Phase Formation, Mechanical Strength, and Bioactive Properties of Lithium Disilicate Glass–Ceramics with Different \( \text{Al}_2\text{O}_3 \) Contents

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Abstract: Owing to its excellent mechanical properties and aesthetic tooth-like appearance, lithium disilicate glass–ceramic is more attractive as a crown for dental restorations. In this study, lithium disilicate glass–ceramics were prepared from \( \text{SiO}_2–\text{Li}_2\text{O–K}_2\text{O–P}_2\text{O}_5–\text{CeO}_2 \) glass systems with various \( \text{Al}_2\text{O}_3 \) contents. The mixed glass was then heat-treated at 600 °C and 800 °C for 2 h to form glass–ceramic samples. Phase formation, microstructure, mechanical properties and bioactivity were investigated. The phase formation analysis confirmed the presence of \( \text{Li}_2\text{Si}_2\text{O}_5 \) in all the samples. The glass–ceramic sample with an \( \text{Al}_2\text{O}_3 \) content of 1 wt% showed rod-like \( \text{Li}_2\text{Si}_2\text{O}_5 \) crystals that could contribute to the delay in crack propagation and demonstrated the highest mechanical properties. Surface treatment with hydrofluoric acid followed by a silane-coupling agent provided the highest micro-shear bond strength for all ceramic conditions, with no significant difference between ceramic samples. The biocompatibility tests of the material showed that \( \text{Al}_2\text{O}_3 \)-added lithium disilicate glass–ceramic sample was bioactive, thus activating protein production and stimulating the alkaline phosphatase (ALP) activity of osteoblast-like cells.

Keywords: lithium disilicate; heat treatment; glass-ceramic; crack propagation; \( \text{Al}_2\text{O}_3 \); crystals

1. Introduction

Glass-ceramics are made of crystalline phase and glass. They are specially formed composite that are created through high-temperature melting, shaping, and heat treatment. Due to their excellent mechanical strength, adjustable thermal expansion, chemical resistance, low dielectric loss, and other properties, glass-ceramics are currently gaining considerable attention in mechanical manufacturing, optics, electronics, aerospace, biomedical, and construction applications [1–4]. In the dental restoration field, glass-ceramics are widely employed due to their biocompatibility, ease of production, and chemical, physical, and mechanical qualities that are extremely similar to those of human enamel [5]. Various materials such as alumina, zirconia, leucite and lithium disilicate glass–ceramics have been developed [6]. Among these materials, lithium disilicate (\( \text{Li}_2\text{Si}_2\text{O}_5 \)) glass–ceramics, which are made of a crystalline ceramic and glass matrix, have drawn the most attention among these materials because of their excellent mechanical characteristics and distinctive translucency [7,8]. There has also been a significant increase in the number of reports on the composition, structure, phase transformations and properties of \( \text{Li}_2\text{O–SiO}_2 \)-based glass
in recent years [9–16]. In this context, this research focuses on LS glass–ceramics, which can be achieved by controlling the nucleation and crystallisation of the Li2O–SiO2 parent glass. The effects of composition and heat treatment on LS glass–ceramics characteristics have been the subject of numerous studies. A high mechanical strength can be achieved in LS glass–ceramics by using P2O5 as a nucleating agent to form fine-grained interlocking microstructures [10]. An improved fracture strength can be achieved by increasing the radii of Rb and Cs ions in the lithium disilicate phase, which facilitates crystallisation [11]. When K2O, Rb2O, and CsO were mixed, the fracture strength of the modified lithium disilicate glass-ceramics significantly increased [12]. CeO2 has a small effect on the Li2Si2O5 phase percentage. The best heat-treatment temperatures for samples containing 1.5 wt% CeO2 were 600 °C for 1 h and 800 °C for 1 h [13]. Lithium disilicate glass–ceramics can be improved by adding K2O and Al2O3, increasing their mechanical properties by up to 201 MPa [14,15]. The concentration of Al2O3 additive oxides on the phase formation and mechanical properties of lithium disilicate glass–ceramics prepared by using the conventional method was also studied by Leenakul and Kraipok [16]. The Al2O3 content affected the crystallisation temperature and phase formation. The highest mechanical strength was correlated with the rod-like crystals of Li2Si2O5. Despite the many studies related to the effect of Al2O3 on the mechanical properties of Li2O–SiO2 glass systems, to the best of our knowledge, there is no study of this effect on the modified Li2O–SiO2 glass systems with CeO2 yet.

This study aims to increase the Al2O3 concentration and find the optimal processing parameters for dental restorations. A SiO2–Li2O–K2O–P2O5–CeO2 glass system prepared by using the conventional melt-quenching method was used as the base glass to examine the effects of Al2O3 on the crystallisation behaviour, microstructure, mechanical properties, and bioactivity of lithium disilicate glass-ceramics. The thermal properties of these glass–ceramics were studied and managed using differential thermal analysis (DTA) to determine the crystallinity of the major phase. To better understand how the Al2O3 content affects the glass–ceramic properties, scanning electron microscopy (SEM) and X-ray diffraction (XRD) were used to examine the heated samples. Furthermore, the phase formation and mechanical and bioactive properties were tested to determine their suitability for dental restorations.

2. Materials and Methods
2.1. Glass and Glass-Ceramic Preparation

The lithium disilicate glass composition had the following mol% composition: 67.4 SiO2, 27.0 Li2O, 2.0 K2O, 2.0 P2O5, and 1.5 CeO2. The LS glass was then supplemented with Al2O3 in concentrations of 0.5, 1.0, and 1.5 mol%. LSA1, LSA2, and LSA3 were assigned to the glass samples, respectively. The raw materials for creating LS glass were SiO2, Li2CO3, K2CO3, (NH4)2HPO4, CeO2, and Al2O3 in the analytical grade from Sigma-Aldrich Pte. Ltd. (Singapore). In a high-temperature furnace, these raw materials were combined and melted in an alumina crucible for 2 h at 1450 °C. In order to create the glass specimens, the molten glass was quenched in a metal mould that had been heated to 500 °C. Afterward, the glass was annealed at 500 °C for 2 h before being cooled to room temperature. After being annealed, the glass samples were heated through a two-stage crystallisation process at 600 °C and 800 °C for 1 h while being heated at a rate of 2 °C/min in an air atmosphere and then cooled to room temperature.

2.2. Differential Thermal Analysis (DTA)

Analytical grade Al2O3 powder served as the reference material, and a differential thermal analyser (TG-DTA 8121, Rigaku, Japan) was used to determine the glass transition temperatures (Tg), crystallisation temperatures (Tc), and melting temperatures (Tm) in an air atmosphere. The alumina crucible was heated at a rate of 10 °C/min from room temperature to 1200 °C with about 10 mg of glass specimen powder.
2.3. X-ray Powder Diffraction Analysis (XRD)

Cu-Kα radiation produced at 30 mA and 45 kV was used in the X-ray powder diffraction analysis using an X-ray diffractometer (XRD) (SmartLab, Rigaku, Japan) with a scanning range of 10° to 60° and a step size of 0.012°. Using JCPDS numbers from the ICDD-PDF2 database, the crystalline phases in the controlled heat-treated glass specimens were identified.

2.4. Field Emission Scanning Electron Microscopy (FE-SEM)

The microstructure of the glass-ceramic specimens was examined using field emission scanning electron microscopy (FE-SEM) (JSM-6335F, JEOL, Akishima, Tokyo, Japan). An ion sputtering device (JFC-1200, JEOL, Akishima, Tokyo, Japan) was used to coat the fracture surface of the specimens with gold for 10 s.

2.5. Mechanical test

Using Vickers hardness tests (Buehler, Karl Frank GMBH Type-38505, Lake Bluff, IL, USA) with a constant load of 1 kg and a dwell time of 15 s, microhardness was used to analyse the mechanical properties of the prepared glass-ceramics [17]. The equation below was used to determine the Vickers hardness (HV):

\[ HV = \frac{1.8544P}{d^2}, \]  \hspace{1cm} (1)

where 1.8544, \( P \), and \( d \) denote the constant geometrical factor for the diamond pyramid, the applied load with the unit of kg, and the diagonal length of the indenter impression in \( \mu m \), respectively.

There are universal strength machines that can be used for ISO 6872 [18] with a universal strength machine (AG-X plus, Shimadzu, Japan). The flexural modulus (\( E \)) was calculated using the following equation [19]:

\[ E = \frac{L^3m}{4bd^5}, \]  \hspace{1cm} (2)

where \( L \) is the support span (mm), \( m \) is the gradient (i.e., slope) of the initial straight-line portion of the load deflection curve, (N/mm), and \( b \) and \( d \) are the width of the test beam and thickness, respectively. A three-point-bending test with the fracture strength (\( \sigma \)) in the unit of MPa and a crosshead speed of 0.01 mm/min, and the test span was 16 mm. The following equation can be used to determine the three-point flexure:

\[ \sigma = \frac{3PL}{2wb^2}. \]  \hspace{1cm} (3)

In this case, \( P \) stands for the breaking load in N, while \( w \), \( b \), and \( l \), respectively, are the width, the thickness of the samples, and the test span in mm.

2.6. Micro-Shear Bond Strength (\( \mu SBS \))

All the lithium disilicate glass-ceramic samples with various Al₂O₃ contents (LSA1, LSA2 and LSA3) were selected and subjected to the \( \mu SBS \) test for the evaluation of their bonding performance. Briefly, 20 ceramic plates (5 × 5 × 2 mm) of each ceramic condition were prepared and embedded in a metal ring using epoxy resin. After polishing with 600, 800, 1000, and 1200 grit silicon carbide paper, the specimens were randomly divided into 4 group according to 4 surface treatments: (1) no surface treatment; (2) silane coating (Monobond N, Ivoclar Vivadent, Liechtenstein); (3) hydrofluoric acid (IPS® ceramic etching gel, Ivoclar Vivadent, Liechtenstein); and (4) hydrofluoric acid and silane coating. The treated ceramic surface was then cemented with 4 resin cement rods (Multilink® N, Ivoclar Vivadent, Liechtenstein) by injecting resin cement into 4 plastic tubes (0.8 mm internal diameter and 0.5 mm height). After removing the tubes and storing in water for 24 h, the
specimens were subjected to a μSBS test using universal testing machine (Instron® 5566 a universal testing machine, Instron Engineering Corporation, Norwood, MA, USA) at the crosshead speed of 0.5 mm/min. Data were analysed using a two-way ANOVA test with Dunnett T3 post hoc test at a significant level of 0.05 (p < 0.05).

2.7. In Vitro Test for Biocompatibility of the Material

2.7.1. Preparation of the Tested Materials

Briefly, 2 × 2 × 2 mm synthetic material was established prior to a 30-min UV irradiation process (257.7 nm, 40 W/cm², ESCO UV-30A, Barnsley, England) on each side. The irradiated material was transferred into each well of a sterile 96-well plate for cell culture.

2.7.2. Culture of Osteoblast-Like Cells

MG-63, an osteosarcoma cell line (ATCC, CRL1427) was used to test with the synthetic material. The cell was cultured in Dulbecco’s modified Eagle’s medium (DMEM) with the supplementation of a 10% fetal bovine serum (FBS), 2% N-(2-hydroxyethyl)piperazine-N’-(2-ethane sulfonic acid) (HEPES), 1% non-essential amino acid, and 1% penicillin/streptomycin until they reached 80% confluence. Then 1 × 10⁴ cells were loaded in the well containing the treated material with a volume of 100 µL and the plate was incubated in a humidified incubator under 37 °C and 5% CO₂ for 24 h. The fresh medium was then replaced and the plate continued to be incubated for 48 and 96 h.

2.7.3. Cell Attachment, Cell Viability, and Cell Proliferation by MTT Assay

Briefly, a 5 mg/mL methyl thiazolyl diphenyl-tetrazolium bromide (MTT) solution was prepared in a sterile phosphate-buffered saline (PBS) solution, pH 7.4 and added into each well at 10 µL. After that, the plate continued to be incubated for 4 h to allow the formation of the formazan crystal by viable cells. The media supernatant was then replaced with 100 µL dimethyl sulfoxide (DMSO). The absorbance at 540 nm was then recorded with a plate reader machine.

2.7.4. Preparation of the Cell Lysate

The cell layer was primarily washed twice with 37 °C sterile PBS prior to the addition of 50 µL cell lysis buffer. The cells were exposed to the lysis buffer at room temperature for 15 min to completely lyse. The lysate was collected and stored in an Eppendorf pipette under −20 °C for further analyses.

2.7.5. Total Protein Determination by Bradford Assay

The total protein content was analysed in 5 µL of the cell lysate by mixing with 100 µL of the working Bradford solution. The complete reaction was allowed to incubate for 15 min at room temperature. The absorbance was recorded with a plate reader at 595 nm. Like the sample, standard bovine serum albumin (BSA) was used to establish the standard curve for the deduction of total protein content in each sample.

2.7.6. Alkaline Phosphatase (ALP) Activity Determination

Briefly, 20 µL of the lysate was mixed with 80 µL of 1 mg/mL p-nitrophenolphosphate in a diethanolamine buffer pH 9.8 for 3 h and the yellow product was determined by with a plate reader to measure the absorbance at 405 nm. Along with the p-nitophenol (pNP) standard, ALP activity could be elucidated and expressed in the specific activity per mg of the total protein.

2.7.7. Statistical Analysis

ANOVA followed by Tukey’s pos-hoc test was used when the ANOVA result showed a significant difference. The p-value was set at 0.05 in all the statistical procedures.
3. Results and Discussion

3.1. Thermal Analysis

Figure 1 illustrates the effect of the Al₂O₃ concentration on the thermal parameters of the glass specimens by performing DTA at a heating rate of 10 °C/min while maintaining an air atmosphere throughout the experiment. The letters Tg, TC₁, TC₂, and Tm indicate the temperatures at which the glass transition, first crystallisation, second crystallisation, and melting temperature occur in the DTA curves. Exothermic peaks indicate that the glass is crystallised, whereas endothermic peaks indicate that crystalline phases dissolve within the glass matrix. In the ranges of 663–668 °C and 825–845 °C, the results for each glass sample showed two exothermic peaks. The Al₂O₃ content was also found to have a negligible effect on the second crystallisation and melting temperature, which corresponds to a previous study [16]. It is possible that this is because Al₂O₃ typically makes the glass more viscous, which is caused by the elimination of nonbridging oxygen sites [14]. In addition, TC₁ is the temperature at which the lithium metasilicate (Li₂SiO₃) phase begins to crystallise. The second crystallisation temperature, TC₂, denotes the phase transition at which the crystallised LM phase changes into the lithium disilicate (Li₂Si₂O₅) phase [20–22]. According to the DTA results, the heat treatment condition was selected as two stages at 600 °C and 800 °C for 2 h at each temperature. It was speculated that the preliminary glass nucleation would occur at the initial stage at 600 °C and then the crystalline phase of Li₂Si₂O₅ would start to occur at the final stage at 800 °C.

![Figure 1. DTA curves of Li-Si glass powder with different Al₂O₃ contents.](image)

3.2. Phase Formation

The phase formation of the glass-ceramic samples was investigated by forming XRD analysis, which showed the precipitating crystalline phase as a function of the various Al₂O₃ contents. The XRD results of all the LSA glass-ceramic samples after heat treatment at 600 °C and 800 °C for 2 h are shown in Figure 2. The XRD results revealed that Li₂Si₂O₅ was the main crystalline phase in all the samples. The highest intensity of the Li₂Si₂O₅ peak was obtained when an Al₂O₃ content of 1%mol was added, and it coexisted with a small amount of cristobalite phase. This finding is consistent with the DTA results at 800 °C; this is close to the crystalline temperature of all the glass samples, especially LSA1, which showed a Tc at 825 °C, while LSA2 and LSA3 were at 830 °C and 845 °C, respectively. Therefore, the crystallisation reaction of Li₂Si₂O₅ was more complete, and the highest
intensity peak was observed. The crystalline phase of Li$_2$Si$_2$O$_5$ was transformed in two steps via the following reaction:

\[
\text{Li}_2\text{O (glass) + SiO}_2\text{ (glass) } \rightarrow \text{Li}_2\text{SiO}_3\text{ (crystal)}, \tag{4}\\
\text{Li}_2\text{SiO}_3\text{ (crystal) + SiO}_2\text{ (crystal) } \rightarrow \text{Li}_2\text{Si}_2\text{O}_5\text{ (crystal)}. \tag{5}
\]

![XRD analysis of glass-ceramic samples with various Al$_2$O$_3$ contents after heat treatment.](image)

Generally, in this Li$_2$O–SiO$_2$ glass system, the first crystalline phase of Li$_2$SiO$_3$ is formed by a reaction between Li$_2$O and SiO$_2$ (Equation (4)) at a lower temperature of 550–650 °C [22–24]. However, when the heat treatment temperature increased, Li$_2$SiO$_3$ became unstable and transformed into the more stable phase of Li$_2$Si$_2$O$_5$, as shown in Equation (5).

The amounts of the Li$_2$Si$_2$O$_5$ phase decreased with the increasing Al$_2$O$_3$ content, while the cristobalite phase disappeared and CeO$_2$ started to form in the LSA2 samples. Moreover, the CeO$_2$ phase remained almost unchanged with a further increase in the Al$_2$O$_3$ content in LSA3. This may also explain why the cristobalite phase is metastable and cannot be retained at higher Al$_2$O$_3$ contents. However, the reaction that transformed to Li$_2$Si$_2$O$_5$ was incomplete and showed impurities in the CeO$_2$ phase in the LSA2 and LSA3 samples. This may be attributed to the addition of Al$_2$O$_3$ leading to a decrease in the crystal growth velocity [25], so when the Al$_2$O$_3$ content exceeded the solution limit (LSA2 and LSA3), a foreign CeO$_2$ phase was residual in the glass-ceramic samples, which led to the impurity of the materials.

3.3. SEM Analysis

Figure 3 shows the microstructure of the surface glass–ceramic samples. The results illustrated that the surfaces of the LSA1 and LSA2 samples showed the formation of a rod-like Li$_2$Si$_2$O$_5$ phase that was randomly dispersed. However, the morphology of the crystalline phase of the LSA3 sample was different. With the addition of Al$_2$O$_3$ to 1.5 mol%, the grain size that was changed to higher increased, and the number of rod-like shapes gradually decreased. Moreover, the shape of the crystals was slightly transformed to the spherical shape of Li$_2$SiO$_3$, presenting a polymorphic microstructure [26].
These results were confirmed by the fracture surface of the glass–ceramic samples in Figure 3, which shows that in the LSA1 samples, the crystalline shape is rod-like and distributed on the glass matrix of the glass–ceramics. Generally, cracks are always formed at the weaker interfaces of the crystals and propagate through the residual glass matrix. However, in LSA1, the crystal morphology is rod-like. It has a closely packed and multi-directional interlocking microstructure of $\text{Li}_2\text{Si}_2\text{O}_5$ that deflects and delays crack propagation [27]. Microcracks were formed inside the matrix of glass–ceramics with curves and intersecting paths. This was related to the interlocking structure of $\text{Li}_2\text{Si}_2\text{O}_5$, which prevented crack propagation in the samples. On the other hand, with increasing $\text{Al}_2\text{O}_3$ content, the density of $\text{Li}_2\text{Si}_2\text{O}_5$ crystals decreased and became spherical coexisting with the rod shape in LSA3. Notably, increasing the $\text{Al}_2\text{O}_3$ content depressed the reaction of $\text{Li}_2\text{Si}_2\text{O}_5$ but consumed the $\text{Li}_2\text{SiO}_3$ crystal.
3.4. Mechanical Properties

The improved mechanical properties of glass–ceramics are also a consequence of their higher crystallinity, appropriate morphology of precipitated crystals and low porosity [16,26]. The flexural strength, the flexural modulus of elasticity, and microhardness values of the glass-ceramic samples with different Al$_2$O$_3$ are shown in Figures 4 and 5. Similar trends can be observed in all the mechanical properties measured in this study with respect to the Al$_2$O$_3$ contents. The highest mechanical strength values were obtained from the glass–ceramics sample upon adding Al$_2$O$_3$ to 1.0 mol% and slightly decreased as the Al$_2$O$_3$ content was increased to 1.5 mol%. A possible explanation is that LSA1 samples contained the highest crystalline phase of rod-like Li$_2$Si$_2$O$_5$, which formed an interlocking microstructure to prevent cracks within the samples. In addition, the increasing Al$_2$O$_3$ had the effect of decreasing the densification of the crystal, which is related to the amount of glassy phase and its viscosity [28]. In the samples with higher Al$_2$O$_3$ content, the amount of glassy phase was reduced, and the viscosity of the glassy phase increased, so the addition of a crystalline phase could inhibit densification [28]. This is consistent with the work of Fernandes et al. [29] reporting that the glass sample with the lowest Al$_2$O$_3$ increased the densification of the crystal which resulted in an improvement in the mechanical properties of lithium disilicate glass-ceramics.

The mechanical strength values of the specimens in the present study were close to those of the commercial lithium disilicate developed by Ivoclar Vivadent company and other studies, as shown in Table 1. Commercial lithium disilicate has a flexural strength and Vickers hardness values of 4400 and 5800 MPa, respectively. These excellent mechanical properties can be attributed to the development of Li$_2$Si$_2$O$_5$ in the sample.

![Figure 4](image-url)  
**Figure 4.** Flexural strength and flexural modulus of elasticity of the glass–ceramic samples with different Al$_2$O$_3$ contents after heat treatment at 600/800 °C.

| Materials | Commercial Leucite (IPS Empress®) | Commercial Leucite (IPS Empress 2) | Commercial Lithium Disilicate | Lithium Disilicate | Lithium Disilicate |
|-----------|----------------------------------|-----------------------------------|-----------------------------|-------------------|-------------------|
| Flexural strength (MPa) | 90–130 | 400 | 400 | 350–450 | 316.72 |
| Vickers hardness (MPa) | 4001–6500 | 6500 | 5800 | 4001–6500 | 6685 |
| Ref. | [30,31] | [32] | [28] | [8,33] | This study (LSA1) |
3.5. Microshear Bond Strength

The cementation of resin cement for ceramic restorations is a crucial process for clinical success [A1]. Various surface treatments have been used before cementation to improve the bond strength of dental ceramics [34,35]. The micro-shear bond strength test was selected in this study to evaluate the bonding performance owing to its benefits of more uniform stress distribution and lack of damage to specimens that are suitable for the glass–ceramic material [36,37].

In addition to the different mechanical properties, there was no significant difference in μSBS among the three group of samples (LSA1, LSA2 and LSA3) of lithium disilicate glass–ceramics. This might be due to the lower effect of the mechanical properties on the bonding ability between the resin cement and the selected ceramics, as shown in Table 2. However, mechanical properties are crucial and must be considered because ceramics with better mechanical properties provide a longer lifetime for all ceramic restorations [38,39].

The mean μSBS of all the groups with different surface treatments was significantly higher than that of the control group. This result could be attributed to the effectiveness of the chemical bond created by the silane coupling agent and the micromechanical retention created through hydrofluoric acid etching.

Silane promotes the adhesion between the functional methacrylate group of resin cement and the hydroxyl group of the silica-based ceramic. Additionally, a hydrophobic surface can be created by the application of a silane-coupling agent, which allows resin cement to flow [40–42].

Table 2. Mean and standard deviation of micro-shear bond strengths.

| Surface Treatment                        | Microshear Bond Strength (Mean ± SD; MPa) |
|-----------------------------------------|------------------------------------------|
|                                         | LSA1          | LSA2          | LSA3          |
| No surface treatment (Control)          | 3.69 ± 0.56   | 3.47 ± 0.58   | 3.23 ± 0.48   |
| Silane coating                          | 34.20 ± 2.57  | 37.21 ± 3.43  | 37.97 ± 3.07  |
| Hydrofluoric Acid                       | 20.92 ± 2.52  | 20.74 ± 2.85  | 19.34 ± 3.48  |
| Hydrofluoric acid + Silane coating      | 45.34 ± 2.40  | 47.57 ± 4.05  | 47.89 ± 3.10  |

Different superscript letters indicate statistical significance ($p < 0.05$).

Hydrofluoric acid can dissolve the glass matrix and increase the surface area for micromechanical retention between resin cement and the etched glass surface [43–45].

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**Figure 5.** Vickers hardness values of the glass–ceramic samples with different Al₂O₃ contents after heat treatment at 600/800 °C.
resulting in increased bond strength. However, the lithium disilicate glass–ceramics used in this study contained Al$_2$O$_3$, which can neutralise the acidity of hydrofluoric acid and impair the etching ability. Moreover, the penetration of resin cement could be interfered with by the insoluble salt formed owing to the interaction between aluminium ions and hydrofluoric acid [46]. This may be the reason for the low $\mu$SBS in the group treated with hydrofluoric acid.

The highest $\mu$SBS value was obtained by etching with hydrofluoric acid, followed by the application of a silane coupling agent. This method has been recommended as the most effective surface treatment for glass–ceramics [45,47–49]. This result might be due to the synergistic combination of mechanical and chemical retention, as the increased surface area created by etching provides more surface area for chemical bonds.

### 3.6. Cellular Activity

Although the results showed lower cell numbers attached to LSA3, there was no significant difference among the others (Figure 6). The attachment of the cells onto the surface of the glass-ceramic samples were not affected by the observation period (6–24 h), indicating that the glass-ceramics allowed MG-63 cell attachment, regardless of the concentration of Al$_2$O$_3$.

![Figure 6. MG-63 cell attachment to lithium disilicate glass-ceramics with various Al$_2$O$_3$. MTT assay of MG-63 cells attached to lithium disilicate glass-ceramics with various Al$_2$O$_3$ for 6 h, 12 h, and 24 h. There was no statistical difference among glass-ceramics types and time points.](image)

Lithium disilicate glass-ceramics with various Al$_2$O$_3$ were biocompatible with MG-63 cells. Although the MG-63 viability cultured on LSA1 significantly decreased compared with that on LSA2 and LSA3 after 4 days of incubation, this difference was dissipated after 7 days of incubation (Figure 7). The viability of the cells significantly increased over time compared with that at the early time point. This result confirmed the biocompatibility of all types of glasses owing to the ability of MG-63 cell proliferation on all types of glasses.

Lithium disilicate glass-ceramic material with various concentrations of Al$_2$O$_3$-activated protein was produced by the MG-63 cells. The difference in the total protein content was significantly higher in the MG-63 cells cultured with LSA1 after 4 days of culture; however, these contents were similar in all material types after 7 days of culture (Figure 8). When compared between time points, the protein content was significantly increased in all the investigated materials. Taken together, the proliferation results confirmed that the glass specimens, regardless of the presence of aluminium, were biocompatible with MG-63 cells.
Figure 7. Viability and proliferation of MG-63 cells. Lithium disilicate glass-ceramics with various Al\textsubscript{2}O\textsubscript{3} were assessed in terms of biocompatibility with MG-63 cells using MTT assay for 4 (D.4) and 7 days (D.7). The results were from a triplicate (n = 3) experiment and the statistical difference was tested when p < 0.05, which is represented by * (among the material types in each investigation period) and by # (between two investigation periods among the same material types).

Figure 8. MG-63 protein contents. Total MG-63 protein production after the culture with lithium disilicate glass (LSA3) or lithium disilicate glass supplemented with aluminium at 0.5% mol (LSA1) and with aluminium at 1% mol (LSA2) for 4 (D.4) and 7 days (D.7), as determined with Bradford assay. The results were from a triplicate (n = 3) experiment and the statistical difference was tested when p < 0.05, which is represented by * (among the material types in each investigation period) and by # (between two investigation periods among the same material types).

In terms of the alkaline phosphatase (ALP) activity of MG-63, the MG-63 cells cultured with lithium disilicate glass-ceramics with various Al\textsubscript{2}O\textsubscript{3} for 4 (D.4) and 7 days (D.7) were assessed for the ALP activity by performing p-nitrophenolphosphate conversion assay, and the results are shown in Figure 9. Regarding the expression of a bone formation marker, the ALP activity was not significantly expressed in the MG-63 cells cultured with different types of lithium disilicate glass-ceramics after 4 and 7 days of investigation. Likewise, the ALP activity expression trend was slightly reduced during prolonged investigation periods in all the tested glass-ceramics. This suggests that lithium disilicate glass-ceramics,
regardless of Al$_2$O$_3$, are biologically active, allowing MG-63 cells to normally express the ALP activity, leading to the regulation of bone formation.

![Figure 9](image-url)

Figure 9. The alkaline phosphatase (ALP) activity. There was no statistical difference among glass-ceramics types and time points.

IPSe. max is a lithium disilicate glass–ceramic ingot that delivers high-strength and highly aesthetic materials for press technology. Figure 10 shows LSA3 before and after heating at 600/800 °C. This sample can be pressed into the shape of a dental crown.

![Figure 10](image-url)

Figure 10. The LSA3 sample before heating (left), the LSA3 sample heated at 600/800 °C and the completely pressed object (right).

4. Conclusions

The aim of this study was to investigate the effect of Al$_2$O$_3$ at the three different contents of 0.5, 1.0 and 1.5 mol% on the mechanical properties of the modified Li$_2$O–SiO$_2$ glass systems with CeO$_2$. The Al$_2$O$_3$ content played a major role in phase formation changes in glass-ceramic samples. With increasing the content of Al$_2$O$_3$, the relative content of the Li$_2$Si$_2$O$_5$ phase decreased. The data showed that the LSA1 had the highest crystallinity of Li$_2$Si$_2$O$_5$ as well as the highest mechanical strength correlated with the
homogeneous crystallisation of interlocked rod-like Li$_2$Si$_2$O$_5$. In spite of the differences in the mechanical strength, of the samples, all the ceramic conditions showed the same micro-shear bond strength for each surface treatment. An MTT assay was used to test the cellular adhesion and biocompatibility of lithium disilicate glass-ceramic sample for all three contents of Al$_2$O$_3$. Even though the results were insignificant among the three samples, can be reasonably claimed that these samples are suitable for dental applications.

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