standard specification, the available materials may be classified according to their temperature of fusion in the dental laboratory as follows (O'Brien, 2002).

- High fusing: 1,288 °C to 1,371 °C - (2,350 °F to 2,500 °F)
- Medium fusing: 1,093 °C to 1,260 °C - (2,000 °F to 2,300 °F)
- Low fusing: 660 °C to 1,066 °C - (1,220 °F to 1,950 °F)

Classification can also be made according to application (Combe and Grant, 1992).

• Core porcelain, which is the basis of porcelain jacket crowns and characterized by high mechanical properties.

• Dentine or body porcelain, which is more translucent than the above and largely governs the shape and colour of restorations.

• Enamel porcelain, which is used in areas requiring maximum translucency, for example, at the incisal edge.

2.3 Biocompatibility of biomaterials

Biomaterials and medical devices constitute an extremely diverse and heterogeneous category of items. Because the use of these products normally entails their direct or indirect contact with patients, there is an obligation on the part of manufacturers to establish the safety of their products before they are marketed. Medical device safety evaluation assesses the risk of adverse health effects due to normal use and likely misuse of a device. Adverse health effects could result from the exposure to the materials from which a device is made. Therefore, biological evaluation such materials or components is needed (Bollen and Svendsen, 1997).

2.3.1 Definition of biocompatibility

The term biocompatibility is defined as the ability of a material to elicit an appropriate biological response in a given application (Williams, 1987). If examined closely, the definition of biocompatibility implies an interaction among a host, a material and an expected function of the material (fig. 2.1). Biocompatibility exists only when all 3 factors are considered and it can change if any of these factors change (Wataha, 2001).

In practice, however, no material is totally inert in a biological environment. Thus, an appropriate host response, which may be a complex series of events, is important. Furthermore, a material may degrade *in situ*, so biocompatibility of its degradation products also needs to be noted (Combe *et al.*, 1999).

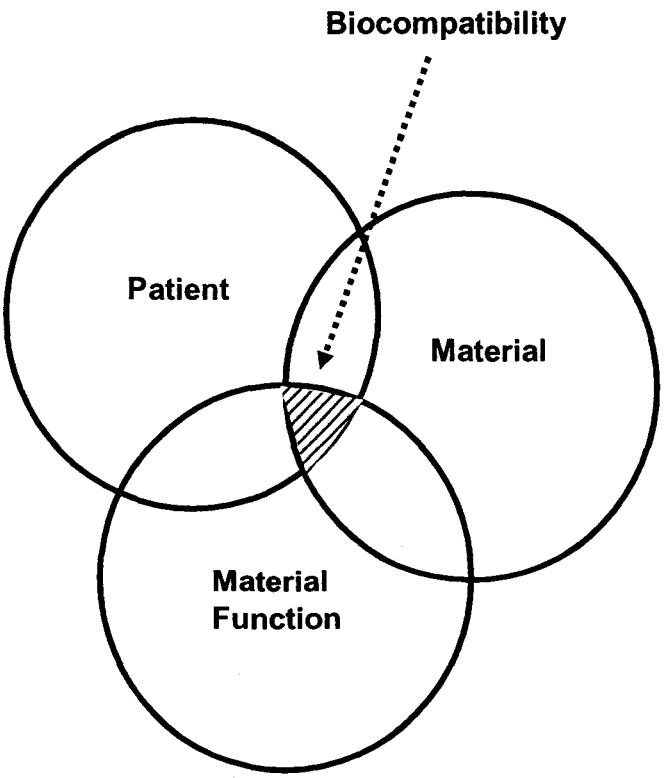

Fig. 2.1: Interactions between host, material and application of material

2.3.2 Assessment of biocompatibility

Measuring the biocompatibility of a material is not simple and the methods of measurement are evolving rapidly as more is known about the interactions between dental materials and oral tissues and as technologies for testing improve. Traditionally, there are three basic types of tests used to measure the biocompatibility of dental materials: the *in vitro* tests, the animal tests and the

usage tests performed either in animals or in humans (Schmalz, 1997). Each of these tests has advantages and disadvantages and each is used to some extent to evaluate a material before it is made available commercially.

2.3.2.1 Primary (*in vitro*) tests

The major category of tests for the initial evaluation of materials is the cytotoxicity test. From the earliest periods of cell culture, there has been an emphasis on establishing standards by gathering data, which could be validated among several labs in the same way that material properties could be validated by chemical and physical testing and understanding of the general nature of the reactions, which take place in response to materials, usually via direct contact. The use of *in vitro* techniques to study the toxicity of various synthetic materials began some 30 years after tissue culture was first established. Various investigators began to apply organ and tissue culture techniques to toxicological problems in the 1950s and 1960s (Hanks *et al.*, 1996).

A critical term in the evaluation of biomaterials biocompatibility is toxicity. A toxic material is defined as something that releases a chemical in a quantity sufficient to kill cells directly or indirectly by inhibiting important metabolic pathways. A cytotoxic material is a term used to define an agent that is "cell killing". The percentage of cell death is an indication of the potency "dose" of the agent. Although several factors may contribute to the toxicity of a chemical, the dose of the agent delivered to the cell is the most important factor (Guelcher and Hollinger, 2005).

Cell culture studies are useful tools for dental materials investigations. Cell culture methods are better standardized and reproducible. They are rapid and easy to perform at relatively low costs (Schmalz, 1994).

The tests of the row materials and formulation must be repetitive and consistent. For ethical and legal reasons, *in vitro* toxicity assessments have been proposed as an alternative approach to animal use (Verhulst *et al.*, 1998). Animal models are essential in providing information on biological reactions to biomaterials but their results are difficult to interpret at the cellular level because of the numerous and complex events that occur at the insertion of a foreign material into a bloody wound site. *In vitro* approaches, however, represent ideal systems for studying cell behavior with materials, thus avoiding the complications and interferences encountered *in vivo* (Josset *et al.*, 1999).

The common conception of cytotoxicity is that the cell is killed by the cytotoxin. Cytotoxins may have reversible or irreversible effects and their effects may be immediate or delayed up to several weeks. There are major differences between (1) physico-chemical damage, which may produce an instantaneous loss of viability, (2) an environmental or pharmaceutical cytotoxin, which may have a slight but progressive effect on metabolism over a period of hours or longer and (3) a loss of reproductive potential, e.g. as a result of irradiation, which may not be immediately apparent in the reduction of cellular viability (Stacey *et al.*, 2001).

The high sensitivity of cytotoxicity tests is due to the isolation of the test cells in cultures and the absence of the protective mechanisms that assist cells within the body. Results of these tests correlate reasonably well with short-term implant studies. However, they do not necessarily correlate with other standard tests of biocompatibility that are designed to examine specific end points (such as sensitization) or that use extracts prepared under more rigorous conditions (for example, at 121°C in saline) (Wallin and Arscott, 1998).

2.3.2.1.1 Cellular functions evaluation

By using cell cultures, the changes of some cellular functions induced by artificial materials or devices are evaluated as described in table 2.2 by Cenni *et al.* (1999). In the screening stage, the functions shared by all types of cells are investigated. In the supplementary tests, specific functions are studied; the cells of the same type that will face the implant *in vivo* are used whenever possible (Cenni *et al.*, 1999).

Table 2.2: Functions common to all cells and evaluation methods

Function	Tests
Enzyme activity	- Reduction of tetrazolium salts (MTT test) - Oxide reduction activity (Alamar blue assay)
Cellular viability	- Neutral red uptake - Propidium iodide staining
Cellular growth	- Cell count - Crystal violet or amidoblack staining - Total protein assay - DNA assay - ^{3}H-TDR uptake

2.3.2.2 Secondary (animal) tests

The obviousness that animals are the key to *in vivo* testing is of striking importance and readily acknowledged by the community of individuals involved in biomaterial testing. Each animal toxicity test measures one of the many aspects of

toxicity, such as acute and chronic systemic toxicity, local irritancy to the skin and eye, teratogenicity, etc (Georgieva, 2003).

Sensitization or hypersensitivity tests are indicated to detect materials, which are capable of interacting with the body's immune system and inducing specific hypersensitivity, such that, on subsequent exposure to the same material characteristic, allergic effects are produced. They are used by exposing the skin to the material or taking extracts from the device or materials and injecting or topically applying them to the animal (ISO 10993-10, 1992).

Irritation tests evaluate the reaction to a single, repeated or continuous exposure of materials that may produce skin, mucosal or eye irritation as a localized non-specific tissue response characterized by the usual signs of inflammations. These tests do not involve the immunological mechanism in the body (ISO 10993-10, 1992).

Systemic toxicity tests evaluate the potential adverse effects of medical devices on the body's organs and tissues that are remote from the site of contact. Depending on the type of device being tested, topical, inhalation, intravenous, intra peritoneal or oral administration of extracts or implantation of the device in the animal is observed for toxicity. There are four categories of toxicity evaluations, which include acute (24 hours), subacute (14 to 28 days), subchronic (90 days or 10% of the animal life span) and chronic (ISO 10993-11, 1993).

Implantation tests are the most direct means of evaluating the potential effects of materials on the surrounding living tissue. Samples are cut to size, if necessary; sterilized and implanted aseptically inside the body of a laboratory animal. After a period of time ranging from weeks to months, the implant sites are examined.

Attention is focussed entirely on local effects that occur in response to the presence of the test material that has been in intimate contact with living tissue. Part six of the biocompatibility standards developed by the International Organization for Standardization, ISO 10093-6, presents the general considerations that must be taken into account when conducting such implant studies. It describes the selection of species, appropriate tissues for implantation, the length of time implants should remain in place, implantation methods and the evaluation of biological responses (ISO 10993-6, 1994).

The tissue and cellular response to biomaterials implanted in animals is screened on the basis of morphological observations on routine histological evaluation. This investigation takes into account the type of cells present and their populations (Butler *et al.*, 2001).

Experiments on animals are very useful and entirely conclusive for the toxicology and hygiene of humans. On this view, the primary function of animal tests is to uncover the casual mechanisms, which produce and direct the course of a disease or condition in animals. These results are then extended by analogy to humans. The resultant understanding of the relevant casual mechanisms in humans empowers scientists to prevent or treat the disease or condition under investigation (LaFollette and Shanks, 1995).

2.3.2.3 Usage tests

Usage tests are performed in animals or in human volunteers. They are distinct from other animal tests because they require that the material be placed in a situation identical to its intended clinical use. The relevance of a usage test to clinical practice is potentially high by definition. However, these tests are extremely

expensive, last for long periods and are exceptionally difficult to control and interpret accurately. Finally, human usage tests may involve many legal liabilities and issues that are not factors for animal and *in vitro* tests (Craig and Powers, 2002 and Anusavice, 2003).

2.3.3 Screening concepts for measuring biocompatibility

For approximately 20 years, researchers have recognized that the most efficient, cost-effective and relevant way to evaluate the biocompatibility of materials is to use a combination of *in vitro*, animal and usage tests (Schmalz, 1996). However, the ways in which these tests are used together and philosophies about the role of each type of test have changed somewhat over the years (Schmalz, 1997).

Schmalz (1997), Wataha (2001) and Craig and Powers (2002) proposed a pyramid scheme of unspecific toxicity tests followed by specific toxicity tests and then clinical trials (fig. 2.2, a). Only materials that passed level 1 were tested further. Unspecific tests were not directly relevant to use of material. Approximately 10 years later, other pyramid scheme were proposed and divided the pyramid into primary, secondary and usage tests and is still commonly used today (fig. 2.2, b). It differed from the previous scheme because the former emphasizes many cellular reactions in addition to toxicity. As in scheme "a", each test level screens for tests above it. Primary tests measure basic biological properties such as toxicity or mutagenicity of material. Secondary tests assess more advanced properties such as allergenicity. Usage tests are equivalent to the clinical trial. Newer schemes have been developed that reflect the complexity of biocompatibility testing of materials (fig. 2.2, c and d). The newer schemes recognize the need to use several

types of tests together and treat evaluation of materials biocompatibility as an ongoing process.

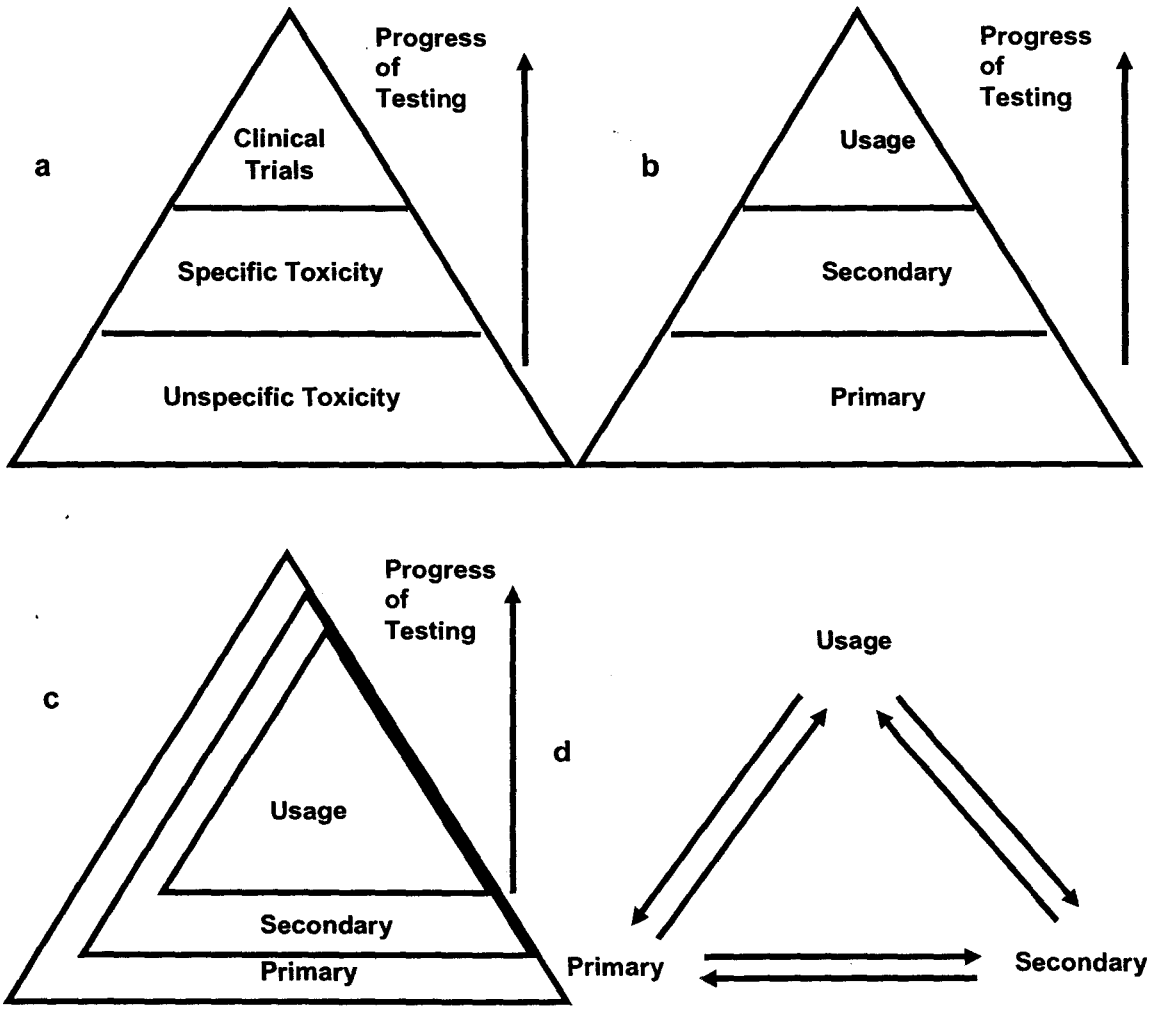

Fig. 2.2: Schemes for testing biocompatibility

2.3.4 Issues in ceramics biocompatibility

2.3.4.1 *In vitro* studies

Ozen *et al.* (2005) conducted a study to determine the influence of various types of dental casting alloys and all-ceramic (In-Ceram) materials on cellular viability and the cytokine 1L-1beta (ß) secretion level in a three-dimensional cell

culture system consisting of human gingival fibroblast cells. Cellular viability was measured by colorimetric tetrazolium (MTT) reduction test and for 1L-1(ß) measurement, assay aliquots were taken from exposed media after different exposure times. According to their investigation on ceramic, the authors reported that the material did not influence cellular viability. Moreover, it did not elevate 1L-1(ß) release from cells at non-toxic levels. The study finding suggests that the cytotoxicity level of the ceramic material tested is in parallel to their 1L-1(ß) level. This finding indicates that the material may not be involved in inflammatory activities at non-toxic levels. The authors recommended the use of 1L-1(ß) parameters, as they provide a better result than a single end point about the biological response of the test materials.

Lin *et al.* (2005) studied the *in vitro* biocompatibility of calcium silicate ($CaSiO_3$) ceramics by examining the adhesion and proliferation of the bone marrow mesenchymal stem cells. The result showed that the ceramics studied supported cells adhesion and proliferation, which indicated good biocompatibility. The authors suggest that $CaSiO_3$ ceramics might be a potential bioactive material as bone implants.

Ryu *et al.* (2004) conducted a qualitative evaluation of the cytotoxic effects of MgO-doped HA / ß-TCP ceramics using murine fibroblast L929 cells. Phosphate Buffered Saline was used as an extractant and Dulbecco`s Modified Eagle Medium supplemented with 10% fetal bovine serum as a negative control. The *in vitro* result showed that L929 cells grew well in the extract of 1wt% MgO-doped HA/ß-TCP ceramics. As compared with the negative control, morphological changes and