Biomedical Materials

PAPER

Biosafety evaluation of Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics

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Abstract

Lithium disilicate (Li$_2$Si$_2$O$_5$) glass-ceramic is a commonly used dental ceramic material. In this study, Li$_2$Si$_2$O$_5$ whiskers were prepared by the hydrothermal method, mixed with Li$_2$Si$_2$O$_5$ glass powders, and Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics were prepared by reaction sintering. The biosafety of the new Li$_2$Si$_2$O$_5$ glass-ceramics were evaluated by in vitro cytotoxicity, hemolysis, oral mucosal irritation, acute systemic toxicity, and subacute systemic toxicity (oral route) tests according to ISO 7405/ISO 10993 standards. The cytotoxicity test results showed that the cell growth of the experimental group was good, and the cell number and morphology were not significantly different from those of the blank group ($P > 0.05$). The toxicity grading for both experimental and blank control groups were 0. The hemolysis rate of the material was 1.25%, which indicated that it did not cause hemolytic reaction. The material was non-irritating to the oral mucosa. In acute systemic toxicity test, animals in the experimental group showed increased body weight, moved freely, with no signs of poisoning. The food utilization rate and relative growth rate (change of the weight) of rats in the subacute systemic toxicity test were not statistically different from those of the control group ($P > 0.05$). Preliminary evaluation of the biosafety of the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics showed that it met the existing regulatory standards, and further biosafety experiments can be conducted, following which the material may be expected to be applied in clinical practice.

1. Introduction

Dental restorative materials need to meet basic prerequisites, including similarity to the tooth structure in terms of the mechanical, physical, and esthetic properties [1]. Compared with metal and other ceramic restorative materials, glass-ceramics have excellent biocompatibility and resistance to wear and corrosion, and they have a similar luster to that of teeth that is unmatched by other materials [2]. Among glass-ceramics, glass-ceramics with lithium disilicate (Li$_3$Si$_4$O$_9$) as the main crystalline phase have excellent esthetic and mechanical properties owing to their unique crystal properties and crystal distribution [3, 4]. However, the low fracture toughness and high brittleness of glass-ceramics limit the scope of their clinical applications [5]. For this reason, toughening of glass-ceramics to decrease their brittleness and increase their fracture toughness has become the core of all-ceramic materials research in recent years [6]. Many researchers have attempted to improve the fracture toughness of Li$_2$Si$_2$O$_5$ glass-ceramics by changing the heat-treatment process and using different nucleating agents [7], but the results are not satisfactory. The properties of Li$_2$Si$_2$O$_5$ glass-ceramics have often been determined by the crystallization phases and microstructure, such as the grain shape, size, and distribution [8, 9]. The particle size of Li$_2$Si$_2$O$_5$ crystals prepared by traditional methods is small. The presence of these small grains leads to a lack of interlocking microstructures in the crystals, resulting in more microcracks and poor crack propagation resistance [10, 11]. Belli et al [12] found that ceramic materials with high-aspect-ratio crystals...
generally have better fracture resistance than materials with low-aspect-ratio crystals, and they proposed to toughen Li$_2$Si$_2$O$_5$ glass-ceramics by appropriately increasing the crystal aspect ratio. It has been found that whiskers have high strength, high modulus, and high elongation, which can effectively improve the strength, toughness, hardness, wear resistance, and heat resistance of composite materials while retaining the main characteristics of ceramic materials \cite{13, 14}. Addition of whiskers to ceramic materials can improve the material properties and fracture strength through crack bridging, crack deflection, and pull-out effects \cite{15}. Therefore, an increasing number of ceramic materials have been enhanced by adding whiskers to improve their mechanical properties. Nevertheless, few studies on whisker-reinforced Li$_2$Si$_2$O$_5$ glass-ceramics have been performed. Therefore, in this study Li$_2$Si$_2$O$_5$ whiskers were creatively synthesized by the hydrothermal method in preliminary experiments. The Li$_2$Si$_2$O$_5$ whiskers were then added to Li$_2$Si$_2$O$_5$ glass-ceramics to achieve enhanced toughening of glass-ceramics, which overcame the problems of traditional glass-ceramics, such as high brittleness and low fracture toughness, and the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics showed good prospects for clinical applications.

Glass-ceramics are a type of biomaterial that require not only excellent physical and chemical properties, but also good biocompatibility to minimize the adverse effects caused by direct contact with tissues. These materials must not only maintain their integrity under such harsh conditions, but they must also maintain these characteristics throughout their function \cite{2}. Although restorative dentistry materials are as durable and inert as possible, restorations can degrade or fail while releasing some components into the mouth and causing harm to the body. Therefore, extensive attention must be paid to the safety and biocompatibility of ceramic materials. Biocompatibility is described as the ability of a biomaterial to perform its desired function without any adverse reactions in the beneficiary of the material. Biocompatibility is a dynamic process, and the biological response may change over time depending on the interactions between the host and the material, and the function of the material \cite{16}. Dental materials are considered to be biomaterials and they are expected to be non-toxic in living tissues. Dental materials are strictly tested by regulatory agencies before they are allowed to be used in clinical practice. The test methodologies are categorized as in vitro, animal, and usage tests \cite{17}. Therefore, the biosafety of the new Li$_2$Si$_2$O$_5$ glass-ceramics were initially evaluated by in vitro cytotoxicity, hemolysis, oral mucosal irritation, acute systemic toxicity, and subacute systemic toxicity (oral route) tests according to ISO 7405/ISO 10993 standards to investigate the possibility of its application in clinical practice and provide an experimental basis for later clinical application.

\begin{table}
\centering
\caption{Composition of the base glass (mol.\%).}
\begin{tabular}{ccccccc}
\hline
  & SiO$_2$ & Li$_2$CO$_3$ & NH$_4$H$_2$PO$_4$ & K$_2$CO$_3$ & Al$_2$O$_3$ & La$_2$O$_3$ \\
\hline
  & 65.5 & 27.5 & 1.2 & 1.8 & 2 & 2 \\
\hline
\end{tabular}
\end{table}

\section{2. Materials and methods}

\subsection{2.1. Preparation of the materials}

\subsubsection{2.1.1. Preparation of Li$_2$Si$_2$O$_5$ glass powders}

The composition of the ingredients of the base glass was shown in table 1. The raw materials were weighed and grounded thoroughly for 30 min, heated at 1450 °C in a muffle furnace, held for 30 min, water quenched, grounded into glass powders, sieved, washed and dried.

\subsubsection{2.1.2. Preparation of the Li$_2$Si$_2$O$_5$ whiskers}

LiOH-H$_2$O and nanoscale SiO$_2$ were mechanically mixed in a ratio of 1:1 with 75 ml of deionized water for 4 h. The slurry was transferred to a stainless steel autoclave lined with Teflon and heated at 150 °C for 6 h. The autoclave was then rapidly cooled to room temperature and the white product was collected by filtration, washed several times in turn with distilled water and ethanol, and finally dried at 80 °C for 24 h to produce Li$_2$Si$_2$O$_5$ whiskers.

\subsubsection{2.1.3. Preparation of the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics}

A mixture of Li$_2$Si$_2$O$_5$ whiskers and glass powders was wet mixed with ZrO$_2$ balls in 99.7% anhydrous alcohol for 2 h. After drying, the mixture was pressed in a hardened steel die under 20 MPa, then the sample was sintered in a vacuum furnace at 900 °C for 1.5 h. Finally, the sample was cooled to ambient temperature to obtain the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics.

\subsubsection{2.1.4. Characterization}

The flexural strength and fracture toughness of the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics were measured with a universal mechanical testing machine and single-edge notched beam. X-ray diffraction (XRD) was performed to investigate the crystalline structure. Scanning electron microscopy (SEM) was performed to analyze the microstructure (5% hydrofluoric acid, 1 min).

\subsection{2.2. Preparation of the specimens}

The specimen size was 5 mm in diameter and 1 mm thick.

\subsection{2.3. Preparation of the extracts}

The specimens were ultrasonically washed in 99.7% anhydrous ethanol for 20 min, rinsed with distilled water, routinely disinfected, and dried. The specimens were placed in physiological saline (the ratio of the surface area of the test piece to the extraction
medium was 3 cm² ml⁻¹) and kept in a constant-temperature water bath at 37 °C for 24 h.

2.4. Biosafety tests

2.4.1. In vitro cytotoxicity test [18] (table 2)

L-929 mouse fibroblasts were revived and passaged, and the cells were lysed with 0.25% trypsin to make a single cell suspension of 1 × 10⁶ cells ml⁻¹ and inoculated on a 96-well plate (200 µl well⁻¹). The cells were incubated in a cell-culture incubator at 37 °C and 5% CO₂ for 24 h. The original culture medium was discarded after observation of cell apposition. The cells were randomly divided into seven groups with six wells of samples in each group for exchange of immersion solution (200 µl well⁻¹). The experimental group was the extracts of the new Li₂Si₂O₅ glass-ceramics with Dulbecco’s modified Eagle medium (DMEM) culture medium containing 10% embryonic bovine serum as the immersion medium, and it was diluted to 25%, 50%, 75%, and 100%. The positive control group was DMEM culture solution containing 0.64% phenol and 10% embryonic bovine serum (a separate culture plate was set up for the phenol-containing control group to prevent phenol volatilization from affecting the experimental group.). The negative control was alumina ceramic discs with DMEM culture fluid containing 10% embryonic bovine serum as the immersion medium, and the blank control was DMEM culture fluid containing 10% embryonic bovine serum. Three 96-well plates were inoculated under the same conditions and incubated in a thermostat for 2, 4, and 7 d.

A 96-well plate was removed on days 2, 4 and 7 of the extract exchanges to observe the cell morphology under an inverted microscope. Subsequently, 20 µl of 0.5% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well and incubation was continued for 4 h. The supernatant was aspirated and 150 µl of dimethyl sulfoxide (DMSO) was added to each well, and the plate was then uniformly shaken for 10 min to dissolve the crystals. The absorbance value (OD) of each well was measured at 570 nm using an enzyme-linked immunoassay (ELISA), and the relative growth rate (RGR) of the cells was calculated (RGR = mean OD value of each experimental group/mean OD value of the blank group × 100%). The grading of the cytotoxicity was determined according to the grading criteria of ISO 10993-5.

2.4.2. Hemolysis test [19]

Four-weeks-old New Zealand rabbit blood (20 ml) was added to 1 ml of 1% potassium oxalate solution for anticoagulation. The anticoagulated rabbit blood (8 ml) was added to 10 ml of 0.9% saline for dilution. 0.2 ml diluted anticoagulant rabbit blood was added into 10 ml distilled water and was placed at 545 nm of spectrophotometer. The absorbance was 0.8 ± 0.3 [20]. The experimental group was the new Li₂Si₂O₅ glass-ceramics (5 g) added to 10 ml of saline. Physiological saline (10 ml) and distilled water (10 ml) were set as the negative control group and positive control group, respectively. Three parallel experiments were performed for each group. All test tubes were put in 37 °C constant-temperature water bath for 30 min. Then 0.2 ml diluted fresh anticoagulant rabbit blood was added to each test tube, mixed gently, and kept in constant temperature water bath for 60 min. The solution was centrifuged at 750 g for 5 min. The absorbance of the supernatant was then measured at 545 nm and the hemolysis rate was calculated:

\[
\text{Hemolysis rate (\%)} = \frac{[\text{ODtest} - \text{ODnc}] - [\text{ODpc} - \text{ODnc}]}{\text{ODnc}} \times 100\%
\]

where ODtest, ODpc and ODnc are the absorbance value of the test sample, the positive control, and the negative control, respectively.

2.4.3. Oral mucosa irritation test

Ten Specific Pathogen Free (SPF)-grade male Chinese gophers (20–25 g) of 6–8 months with no mucosal damage to the bilateral buccal sacs were used in the test. The gophers were anesthetized with 2% sodium pentobarbital (at a dose of 20 mg kg⁻¹). The mouths of the gophers were disininfected inside and outside with iodophor. One side of the oral mucosa of each gopher was sutured and fixed with the new Li₂Si₂O₅ glass-ceramics specimen. The other side of the buccal mucosa, the control, was sutured and fixed with an identically sized adhesive disc. Postoperatively, the
mucosal sutures were observed daily for detachment, signs of toxicity in the animals, and other abnormalities of the mucosa surrounding the specimen, such as congestion, swelling, erythema, and erosion. Gophers were executed by overdose anesthesia 14 d after surgery. The mucosa and its surrounding tissues were fixed, embedded and sectioned, and stained with hematoxylin-eosin (HE) for histopathological examination.

2.4.4. Acute systemic toxicity test
Twenty healthy (SPF grade) C57 mice weighing 16–22 g were randomly divided into two groups: an experimental group and a control group (half male and half female). The mice in the experimental group were injected intravenously with the new Li$_2$Si$_2$O$_5$ glass-ceramics extract at a dose of 50 ml kg$^{-1}$, while the mice in the control group were injected with the same amount of saline. The animals were immediately observed, then at 4, 24, 48 and 72 h after treatment for adverse signs, such as prostration, cyanosis, and dyspnea, and they were weighed daily. The animals were anesthetized with 2% sodium pentobarbital at a dose of 20 ml kg$^{-1}$ before execution, and blood was collected from the heart for hemato pathological examination. At the end of the experiment, the mice were executed under excessive anesthesia, observed for abnormal changes in their vital tissues and organs and subjected to histopathological examination.

2.4.5. Subacute systemic toxicity test: oral route
Twenty SPF-grade Sprague-Dawley (SD) rats (130–150 g mass, 5 weeks old) were randomly divided into experimental and control groups of ten rats each (half male and half female). The animals were acclimatized to the laboratory environment 7 d before the experiment, and they were required to fast overnight without restricting their water intake before injection of the extract. The extract of the new Li$_2$Si$_2$O$_5$ glass-ceramics (5 ml kg$^{-1}$) was administered daily to the experimental group of SD rats by gavage needle. The control group received the same dose of physiological saline by gavage once a day for 28 d. After the end of administration, all of the animals were observed for a week. The food consumption and weight of the rats was measured at least once a week, and the food utilization rate and RGR were calculated. At the end of the experiment, the animals were executed under excessive anesthesia to observe any abnormal changes in their vital tissues and organs and to perform histopathological observation:

Food utilization rate (%)  
= weight gain (g) / total food consumption (g)  
× 100%

Relative growth rate of weight (%)  
= weight gain (g) / original weight (g)  
× 100%.

3. Results

3.1. Characterization of the Li$_2$Si$_2$O$_5$ whiskers
The SEM morphology and XRD pattern of the Li$_2$Si$_2$O$_5$ whiskers synthesized by the hydrothermal method are shown in figures 1(A) and (B). The whiskers were rod-like crystals with an average length of 1.37 µm and an average width of 0.13 µm, and the aspect ratio of the whiskers was 5–12 (figure 1(A)). XRD analysis showed no impurity peaks, indicating that the obtained sample had high purity, and the peaks were consistent with the diffraction peaks of ICDD PDF 33-0816. Therefore, synthesis of the Li$_2$Si$_2$O$_5$ whiskers by the hydrothermal method laid the foundation for preparation of glass-ceramics.

3.1.1. Characterization and mechanical properties of the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics
The XRD pattern of the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics are shown in figure 2(A). The main precipitated crystalline phases were Li$_2$Si$_2$O$_5$ (ICDD PDF#40-0376). The SEM morphology of the etched Li$_2$Si$_2$O$_5$ glass-ceramics are shown in figure 2(B). Addition of Li$_2$Si$_2$O$_5$ whiskers led to the coexistence of multi-scale crystals in the glass-ceramics. Large rod-like crystals formed by epitaxial growth of the Li$_2$Si$_2$O$_5$ whiskers, while small rod-like crystals formed by self-crystallization of the Li$_2$Si$_2$O$_5$ glass-ceramics powder. Addition of Li$_2$Si$_2$O$_5$ whiskers improved the mechanical properties of the glass-ceramics. The flexural strength (389.5 ± 11.77 MPa) and fracture toughness (3.46 ± 0.10 MPa m$^{1/2}$) met the requirements of restorative materials, which has important practical significance for their large-scale application in the field of dental restoration.

3.2. In vitro cell-culture study
After 7 d of cell culture of each group, the number of fibroblasts was significantly reduced in the positive control group on days 2, 4 and 7 of cell culture and the cells were scattered and possessed a strip or circular shape. The cells in all of the experimental groups grew normally, and the cells were shuttle- or long-stripe-shaped, similar to the blank control group (figure 3). The absorbance values of each group are shown in figure 4. There was a significant difference between the positive control group and the other groups ($P < 0.05$), while there was no significant difference between the experimental group and the negative control group and blank control group ($P > 0.05$). The RGR of the cells in each experimental group was significantly higher than that of the positive control group at each time point of 2, 4 and 7 d of culture ($P < 0.05$), but there was no significant difference between the negative control group and blank control group ($P > 0.05$) and the cytotoxicity grade (CTG) was 0 (table 3). The experimental data indicated that the new Li$_2$Si$_2$O$_5$ glass-ceramics were non-cytotoxic.
3.3. Assessment of hemolysis
According to ISO 10993-4 hemolysis rate of less than 5% implies that the test material can be used for biomedical applications. The supernatant of the positive control group was red, indicating that the red blood cells had been destroyed. In contrast, the supernatants of the other groups were colorless and almost no hemoglobin could be observed (figure 5). The hemolysis rate measured in this experiment was 1.25% (table 4), which was lower than 5%, indicating that the new Li$_2$Si$_2$O$_5$ glass-ceramics had excellent hemocompatibility.

3.4. Mucosal irritation analysis
All of the gophers ate normally and moved freely during the experiment. No abnormalities or adverse reactions were observed. Three pieces of specimens were lost (one ceramic specimen and two dental discs), and the rest of the specimens were well fixed. When the test piece was removed, there was no significant difference between the two sides of the buccal mucosa, and the surrounding tissues were not observed to be congested, swollen, or eroded. Histological examination of the buccal mucosa and its surrounding tissues showed normal cell morphology and structure with no inflammatory cell infiltration, hyperkeratosis, granular layer changes, or other undesirable changes (figure 6). According to YY/T 0127.13-2018, the new Li$_2$Si$_2$O$_5$ glass-ceramics were non-irritating to oral mucosa.

3.5. Acute toxicity analysis
The mice in the experimental group moved freely during the observation period, with good mental status and no signs of poisoning. The body weights of all of the animals increased normally with no statistically significant difference in the body weight change ($P > 0.05$) (figure 7). There were no abnormalities
in hematological examination of the mice, and the differences between the experimental group and control group were not statistically significant ($P > 0.05$). Gross anatomical examination of the experimental group showed no abnormal changes, and histopathological examination of the important organs of the mice showed no pathological changes such as degeneration, atrophy, or necrosis (figures 8(A) and (B)). The experimental results showed that the leaching solution of the new Li$_2$Si$_2$O$_5$ glass-ceramics had no acute toxic effects, and the acute systemic toxicity test of the new Li$_2$Si$_2$O$_5$ glass-ceramics were qualified.

### 3.6 Subacute toxicity analysis

In all of the groups of rats during the experiment, there was no death, the rats showed normal appetite, and there was no adverse reaction or poisoning.
Table 4. OD values of the hemolysis test.

| Grouping           | OD value |     |     | Average OD value | Hemolysis ratio (%) |
|--------------------|----------|-----|-----|------------------|---------------------|
|                    | 1        | 2   | 3   |                  |                     |
| Experimental group | 0.0358   | 0.0313 | 0.0366 | 0.0346          | 1.2502             |
| Negative control   | 0.0203   | 0.0265 | 0.0246 | 0.0238          | —                   |
| Positive control   | 0.9326   | 0.8471 | 0.8753 | 0.8850          | —                   |

The RGRs and food utilization rates of the rats in the experimental and control groups were calculated based on the changes in the food consumption and body weight, and the differences were not statistically significant by the t-test (figure 9). No congestion, hemorrhage, edema or other abnormal changes were observed in the anatomy of the important organs of the rats in the experimental group. Histopathological examination of the vital organs of the rats did not show pathological changes such as degeneration, atrophy, or necrosis (figures 8(C) and (D)). The results showed that the new Li$_2$Si$_2$O$_5$ glass-ceramics materials had no toxic effects in the subacute systemic toxicity test.

4. Discussion

Li$_2$Si$_2$O$_5$ glass-ceramics are the strongest class of available dental glass-ceramics. Li$_2$Si$_2$O$_5$ whiskers were prepared by the hydrothermal method, mixed with Li$_2$Si$_2$O$_5$ glass powders, and the Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics were prepared by reaction sintering. Li$_2$Si$_2$O$_5$ whiskers are micronano scale short fibers with small diameters and high
aspect ratios grown from high-purity single crystals, and they have highly ordered atomic arrangements without defects, such as grain boundaries, dislocations, and cavities. The ideal interfacial bonding state and very high aspect ratio are conducive to the action of the crystals. They prevent crack expansion by the bridging mechanism, crack-deflection mechanism, pull-out mechanism, and they enhance the fracture toughness of the material. Their strength is close to the theoretical strength of the interatomic valence bonds of the material, and their strength greatly exceeds those of currently used reinforcing agents [21]. Li$_2$Si$_2$O$_5$ whiskers can be used as a modifier for plastics and ceramics, and they have excellent physical, chemical, and mechanical properties. In addition, Li$_2$Si$_2$O$_5$ whiskers can withstand large strain without permanent deformation, show no fatigue effect, and show no loss of strength even when ground into powder [22]. By adding high-aspect-ratio whiskers to the matrix of glass-ceramics, on the
one hand, the high-strength whiskers can share the applied load, and on the other hand, the weak interfacial bond between the whiskers and the ceramic interface can be used to create an external energy absorption system, thus reducing the brittleness of the ceramic materials [23]. In addition, reaction sintering eliminates the incompatibility between the boundary phases and forms a good bonding interface, while the presence of external pressure prevents rapid growth of the grains, resulting in a uniform distribution of the grains and formation of an interlocking microstructure. The strength of the Li₂Si₂O₅ glass-ceramic are higher for more uniform and tightly arranged rod-shaped crystals that form the interlocking microstructure. Biomaterials need to undergo rigorous testing before they can be used in humans. The mechanical and physicochemical properties of the new Li₂Si₂O₅ glass-ceramics were optimized through preliminary experiments. Biomaterials must also be safe and reliable before they can be used in the clinical setting, which requires that the reactions that occur after contact with the human body do not cause changes to the material itself or cause harm to the human body. Therefore, it is necessary and important to evaluate the biosafety of new biomaterials. The biosafety evaluation of biomaterials includes both in vitro and in vivo tests. Usually, in vitro studies are conducted first, followed by in vivo studies, and clinical evaluation is usually the final evaluation [24]. In vitro cells are usually more sensitive to harmful substances than in vivo tissues. The in vitro cytotoxicity test is one of the basic experiments of biosafety evaluation, and it is widely used in screening of biological materials and can reduce unnecessary animal experiments [25]. Commonly used assays for cytotoxicity are the MTT method and agar overlay method. MTT assay is based on the principle that the succinate dehydrogenase in the mitochondria of living cells can reduce exogenous MTT to water-insoluble formazan and deposit it in cells, while dead cells do not have this function. DMSO can dissolve the formazan in the cells, and its light absorption value is measured at 570 nm by ELISA, which indirectly reflects the number of living cells. According to ISO 10993-5, a cell survival rate of more than 90% for 1 week indicates that the material is non-cytotoxic. In this study, all of the experimental groups showed normal cell morphology at 2, 4 and 7 d, the relative cell growth rate was more than 100%, and the toxicity rating was 0, indicating that the material was not cytotoxic. The hemolysis test is commonly used as a screening test of biomaterials. The hemolysis test is based on the principle that a biological material will lyse red blood cells and free hemoglobin upon contact with blood cells, allowing the hemolytic properties of the material to be assessed in vitro. The hemolysis rate represents the extent to which red blood cells are broken by contact of the material extract with blood. According to ISO 10993-4, a test material with hemolysis rate less than 5%, can be used for biomedical applications. The hemolysis rate of the new lithium disilicate glass ceramics was 1.25%, indicating that the material had good hemocompatibility. The gopher was chosen for oral mucosal stimulation experiments because the buccal sac of gophers is wide, suturing and fixation of the material does not interfere with feeding, and the site is an immunologically specific area with glandular and lymphatic pathways and without histocompatibility antigens. This makes it a good area for tissue culture, observation of microcirculatory changes, and human tumor transplantation. The specimen was sutured and fixed with the buccal capsule to ensure that it remained in full contact with the mucosa throughout the experiment, which can fully simulate the contact mode between porcelain teeth and the human body. In the oral mucosal stimulation experiment, the irritation reactions of the new Li₂Si₂O₅ glass-ceramics and dental adhesive discs on the oral mucosa were compared. No significant inflammatory reactions were observed by the naked eye and histopathological examination, indicating that the material was non-irritating to the oral mucosa.
The acute toxicity of ceramic materials was tested by intravenous injection of extracts to assess the clinical compliance of the materials because small amounts of low-molecular-weight substances in the materials leached into the body and may have harmful effects on the body upon contact with the oral mucosa. The results of the acute systemic toxicity test were evaluated based on signs of toxicity such as lethargy, hyperactivity and convulsions, weight loss or death of the animal. The new ceramic material would be considered as toxic if: (a) two or more mice die, or (b) abnormal behavior (such as convulsions or prostrations) occurs in two or more mice, or (c) a body weight loss greater than 2 g occurs in three or more mice, during the procedure. No mortality was reported in the experimental group with normal appetite, weight gain and free movement exhibited by the animals throughout the experiment; and no abnormalities were found in the hematological and histopathological examinations. Hence the material was considered as non-toxic based on the acute systemic toxicity test.

All-ceramic restorations are in direct contact with the soft tissues of the oral cavity for a long time. Some of the components of the restorations can precipitate, and the precipitated components inevitably enter the body through the digestive tract. In this study, the extract of the restorative material was injected into the rats by gavage, which simulated the process of the precipitated components of the restorative material entering the digestive tract. The dose of the drug can be controlled by the gavage method, and the toxicity of the material to the organism after entering the digestive tract can be accurately evaluated. During the experiment, the animals did not die, their appetite was normal, and they moved freely. The differences between the food utilization rates and RGRs of the rats in the experimental and control groups were not statistically significant, indicating that the material showed no subacute systemic toxicity.

5. Conclusions

The Li$_2$Si$_2$O$_5$ whisker-reinforced glass-ceramics have been prepared by mixing Li$_2$Si$_2$O$_5$ whiskers with Li$_2$Si$_2$O$_5$ glass powder and performing hot-pressure sintering, and its biosafety was preliminarily evaluated. The results of cytotoxicity, hemolysis, oral mucosal irritation, acute systemic toxicity, and subacute systemic toxicity tests indicated that the biosafety of the material met the existing regulatory standards, and further biosafety tests can be conducted, following which the material may be expected to be applied in clinical practice.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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Affirms

All animals were kept in a pathogen-free environment and fed ad lib. The procedures for care and use of animals were approved by the Ethics Committee of the Shanxi Medical University School and Hospital of Stomatology and all applicable institutional and governmental regulations concerning the ethical use of animals were followed (Authorization Number 2021SLL016).

Author statement

Xiaoming Liu: Investigation; Data curation; Validation; Formal analysis; Methodology; Roles/Writing—original draft. Jingyu Yan: Data curation; Investigation; Formal analysis; Validation. Xiuping Wu: Data curation; Investigation; Formal analysis; Validation; Writing—review and editing. Xiao Wu: Formal analysis; Writing—review and editing. Yanjie Zhang: Conceptualization; Formal analysis; Resources; Methodology; Supervision; Funding acquisition; Writing—review and editing. Bing Li: Conceptualization; Formal analysis; Methodology; Resources; Supervision; Roles/Writing—original draft; Writing—review and editing; Funding acquisition; Project administration.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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