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Zirconia toughened mica glass ceramics for dental restorations: Wear, thermal, optical and cytocompatibility properties

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ABSTRACT

Background. In an effort to design novel zirconia reinforced mica glass ceramics for dental restorations, clinically relevant properties such as wear, coefficient of thermal expansion, optical transmittance, and cytocompatibility with human gingival fibroblast cell lines were investigated in the present study.

Materials & Methods. Microstructure analysis of two body wear of heat treated mica glass ceramic ceramics (47.2 SiO_2–16.7 Al_2O_3–9.5 K_2O–14.5 MgO–8.5 B_2O_3–6.3 F wt.%) reinforced with 20 wt.% YSZ, were evaluated against a steatite antagonist in a chewing simulator following Willytec Munich method. In addition, Coefficient of thermal expansion (CTE), total transmittance, scattering coefficient and cytocompatibility on human gingival fibroblast cell lines were performed and compared to the commercially available dental ceramic systems.

Results. The experimental mica glass ceramic demonstrate micro-ploughing, pull out and debris formation along the cutting surface, indicating abrasive wear mechanism. Thermal expansion of mica glass ceramic composite was recorded as $5 \times 10^{-6}/°C$, which is lower than the thermal expansion of commercially available core and veneering ceramics. Further, significant differences of transmittance and scattering coefficient of mica glass ceramics with 20 wt.% YSZ with commercial dental ceramics was found and extensive fibroblast cell spreading with filopodial extension, cell-to-cell bridges and proliferation with human gingival fibroblast cell lines.

Conclusion. With acceptable cytocompatibility with human gingival fibroblast cells and better wear properties with respect to commercial IPS emax Press, the mica glass ceramic composites (47.2 SiO_2–16.7Al_2O_3–9.5 K_2O–14.5 MgO–8.5 B_2O_3–6.3 F wt.%) with 20 wt.% YSZ have the potential for dental restorative applications as machinable veneering ceramics.

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1. Introduction

A wide variety of materials in dentistry have been explored for replacing a patient's missing teeth with the goal of restoring his or her original teeth's function and esthetics. Amongst the dental materials, dental ceramics have been popular due to their ease of fabrication, good aesthetics and chemical durability [1]. Ceramics such as feldspathic porcelains, glass ceramics and zirconia have been in common use in dental practice. With glass ceramics and zirconia, having limitations, ranging from moderate mechanical properties of glass ceramics to the relative opacity, veneer fractures, and low temperature degradation of zirconia ceramics, new material combinations must be explored to overcome their drawbacks. The present paper primarily evaluates the potential of zirconia toughened mica glass ceramic composite for indirect restorative materials. Mica glass ceramics are known for their machinable and bio-active nature of mica glass ceramics, however owing to their moderate mechanical properties, various strategies have been explored to strengthen mica [2–19]. In the previous work done by the authors, mica containing glass ceramics with 20 wt.% YSZ have demonstrated Vickers hardness of 9.2 GPa, elastic modulus of 125 GPa, indentation toughness of 3.6 MPa $m^{1/2}$ with an optimum chemical solubility [9]. We intend to further evaluate clinically relevant properties of zirconia toughened mica glass ceramics such as wear, coefficient of thermal expansion, optical transmittance and cytocompatibility with human gingival fibroblast cell lines.

A dental ceramic crown can be designed as a monolithic or a core-veneer bilayered restoration. In a bilayered ceramic restoration, thermal compatibility of core and veneer is critical for the success of the dental restoration. Sintering is one of the most common methods of veneering high strength core ceramics. During the process of sintering, as the layers of dental ceramics cool from glass transition temperature to room temperature, residual stresses occur at the core-veneer interface. Such high stresses eventually can result in veneer delamination and catastrophic restoration fractures [20,21]. However, low expansion veneer ceramics which were believed to induce compressive stresses at the interface and contribute to the improved strength of the bilayered restoration, have also been reported with veneer fractures [22]. Hence, similar coefficients of thermal expansion of core and veneer are recommended to prevent the occurrence of stresses at the interface and therefore, evaluation of coefficient of thermal expansion is an important property and requires investigation for an experimental dental ceramic.

One of the other critical properties of dental ceramics is their ability to mimic the tooth color. Color of a restorative material depends on the interaction of the incident light with the material. With a polychromatic source of light, appearance of a restorative material, depends on the interaction of the incident light and the phenomena of reflection, scattering, absorption and transmittance [1]. Transmittance results from the amount of scattering and the amount of light passing through it [23–25]. Optical properties can be studied by investigating direct transmittance, total transmittance and spectral reflectance. Transmittance can be defined as amount of the segment of incident light passing through a sample at a definite wavelength. Optical properties of dental porcelains can be studied by measuring direct transmission (when light goes through without a change in direction or quality), total transmission (combination of direct and diffuse light transmission) and spectral reflectance (fraction of incident light that is reflected at an interface such as porosity) [26]. Transmittance is dependent on factors such as thickness of the ceramic, its porous nature, particle size and wavelength. Therefore, for an improved transmittance, low porosity and small particle size are desired as porosites in a ceramic and large particle size increase scattering. Thickness of the ceramic is inversely proportional to transmittance [23]. Investigations on transmittance of dental ceramics can help in understanding the spectral distribution of the incident light and its relationship to transmittance [27]. Dental materials, can also be considered as turbid substances responding to incident light through absorbing, transmitting, or scattering phenomena. Absorption essentially, measures the rate of reduction in the light intensity, in an elementary layer of a substance. Scattering coefficient, on the other hand reflects the fraction of incident light lost by reversal direction in the elementary layer. Both absorption coefficient and scattering coefficients are influenced by factors such as thickness of the material, the wavelength of the incident light and the nature of the colorants added to the material. Materials with higher values of absorption coefficient and scattering coefficient are known to be opaque and more deeply colored [28]. Various methods have been proposed to calculate the coefficients through theoretical and experimental methods. Kubelka Munk theory with its theoretical approximation model explains the phenomenon of diffuse reflectance in inhomogenous media. The response of the geometric inhomogenities in the material has been attributed to its scattering coefficient [29,30]. Typically, high scattering and low transmittance occurs at lower wavelengths [31].

Any dental material placed in the patient creates a dynamic interface that affects the tissue response and the material interaction [1]. Biocompatibility is an important property to be considered as it involves patient safety and ethical concerns [32]. Specific tests have been developed to evaluate the restorative materials for their adverse effects and toxicity. Ceramics are known to be highly biocompatible and chemically durable. Zirconia is well-known to be bio-inert with no reported systemic or local adverse effects [1]. *In vitro* cyto compatibility of mica based glass ceramics have demonstrated increased alkaline phosphatase (ALP) synthesis by Saos-2 cells, osteoconductivity, cell spreading and adhesion [19,33]. Considering the gingival interface of the dental restoration, relevant cell lines such as human gingival mesenchymal stem cell line were used for evaluating the cytocompatibility of zirconia reinforced mica glass ceramics [34].

Another issue that needs to be considered in a potential dental restorative material is that of wear. Wear can be described as a progressive loss of a restorative material during contact with its antagonist natural teeth [35]. The high hardness of dental ceramics can cause appalling damage to the opposing enamel. The wear pattern of a ceramic is often affected by the properties of its crystal phase and glass matrix. The interface of crystal glass phase and their mechanical properties can influence the occurrence of asperities, gouging and the resultant microfractures. A uniform breakdown of a ceramic surface is generally desirable, as it prevents asperities projecting out of the surface [1]. Hence, investigations on the wear pattern of an experimental ceramic can help in understanding the mechanism of breakdown, and predict its long term clinical performance.Though several reports on the wear behaviour of dental ceramics exist in the literature, the current study evaluates the wear damage in zirconia–mica glass ceramic composites against steatite antagonist in a dual axis chewing simulator [36].

2. Materials and methodology

2.1. *Glass preparation & heat treatment schedule*

The precursors of targeted base glass composition (47.2 SiO_2–16.7 Al_2O_3–9.5 K_2O–14.5 MgO–8.5 B_2O_3–6.3 F (wt.%) were ball milled in an agate jar with ethanol as milling medium at 300 rpm for 6 h to obtain a homogenous mixture. The mixed powders were preheated at 950 °C for 1 h and melted at 1500 °C for 2 h in a platinum crucible in a muffle furnace. The glass melt was then quenched in deionised water at 4 °C to obtain the glass frit. The glass frit was powdered and ball milled with varying amounts of 3 mol % YSZ (20 wt. % YSZ) ($D_{50} \approx 50$ nm) (TOSOH, Japan). The glass–zirconia mixtures were compacted in a cylindrical die (15 mm diameter) at 50 kN using a uniaxial hydraulic press to obtain green compacts. A two stage heat treatment sequence was followed to densify the glass ceramic–ZrO_2 powder compact. The first stage consisted of heating the green compact to 800 °C at a heating rate of 25 °C/min in a muffle furnace, holding time for 2 h to relieve thermal stresses and to initiate nucleation of crystal phases. Further, heat treatment was carried out at 1080 °C with a slower heating rate of 10 °C/min for 48 h to complete the crystallization and densification of the samples. The samples will be addressed as G20Z throughout the paper. It is worthwhile to mention that among all the investigated glass ceramics with YSZ reinforcement, G20Z was found to exhibit a better combination of hardness and indentation toughness, as reported in a recent paper by our group [9].

2.2. *Dental wear simulator*

Evaluation of wear was done as a two body wear, where in the polished disc specimens (12 mm × 2 mm) and steatite ball antagonists (6 mm) were mounted in the standard sample holders of the chewing simulator (CS Economy line, SD Mechanotrik) in a phosphate buffer medium. The specimens were subject to 50 N load at a vertical speed of 25 mm/s and horizontal speed of 20 mm/s, sliding distance of 2 mm, 120,000 cycles with a frequency of 1.6 Hz against a steatite antagonist, with a similar hardness to human enamel. The proposed wear methods in the literature, so far have utilized various wear devices, with varying variables of load, force profile and cycles under conditions of thermocycling or two body or three body abrasive mediums. The wear device in the present study is SD Mechanotrik chewing simulator device, which is based on the Willytec Munich technology. The advantages of Willytec wear device are that it satisfies the requirements of GLP and FDA, with reports of its reproducible wear variables, reduced variability and less fabrication time of using steatite antagonist in the device compared to human enamel [37,38]. Ivoclar wear method, has been in practice since 1998, using Willytec simulator device and following certain adjustable wear variables with its tailored IPS Empress antagonist. Wear parameters in the present study such as number of cycles, weight and frequency were set according to the adjustable variables of Willytec chewing simulator used for the Ivoclar wear method [35,39]. Qualititative evaluation of wear mechanism was performed on zirconia toughened mica glass ceramics (G20Z) and commercially available glass ceramics IPS emax Press with scanning electron microscopy analysis. Surface roughness of multiple sites on specimens (n = 3) and quantitative analysis of material loss were conducted using an optical profilometer (Taylor Hobson Precision, TalySurf CCI, Leicester, United Kingdom). The optical profilometer uses the principle of coherence correlation interferometry, with a maximum vertical resolution of 0.01 μm, repeatability of surface of less than 0.02 nm, noise floor of less than 0.8 nm, measurement points of 1024 × 1024, with a surface reflectivity of 0.3–100%. Stitching data points method was used for calculating the volume loss per unit surface area. Statistical analysis wtih independent t-test was used to compare the surface roughness amplitude parameters of G20Z with IPS emax Press ($p < 0.05$).

2.3. *Coefficient of thermal expansion*

Cylindrical specimens of 10 mm × 3 mm × 3 mm (n = 3) were placed in an Electronic Dilatometer (TA Instruments/UB der Waters GmBH), which was calibrated with a standard quartz specimen. Specimens were heated at a rate of 5 °C/min from room temperature to 900 °C. Coefficient of thermal expansion was calculated from the linear part of thermal expansion curves recorded in the temperature range of 400–500 °C. Statistical analysis of student *t*-test, was used to compare the means of thermal expansion values of G20Z with commercial ceramic samples ($p < 0.05$).

2.4. *Optical transmittance*

The optical transmittance of the polished rectangular specimens of G20Z, commercially available IPS emax Press glass ceramic (A1 shade) and zirconia (Katana), were measured with a double beam spectrophotometer with an integrating sphere (Lambda 20-Perkin Elmer, Orwalk, CT, USA) between 380–780 nm with 1 nm integration. The transmittance values were calculated based on the following equation.

$$T(\%) = \frac{Sum\ \ of\ the\ intensity\ at\ \ each\ wavelength}{Total\ \ number\ \ of\ \ wavelength} = \int \frac{I(\lambda)d\lambda}{d\lambda}$$

In the above equation, intensity was calculated as the percentage of transmitted light at each wavelength [21]. The experimental groups of G20Z specimens were tinted with nearest dentin shades of A2 and B2 (A2/TI1 Dentin,e.max Ceram, B2/TI1 Dentin, Ivoclar Vivadent) in order to closely simulate the shade of commercially available IPS empress system. Transmittance values of all the groups were derived from the values of percentage transmittance at a specific wavelength of 525 nm.

The instrument, spectrophotometer, was calibrated by recording the reflectance (R) from a standard white reference, with a calibration coefficient of 0.94. Spectral mean reflectance values of each of the ceramic samples against white (R_g) with a barium sulphate background and black backgrounds (R_0) were measured. Optical constants a and b were further obtained from their reflectance values in the range of 380–780 nm using Kubelka Munk algebraic equations.

$$a = (\frac{1}{2})\{R + \frac{R_o - R + R_g}{R_o R_g}\}) \quad (1)$$

$$b = (a^2 - 1)^{1/2} \quad (2)$$

Further, absorption coefficient (k) and scattering coefficient (s) were derived from the optical constants. The scattering coefficient (s) (mm^{-1}) for a unit thickness of a material was obtained by the equation as:

$$s = \frac{1}{bx} Ar\,ctgh \frac{(1 - aR_o)}{bR_o} \quad (3)$$

where X is the actual thickness of the specimen, ctgh is a hyperbolic cotangent and Ar ctgh is an inverse hyperbolic cotangent. Absorption coefficient (k) (mm^{-1}) was obtained from the following equation:

$$k = s\,(a - 1) \quad (4)$$

Means and standard deviations of transmittance, scattering and absorption coefficients of G20Z was compared to the commercial ceramics and subject to simple student t-test ($p < 0.05$).

3. Cell viability and proliferation

The compatibility of G20Z with human gingival fibroblast derived mesenchymal stem cell lines (National Centre for Cell Sciences (NCCS), Pune) was tested to assess its potential for use in dental restorative applications. Prior to cell culture, the glass ceramic samples were ultrasonicated, sterilized in an autoclave followed by exposure to UV light. The sterilised samples were then soaked in 70% ethanol solution and washed with Phosphate Buffer Saline (PBS). The cells were revived from cryo preserved vials and cultured in Dubelco's modified Eagle medium (DMEM,Gibco,USA), with 1% antibiotic/antimycotic cocktail and 15% fetal bovine serum (Gibco,USA) at 37 °C in a 5% CO_2 incubator, on reaching 70–80% confluency, the monolayer of cells was detached from the culture flask with 0.05% Trypsin–EDTA (Invitrogen) then neutralized by complete medium and harvested by centrifugation at 450×g for 5 min. The cells were re-suspended in complete medium and 3000–5000 cells were seeded followed by trypsinisation with 0.5% trypsin–EDTA (Invitrogen, USA) solution for subsequent subculture. The cells were seeded on the experimental samples at a density of 3000–5000 cells per ml in a 24 well plate and glass coverslips were used as the control.

The seeded samples were subject to cell viability test using the MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich) assay to assess the cell proliferation on glass ceramic sample at different time points during culture (Day 3, 5 and 7) with respect to the glass coverslips which are used as control samples. MTT is a colorimetric assay which provides an estimate of the number of metabolically active cells on the sample after reacting with the mitochondrial dehydrogenase in live cells resulting in the formation of insoluble formazan crystals. After the required duration of time in culture, the samples with cells were washed with PBS and 15% reconstituted MTT in Dubellco's Modified Eagles' medium was added and incubated for 4 h. The formazan crystals that were at the end of the time period were solubilized in dimethyl sulfoxide to give a purple solution after incubation. The absorbance was measured at 595 nm with a reference wavelength of 750 nm in a micro plate reader (i-mark, BioRad laboratories, India). The cell viability was determined by comparing the absorbance of the sample to that of control by the following relation,

$$\%\text{Cellviability} = (\text{Absorbanceofthesample}/\text{Absorbanceofcontrol}) \times 100 \quad (5)$$

For florescence microscopy, the samples with adhered cells were washed twice with 1 × PBS(Phosphate buffer saline) and fixed with 4% paraformaldehyde for 20–30 min. After fixation, the cells were washed with 1 × PBS followed by permeabilization with 0.1% Triton X-100 solution for 8–10 min. To inhibit non-specific binding, 2% Bovine Serum Albumin (BSA) was used as a blocking agent for 1 h. The samples were stained with Alexa Fluor 488® phalloidin (1:200 dilution, Invitrogen) for 1 h min to visualize actin filaments, and counterstained with 2 μg/ml Hoechst stain (Invitrogen) to visualize cell nuclei under fluorescence microscope (Nikon LV 100D, Japan).

Statistical analysis was performed by using SPSS-20.0 (IBM, USA) commercial software. The data is represented as mean value with standard deviation as error bars. Cell viability analysis on different samples was carried out with student t-test. A value of $p < 0.05$ was considered as statistically significant.

4. Results

Although it is not reported in this study, it is important to mention a few salient points on the investigation of zirconia strengthened glass ceramics from our recent study [9]. With the objective of developing mica containing glass ceramics with 20 wt.% Yttria Stabilized Zirconia (G20Z) for low stress bearing inlays, onlays and veneers, a spectrum of mechanical and physical properties such as a high Vickers hardness of 9.2 GPa, elastic modulus of 125 GPa, good indentation toughness of 3.6 $MPa.m^{1/2}$ with an optimum chemical solubility was recorded. Phase and microscopic analysis of sintered glass ceramic-YSZ composites revealed the characteristic peaks of fluorophlogopite (FPP) and tetragonal zirconia and plate and lath-like interlocking mica crystals with embedded zirconia. In the present study, the focus is more towards determining clinically relevant properties such as wear behaviour, optical transmittance, thermal expansion co-efficient and cell response for the zirconia–glass ceramic composite.

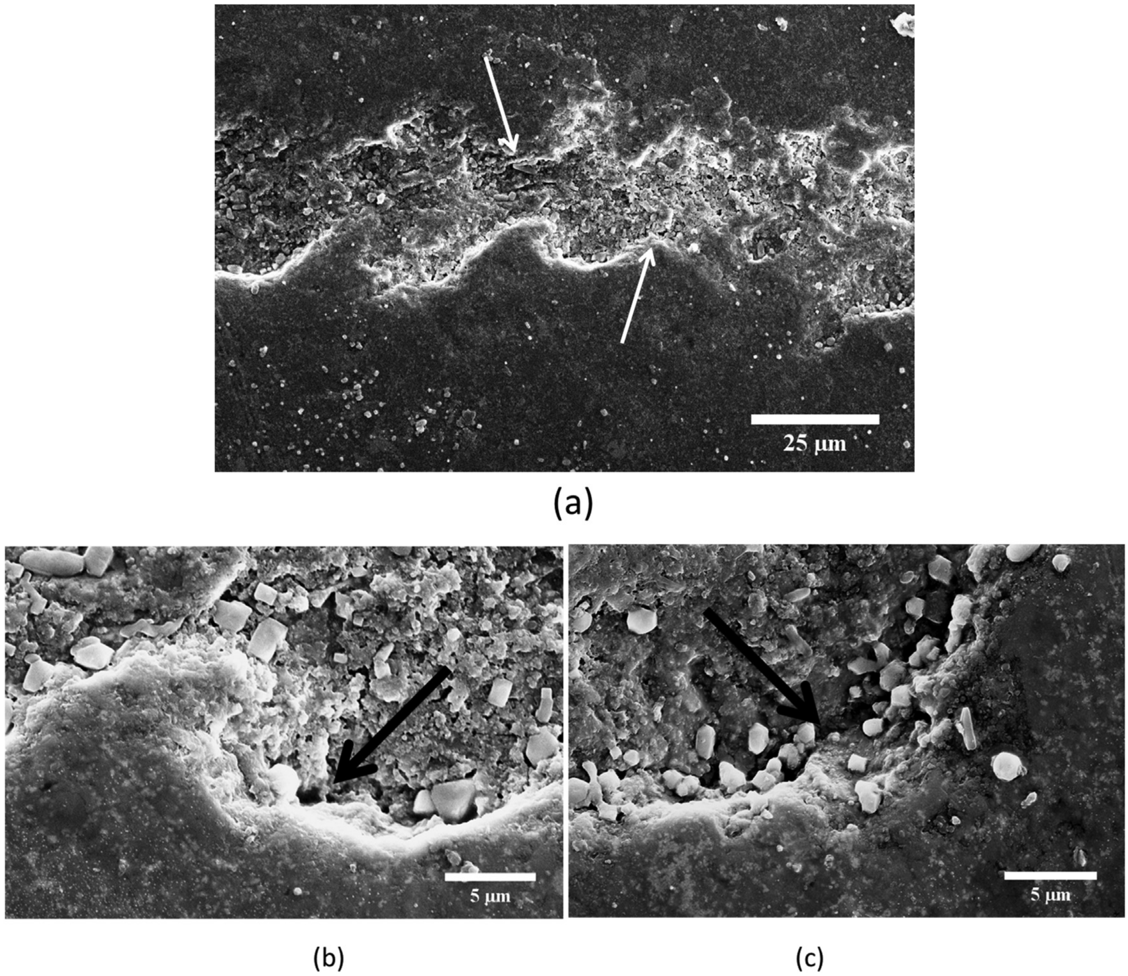


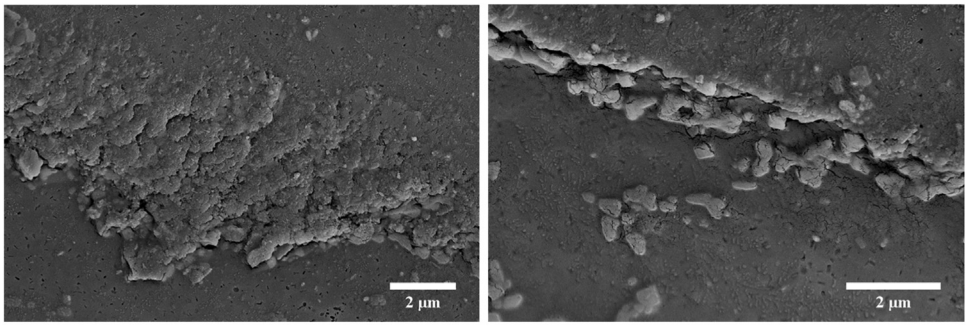
Fig. 1 – SEM micrographs illustrating (a) the wavy wear track showing rupture of G20Z (b) higher magnification of the wear tracks of G20Z demonstrating micro-cutting mode in the form of material removal and spilling of mica platelets as chips (c) reveals the subsurface regions of the glass matrix.

4.1. *Wear behavior*

The wear behaviour of G20Z was conducted using a two body wear configuration. The qualitative analysis of wear behaviour was conducted through electron microscopy of the surface to identify the wear mechanism. As evident in Fig.1, wear track of G20Z composites demonstrates micro-ploughing, pull out and debris formation along the cutting surface indicate abrasive wear at 50 N load. Wear mechanism of IPS emax Press consisting of lithium disilicate glass ceramic under similar testing conditions, was also evaluated. These ceramics also exhibited similar wear mechanism with micro-cutting, debris formation as evident in Fig. 2. A more detailed quantitative analysis of wear was carried out through optical profilometer to evaluate the surface after the dental wear in terms of surface roughness and net material loss in terms of volume of the material ejected from the surface. The surface roughness parameters along with the values of volume loss and the 3D surface profile are presented in Table 1, Fig. 3(a) and (b) respectively. This volume loss of the worn surfaces has been calculated based on the average volume of holes or valleys in the wear facet against the profile base line and were normalized with total wear surface area. Statistical significant difference in surface roughness amplitude parameters such as Ra, Rq and Rz was found between G20Z and IPS emax Press ($p < 0.05$).

4.2. *Coefficient of thermal expansion*

The mean coefficient of thermal expansion of mica glass composites was recorded as $5 \times 10^{-6}/^{\circ}C$ using an electronic thermo-dialatometer at a heating rate of 5 °C/min with quartz as reference. The co-efficient of thermal expansion was obtained from the slope of linear fit of the elongation v/s temperature data recorded by the instrument. As seen in Table 2, it is evident that thermal expansion of G20Z at $5 \times 10^{-6}/^{\circ}C$ is lower than the thermal expansion of commercially available core and veneering ceramics with values in the range of $9.4–10.4 \times 10^{-6}/^{\circ}C$. A statistical significant difference was found between G20Z and the commercially available IPS emax Press and Zirconia at $p < 0.05$.



(a) (b)

Fig. 2 – SEM micrographs of IPS emax Press after Dental wear showing (a) micro-ploughing with wear debris and (b) material transfer to sides of the groove.

Table 1 – Volume loss and surface roughness parameters of G20Z and IPS emax Press.

	G20Z[a]	IPS emax Press
Volume loss per unit surface area ($\mu m^3/\mu m^2$)	4.44 ± 0.72	14.46 ± 0.93
R_a (µm)	0.34 ± 0.14	0.88 ± 0.15
R_q (µm)	0.47 ± 0.20	1.03 ± 0.12
R_z (µm)	2.62 ± 1.15	4.10 ± 0.31

[a] G20Z was significantly different from IPS emax Press ($p < 0.05$).

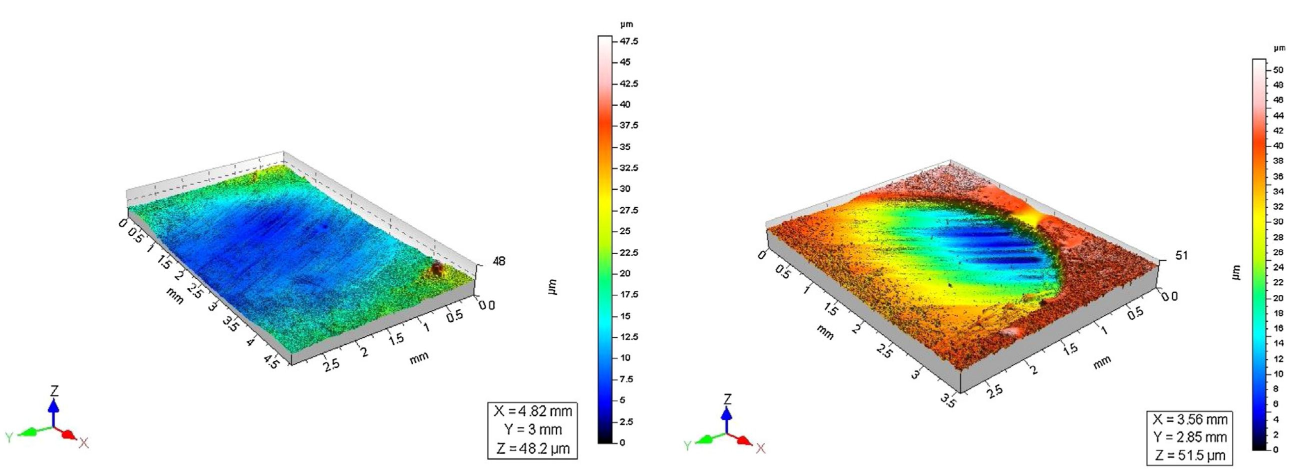
Table 2 – Values of coefficient of thermal expansion of G20Z and commercially available dental ceramics (*Data from scientific documentation of IPS emax and Zirconia systems).

Material	CTE (10^{-6} /C)
G20Z[a]	5.07 ± 0.19
IPS emax Press	10.5 ± 0.4
IPS emax Ceram	9.4 ± 0.1
Zirconia (Katana)	10.4 ± 0.05

[a] G20Z was significantly different from each of the ceramic groups ($p < 0.05$).

4.3. *Optical transmittance*

The transmittance (T%) of G20Z have been compared to the core glass ceramic of IPS emax Press system and dental zirconia (Katana). The experimental group of G20Z have been tinted with dentin shades of A2 and B2 in order to closely mimic the clinical scenario. As seen in Fig.4, the intensity of transmitted light varies with wavelength [27]. The transmittance values in Table 3, demonstrate and highest value of 23% by A2 shaded G20Z in the wavelength range of 380–780 nm. It can also be seen that B2 shaded G20Z, and IPS emax glass ceramics (A1 shade) as a core ceramic tend to demonstrate closer values of transmittance of 13%, with the values of unshaded G20Z of 16% falling in between. In Table 3, pigmented A2G20Z, B2G20Z, and IPS emax Press demonstrate high scattering and absorption coefficients, compared with G20Z and Zirconia. The plots of scattering coefficients with wavelengths are presented in Fig.5. Statistical significant difference in transmittance values, scattering and absorption coefficients was found between



(a) (b)

Fig. 3 – 3D surface profile of a wear facet on (a) G20Z and (b) IPS emax Press specimens depicting roughness profile and material loss after 120,000 cycles of two body wear with steatite antagonist.

Table 3 – Transmittance (T%), scattering coefficient (μ_s) and absorption coefficient (μ_a) values of the investigated ceramic groups.

Material	T%	μ_s (mm^{-1})	μ_a (mm^{-1})
G20Z[a]	16.49 ± 0.71	0.39 ± 0.04	0.03 ± 0.01
A2-G20Z	22.6 ± 0.77	0.60 ± 0.21	0.17 ± 0.19
B2-G20Z	12.75 ± 0.62	0.59 ± 0.10	0.27 ± 0.22
IPS emax Press	12.78 ± 0.60	0.47 ± 0.09	0.14 ± 0.11
Zirconia (Katana)	11.85 ± 0.64	0.37 ± 0.05	0.07 ± 0.04

[a] G20Z was significantly different from IPS emax and Zirconia ceramics ($p < 0.05$).

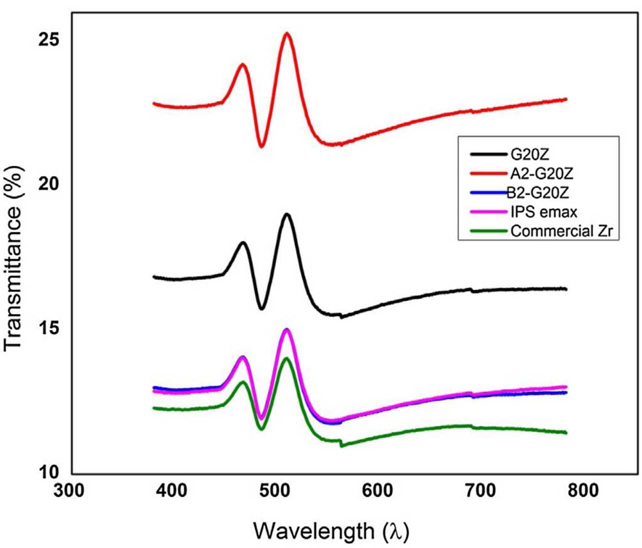


Fig. 4 – Transmittance spectra of G20Z, A2, B2 shaded G20Z, IPS emax Press and commercially available ZrO_2 in the visible wavelength of.380–780 nm.

G20Z and the commercial ceramics, IPS emax Press and zirconia.

4.4. *Cell viability and proliferation*

The results of cell viability studies with human gingival fibroblast derived stem cells using MTT assay is presented in Fig.6, in the form of optical density of the formazan product that is formed at the end of the 4 h incubation period. The optical density data shows that the cell number on the G20Z sample increases from day 3 to day 7 and is similar to the cell numbers in control on both the days indicating that the glass ceramic supports cell growth as well as the glass coverslips used as the control sample in the experiment. Samples with $p < 0.05$ has been treated as significant. The absorbance values do not show an increase after day 5 in both the samples, more importantly, the optical density readings do not show a statistically significant difference between the control and G20Z samples at any point in culture (Day 3, 5, and 7). The cell viability determined according to Eq. (1) for Day 3 (101.25), Day 5 (97.29) and Day 7 (105.7) indicate that the glass ceramic composite is a good substrate that supports cell growth and spreading. This positive cell response to G20Z can also be observed in the representative fluorescence images in Fig.7. The image for G20Z (Fig.7(b)) shows a better morphology than that of control (Fig.7(a)) indicating that a long term study might even favour the glass ceramic substrate for cell proliferation and differentiation in comparison with control.

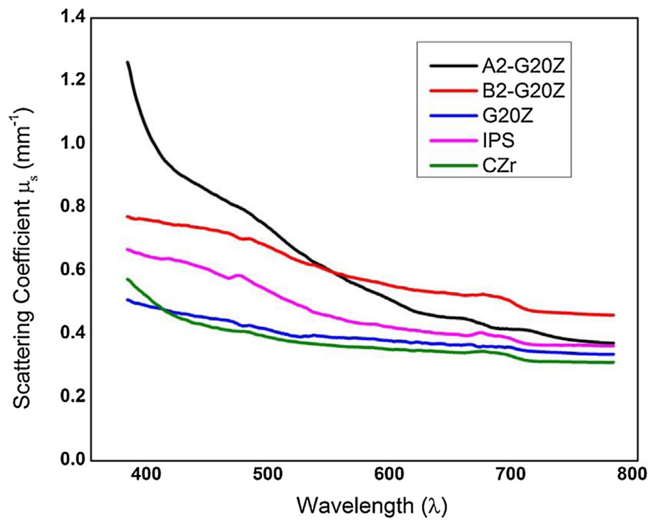


Fig. 5 – Wavelength dependent scattering coefficients of the ceramic groups.

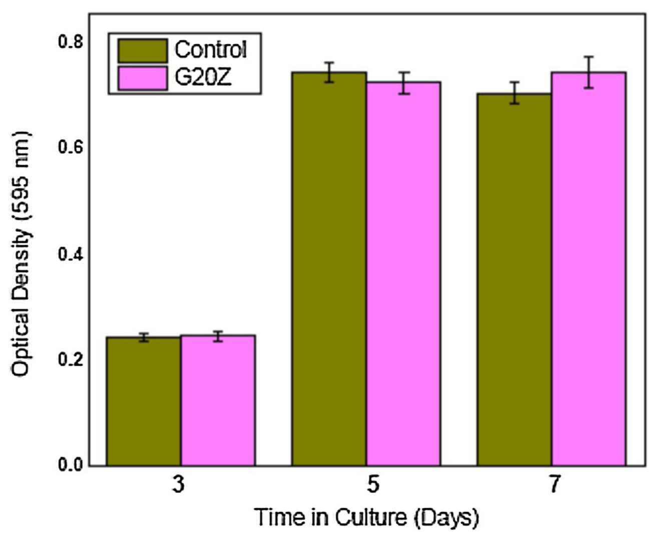
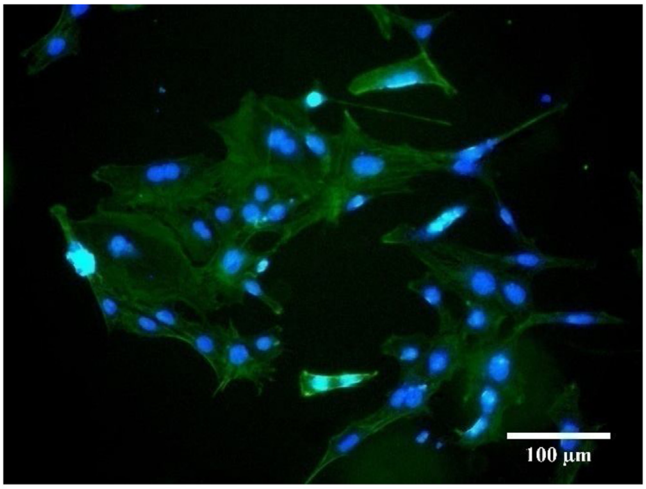


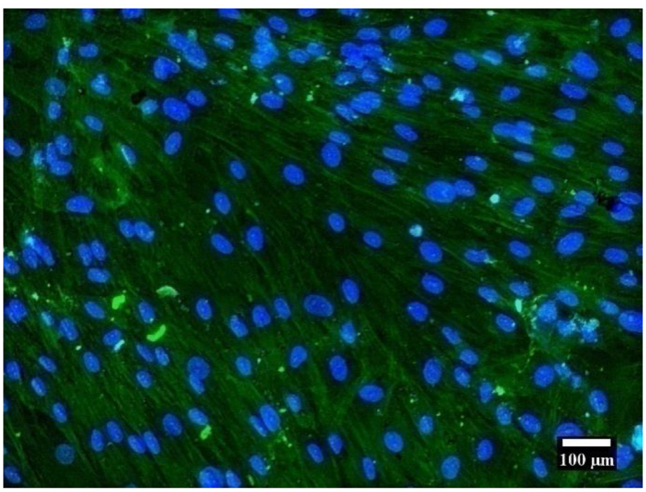
Fig. 6 – Comparison of human gingival fibroblast cell line viability on control and G20Z samples on days 3, 5 and 7 showing good cytocompatibility properties. There was no statistical significance in the Optical density of G20Z with respect control for the same time in culture ($p < 0.05$).

5. Discussion

Though significant progress has been accomplished in the field of glass ceramics and zirconia, yet each of the above dental ceramics have limitations. While glass ceramics have moderate mechanical properties, restricting to anterior teeth and as monolithic restorations for premolars, zirconia too presents with problems of opacity, frequent veneer fractures,



(a)



(b)

Fig. 7 – Representative fluorescence images showing stained nuclei and cytoskeleton of human gingival fibroblast derived stem cells on (a) control and (b) G20Z after day 7.

and long term low temperature degradation. Considering the frequent veneer fractures of bi-layered zirconia restorations, developing tougher and thermally compatible veneering ceramics can further strengthen their longevity [21]. With the limitations of the current dental ceramics and a desire for high strength and esthetic ceramics, novel and alternate material combinations must be explored with a goal of improving the success rate of all ceramic restorations [40].

5.1. *Wear properties*

A number of factors influence wear of a dental restoration, such as interaction of a restorative material properties with surface finish, hardness and pH of food, and patient's masticatory forces. Wear essentially becomes an important criteria amongst dental ceramics, as it affects opposing natural dentition and restorations. It is expected that a restorative dental material must not cause any iatrogenic damage to the opposing tooth structures. Therefore, evaluation of wear of ceramic restorative materials is important [41]. Qualitative characterisation of wear of various dental ceramics has been studied extensively in literature using scanning electron microscopic analysis. In general, wear pattern of dental ceramics, particularly glass ceramics, reveals a microfracture mechanism, compared to the adhesion wear pattern of dental enamel or composites. In a similar trend, abrasive wear of G20Z with micro-cutting, and spill out of mica platelets along the cutting surface can be seen in Fig.1. The wear mechanism of IPS emax Press under similar testing conditions exhibits wear mechanism of micro-ploughing with debris as evident in Fig. 2 under 5 N load. Furthermore, the reduced wear volume loss of G20Z and the lower surface roughness values of R_a, R_q and R_z in G20Z can be interpreted from Table 1.

Studies on wear tracks of mica glass ceramic under high loads reveal spallation with wide crack gaps, extensive fractures and faint, localised fractures with crack gaps under low loads [42]. Wear mechanism studies in lithium disilicate based glass ceramics also indicate the presence of large cracks with plastically deformed layers under loads of 5 N and 25 N in unpolished specimens, and microcracks, with nanoparticles at 5 N and 25 N in polished specimens respectively [21]. In two-body wear process, the relative hardness of the antagonists influence wear mechanism, resulting either in fracture of the asperities of the harder material or ploughing of the softer material. Furthermore, individual factors, such as frequency of tooth brushing, type of diet, surface conditions and material factors such as microcracks and surface roughness play an important role in the wear process of a dental restoration [43]. Microcracks in dental ceramics have been correlated with the mechanical properties, such as fracture toughness [44]. Wear of noncrystalline ceramics has been closely related to physical factors such as plastic deformation and fracture of glassy matrix, frictional resistance and fracture toughness, microstructural factors such as porosity, surface finish and environmental factors [45]. Resistance to micro-fractures and surface breakdown in the wear process, can be related to the interface of plate and lath like interlocking crystals in the glass matrix of G20Z and needle like lithium disilicate crystals of IPS emax Press. The comparable hardness and fracture toughness of G20Z and IPS emax Press can be further corroborated to micro-fractures, often seen in glass ceramics [9].

Surface characteristics of a dental restorative material is one of the vital parameters that reflects the clinical performance of a dental material in terms of hygiene, strength and esthetics [46]. The complexity of surface roughness profiles of materials in terms of valleys and peaks across the scales makes its measurement more challenging. Hence, surface roughness has been characterized with multiple roughness amplitude parameters such as Ra, the arithmetic average of all absolute distances of roughness profile, Rq, the root mean square value of the profile coordinates within the measured sections and Rz, the maximum distance between the peak and the valley [47].The roughness amplitude parameters, reflect the average surface roughness of the material. The Ra value defined as the mean deviation of the asperities from the centre line, is the most commonly used parameter to define surface roughness, easy to measure and provides an average description of height variations [48]. Various instruments have been used to measure surface roughness profiles of dental materials such as contact stylus tracing, laser reflectivity method, non contact lasers, scanning electron microscopy and non contact optical profilometry [47]. The accuracy of measuring surface roughness can be influenced by the type of scanning instrument either as contact or non-contact, the surface of the material and the scanning angle. Contact profiling systems are relatively affordable and accurate, but may not accommodate an even contact on non-rigid surfaces. Non-contact systems, have the advantage of no contact with the rigid or non-rigid surfaces, but have limitations of being influenced by the reflecting surface of the material, inclination of the sample, and its geometry. The accuracy of optical profilometers depend on features such as maximum resolution, its measurement technique and the measurement points [49,50]. The optical profilometer in the present study as mentioned in the methodology section, works on the principle of coherence correlation interferometry, has a maximum vertical resolution of 0.01 μm. The 3D surface profiles of a wear facets of G20Z and IPS emax Press can be interpreted in Fig. 3(a) and (b). The three fold increase in the surface roughness of IPS emax Press compared to G20Z in Table 1, can be explained to higher hardness and better fracture toughness of G20Z due to presence of zirconia in contrast with the lithium disilicate glass ceramic system of IPS emax Press [9].

5.2. *Coefficient of thermal expansion*

Coefficient of thermal expansion is an important parameter for dental ceramics. As the common method of processing ceramics is sintering, it is essential that CTE of veneer ceramic be compatible with that of high strength core ceramics. The apparent incidence of residual stresses, both in the core and veneer can arise due to the influence of heating and cooling rates during sintering in case of CTE mismatch [21]. Many studies have investigated the occurrence of residual stresses in bilayered ceramic restorations. Residual stresses are determined through several methodologies such thermal shock evaluation, crazing resistance, strain gauges for surface evaluation, optical and profilometric methods [51]. Based on the large mismatch and a comparatively lower value of thermal expansion co-efficient to the contemporary dental ceramics, G20Z may not be suitable for sintering as a ceramic veneer. Therefore, we intend to recommend them to be explored as a veneering ceramic for adhesive bonding to core ceramics as 'Rapid Layer Technique' [51]. The concept of adhesive bonding can eliminate the risk of residual stresses associated with the sintering techniques and can provide a favourable stress state at the core-veneer interfaces, improving the longevity of the all-ceramic restorations.

5.3. *Optical transmittance*

It is crucial that optical property of a dental restorative material must match to that of human tooth [52,53]. Optical properties generally result from the interaction of spectral incident light on the object in the form of reflection, transmission and absorption [54]. An important requirement of dental ceramics is to match their optical properties to that of human enamel and dentin for aesthetic considerations [55]. A dental material, in general, allows the passage of incident light with a certain amount of scattering so that the restorative material appears to be of a similar color to enamel [21]. So far in the literature, optical response of the dental material has been evaluated through measurements of direct transmittance, total transmittance including scattering and spectral reflectance [56]. Direct and total transmittance have more relevance with respect to dental ceramics as they represent important criteria, color of the ceramic and its opacity [46,57,58]. Total transmittance is the amount of light transmitted through a solid, inclusive of scattered light. The amount of light transmitted through a material is generally calculated as the ratio of intensity of incident light with the intensity of transmitted light for a given thickness of material with transmission coefficient (t_c) [56]. In an evaluation of the transmittance of newly developed lithium disilicate glass ceramics, they showed higher values than the translucent zirconia ceramics [26]. Microstructural factors, such as grain size, crystal size, porosities and surface roughness often determine optical properties [59,31,25]. Ceramics with small grain size, diminutive porosity with pint-size difference in refractive indices of the constituents are transparent [60].

Most veneering ceramics demonstrate total transmittance at 525 nm in the range of 18%–41% at a thickness of 1 mm [56]. However, the ceramics investigated in the present study, as potential core ceramics are expected to demonstrate lesser transmittance values compared to the veneering ceramics. The results of transmittance values in the present study, corroborate with similar studies on lithium disilicate glass ceramics and zirconia [26,61]. As can be seen in Table 3, the transmittance values of the commercially available dental ceramics such as Zirconia (Katana), IPS emax Press glass ceramic and B2-G20Z demonstrate similar values, at a thickness of 1 mm. High transmittance values of A2-G20Z than B2-G20Z, can be reasoned to the A2-G20Z being lighter, with less concentration of pigments to B2-G20Z.

The response of the incident light on the dental substrate, scattering in particular, depends on material characteristics such as porosity, pore size and certain inclusions in the material such as fillers [62]. Essentially, scattering occurs due to the phenomenon of refraction and reflection at the interface between the particles and voids. Scattering coefficient of a material, can vary depending on the particle size and wavelength of the incident light [29]. Generally, opaque and porous dental materials tend to exhibit high scattering and absorption coefficients [28]. In Table 3, high scattering coefficients and absorption coefficients can be observed for pigmented or intensely colored A2-G20Z, B2-G20Z followed by IPS emax Press. The findings in Fig.5, further elucidates the influencing factors of shades and wavelengths on scattering coefficients. With the addition of external pigments to G20Z, with A2 and B2 shades, the high absorption coefficients of A2-G20Z and B2-G20Z can be explained to the pigments with a tendency to absorb the respective color. G20Z, as mica glass ceramic matrix, with its inherent porosities present in the glass ceramic composite matrix, attributed to the pressure less sintering technique employed in its processing, are relatively white in color similar to zirconia, which can be explained to their relatively close values of scattering and absorption coefficients. In the context of the present work, the crystalline phase of flourophlogopite in the microstructure of glass matrix and its porous nature may have contributed to the scattering effect of G20Z compared to the commercially available IPS emax Press and zirconia (Katana) systems. In clinical settings, coloring pigments ranging from iron oxide, nickel oxide (brown), copper oxide (green), titanium oxide (yellowish brown), manganese oxide(lavender), and cobalt oxide (blue) have been added to porcelain to mimic the natural teeth shades. Cerium oxide, zirconium oxide, titanium and tin oxides have been used to induce opacifying effect in dental ceramics [56]. It can be seen that upon external application of B2 dentin shade to the G20Z composites, the transmittance values were closely similar to the IPS emax Press system. The rationale of applying the dentin shades to the experimental G20Z, is to provide an insight on the possible pigments that can be added to the zirconia mica glass ceramic composite, G20Z, to obtain clinically relevant optical properties.

5.4. *Cell viability and proliferation*

Amongst the existing dental restorations, dental ceramics are the most biocompatible and chemically inert [1]. Several authors have conducted cytotoxicity testing of various dental ceramics such as elemental ion release with inductive coupled plasma mass spectroscopy, trypan blue assay for cell viability, suppression of mitochondrial activity with MTT assay on fibroblast cell lines, and in accordance with ISO 10,993 [64,65]. In the present context, mica based glass ceramics can support favourable cellular activities of cell spreading and cell viability [19,33].

Dental lithium disilicate based glass ceramics have demonstrated suppression of the mitochondrial activity on 3T3 mouse fibroblasts (MTT assay), compared to the traditional feldspathic porcelain [66]. However, they have also shown cytotoxic response with mouse fibroblasts [63]. Assessment of their cytocompatibility to human gingival fibroblast cell lines like in the present study, is more relevant due to the interface of the dental ceramic and gingiva. Hence, the cell viability was quantified using MTT assay of human gingiva derived mesenchymal stem cells. It is recognised that MTT assay evaluates the mitochondrial activity of the cells, reflecting the viability of the cells against the experimental samples. Cellular response such as morphological characteristics such as extensive fibroblast cell spreading with filopodial extension, cell-to-cell bridges and proliferation confirms the zirconia mica glass ceramic composites, G20Z, compatibility as shown in Fig.7.

Generally, microstructural and compositional aspects of a biomaterial generally influence cellular adhesion to a substrate [19]. It has been shown that fluoride ions are known to enhance alkaline phosphatase activity and bone formation [67].

6. Conclusions

New material combinations of dental restorations should have a spectrum of properties including wear, transmittance and cytocompatibility. Yttria-stabilised zirconia (20 wt.%) toughened mica glass ceramic composites demonstrate a two body abrasive wear, with acceptable cytocompatibility with human gingiva derived mesenchymal cell lines. While future research can aim to enhance coefficient of thermal expansion of the mica glass ceramic composites, further modifications in its composition and processing are recommended for optimum optical and thermal properties as a suitable dental ceramic.

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