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Effect of eluates from zirconia-modified glass ionomer cements on DNA double-stranded breaks in human gingival fibroblast cells

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ABSTRACT

Objective. To formulate novel glass ionomer cements (GICs) containing zirconia (nanoparticles (NPs) and micro-particles (MPs)) and investigate the genotoxic effect of their eluates on DNA double-strand breaks of human gingival fibroblasts (HGFs) *in vitro* using a γ-H2AX fluorescent assay.

Methods. GIC (control, C), 10%ZrO_2NPsGIC (T1) and 10%ZrO_2MPsGIC (T2) were prepared per the manufacturer's instructions (hand-mixed, P/L = 3.4:1 w/w%). Dulbecco's modified Eagle's medium (DMEM) was used as the culture medium for HGFs and for eluate preparation. Eluates were collected from all specimens (n = 5/g, 5 × 2 mm) after 24 h and used for XTT to obtain the EC_{50} using Graph Pad Prism4. A γ-H2AX immunofluorescence assay was performed to detect DSBs in HGFs. The mean foci per cells and percentage of free foci cells were statistically compared (one-way ANOVA with Tamhane's *post hoc* and Chi-square respectively) ($p < 0.05$).

Results. (1) EC_{50} ranged from 31 to 36%. 5% and 20% eluate concentrations were selected for the genotoxicity test. (2) Cells exposed to eluates from T1 had lower mean foci per cell than cells in T2 and C eluates ($p < 0.05$). Only cells in T1 at 5% had lower mean foci cell than medium ($p < 0.05$). (3) T1 and C at both concentration showed a higher, but not significant, percentage of free foci cells than negative control (medium). At 20% eluate concentration T2 had a lower percentage of free foci cells than C ($p < 0.05$).

Significance. Nano-zirconia GIC and micro-zirconia GIC were formulated. GIC and both zirconia modified GICs had no genotoxic effect on HGFs *in vitro*. Further studies related to physical properties should be performed to determine the future clinical applications for these novel nanomaterials.

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1. Introduction

Glass ionomer cement (GIC) was invented by Wilson in the early 1970's [1] and has been used in a wide range of clinical applications. The cement's properties are easily modified by changing the powder/liquid ratio or the chemical formulation [2]. GIC has specific unique properties and advantages as a restorative and adhesive material, such as adhesion to moist tooth structure and base metals, anti-cariogenic properties, thermal compatibility with tooth enamel, biocompatibility, and low toxicity [3]. However, the wide-spread use of GIC in stress-bearing areas is limited because of its low fracture strength, toughness and wear [4]. The chemistry of current GICs are well understood, which in turn has led to several improved formulations [5]. However, the weak strength and toughness of GIC remains a problem for using it as a permanent restoration.

Currently, the use of nanoparticles (NPs) has become a significant area of research in dentistry, and various NPs are used to improve the physical properties and biological activities of dental materials [6]. The incorporation of NPs into the glass powder of GIC has been reported to increase particle size distribution, resulting in higher mechanical property values. NPs can occupy the empty spaces between the GIC particles and inhibit crack propagation, thus reinforcing the material [7]. However, the toxicology of any material containing NPs should be investigated because NPs are biologically more reactive and potentially more harmful to body tissues [8]. Cytotoxicity has been reported to correlate with particle size, and the smaller the particle, the greater its toxicity [9–11].

There has been increased interest in the use of zirconia (ZrO_2), a white crystalline oxide of zirconium, for dental applications because of its mechanical properties [12]. A study of the toxic effect of ZrO_2NPs *in vivo* demonstrated that zirconia had a significant role in increasing reactive oxygen species (ROS) generation that induced the generation of free radicals that caused tissue damage [13]. In contrast, ZrO_2NPs mixed with calcium-silicate based cement (white Portland cement) induced fibroblast proliferation and accelerated the resolution of the inflammatory reaction in *vivo* [14]. A study evaluating the *in vivo* toxicity of zirconia micro-particles (ZrO_2MPs) released from zirconia dental implants found that ZrO_2MPs demonstrated a lower accumulation in wistar rat organs compared with titanium MPs [15].

There are numerous reports on the biocompatibility of GIC as a restorative material [16]. The only study on a GIC containing ZrO_2NPs evaluated its ability to promote cell proliferation and adhesion and found that the incorporation of <5% ZrO_2NPs induced proliferation. These authors suggested this combination be further investigated as a biomaterial for future dental applications [17].

Although adding zirconia to improve the physical properties of GIC has shown promising results, there are no reports on the genotoxicity of zirconia modified GICs. Therefore, the objectives of this study were to formulate GICs modified with different particle sizes (NPs and MPs) of zirconia and investigate the genotoxic effect of eluates from these modified GICs on DNA double-stranded breaks of human gingival fibroblasts *in vitro* using a γ-H2AX fluorescent assay.

2. Materials and methods

2.1. Cements and eluate preparation

A high viscosity conventional GIC (GC Gold Label 9 HS Posterior EXTRA, GC Corporation, Tokyo, Japan) was used as a control material. ZrO_2NPs (99%; <100 nm) and ZrO_2MPs (99%; 5 μm) were obtained from Sigma–Aldrich, Munich, Germany (Table 1).

The test powders were prepared by mixing ZrO_2NPs or ZrO_2MPs with GIC powder at 10% w/w. The resulting powders were hand-mixed with the GIC liquid at a powder:liquid ratio of 3.4:1 w/w for 30 s, per the GIC manufacturer's instructions. The mixed cements, ZrO_2NPsGIC (T1), ZrO_2MPsGIC (T2), and GIC (C) (n = 5), were placed in cylindrical Teflon molds (5 mm diameter × 2 mm high), and pressed with a glass slide for 6 min at room temperature. The cements were removed and individually placed in the wells of a 24-well plate (BD Falcon, Heidelberg, Germany). Each well with cement was filled with 400 μl Dulbecco's Minimum Essential Medium (DMEM, Pan Biotech, Aidenbach, Germany) supplemented with 1% penicillin/streptomycin (Pan Biotech), 10% Fetal Bovine Serum (Sigma–Aldrich, Munich, Germany) and stored for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. The eluate from each well was collected after 24 h and filtered through a 0.2 μm sterile filter (Rotilabo-syringe filters, Carl Roth, Karlsruhe, Germany).

2.2. XTT cell viability assay

Human gingival fibroblasts (HGFs) were cultured with DMEM in a tissue culture flask (BD Falcon, Franklin Lakes, NJ) at 37 °C in a humidified 5% CO_2 atmosphere. Each well of a 96-well plate (BD Falcon, Heidelberg, Germany) was seeded with 2×10^4 cells in 100 μl medium and incubated for 24 h. The cells were treated with 100 μl of 100%, 60%, 30%, 10%, or 3% eluate solution from the T1, T2, and C samples. DMEM and 1% TritonX-100 (Riedel de Haen, Hannover, Germany) wells served as negative and positive controls, respectively. The cells were incubated at 37◦C in a humidified 5% CO_2 atmosphere.

The XTT labeling reagent was prepared by mixing 5 mg XTT (Sodium 3′-[1-(phenylamino)-carbonyl-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene-sulfonic acid hydrate, Serva, Heidelburg, Germany) with 5 ml labeling reagent (RPMI, Roswell Park Memorial Institute medium, without phenol red, Pan Biotech, Aidenbach, Germany). The XTT assay was performed according to the manufacturer' s protocol (Cell Proliferation Kit II, Sigma–Aldrich, Munich, Germany). The XTT labeling mixture was prepared by adding 100 μl electron-coupling reagent (PMS, N-methyl dibenzopyrazine methyl sulfate in phosphate buffer saline (PBS), Sigma–Aldrich, Munich, Germany) to 5 ml XTT labeling reagent. After 20 h, the cells were washed and 50 μl XTT labeling mixture was added to the cells. Photometric analysis was performed after 4 h to determine formazan values using a spectrophotometer (Multiskan FC, ThermoFisher Scientific, Waltham, MA, USA) at 450 nm (reference wavelength 670 nm). Half-maximum-effect substance concentration (EC_{50}) values were obtained by fitting the data to a dose–effect sigmoid curve using Graph Pad Prism 4 (Graph Pad Software Inc., La Jolla, CA).

Table 1 – Material composition.

Materials	Composition	Company	Batch
Glass ionomer cement powder	Strontium and aluminosilicate glass	GC Corporation, Tokyo, Japan	1603101
Glass ionomer cement liquid	Polyacrylic and polybasic carboxylic acid	GC Corporation, Tokyo, Japan	1603101
Zirconium dioxide nanoparticle	Nanoparticle, <100 nm particle size (TEM)	Sigma–Aldrich, Munich, Germany	MKBV4252V
Zirconium dioxide micro-particle	powder, 5 μm, 99% trace metal	Sigma–Aldrich, Munich, Germany	BCBQ7166V

2.3. γ-H2AX immunofluorescence assay

The γ-H2AX immunofluorescence assay was performed according to Urcan et al. [18]. 12-mm diameter round cover slips (Carl Roth, Karlsruhe, Germany) were cleaned in 1 N hydrochloric acid (HCl) and distributed into a 24-well plate. In each well, HGFs were seeded at 7×10^4 cells/ml and incubated in 1 ml DMEM at 37 °C in a humidified 5% CO_2 atmosphere. The DMEM was removed from the wells after 24 h. In the T1, T2, and C groups, the HGFs were exposed to 500 μl of 5% or 20% eluate solution for 6 h and HGFs in DMEM served as negative control. The HGFs in the positive control well received 500 μl 1 mM hydrogen peroxide (H_2O_2) for 2 min and was then replaced with DMEM.

After 6 h, cells were washed twice with PBS for 5 min and fixed with 4% paraformaldehyde (Sigma–Aldrich, Munich, Germany) in 300 μl PBS for 5 min at 4 °C. The cells were washed four times with ice-cold PBS for 2 min and permeabilized with 500 μl of triton-citrate buffer (0.1% sodium citrate (Fluka BioChemika, Belgium), 0.1% Triton X-100) at 4 °C for 10 min. The cells were washed four times with PBS for 2 min and blocked with 300 μl serum-free blocking buffer (Dako Protein block, Hamburg, Germany) at 25 °C for 20 min, and incubated with 300 μl mouse monoclonal anti γ-H2AX (Millipore, Billerica, MA) (1:1100 dilution in antibody diluent (Dako, Hamburg, Germany)) at 4 °C overnight.

The cells were washed after 24 h four times for 5 min with ice-cold PBS and incubated with 300 μl FluoroLink Cy3-labeled goat anti-mouse secondary antibody (GE Healthcare, Munich, Germany) (1:1100 dilution in antibody diluent) at 25 °C in the dark for 2 h. The HGFs were washed four times for 5 min and cell nuclei were stained with Sybr green 1 (ThermoFisher Scientific, Waltham, MA) at 1:30,000 in TAE buffer (0.4 M tris-acetate in 0.01 M ethylene diamine tetraacetic acid (EDTA), Carl Roth GmbH, Germany) at 25 °C in the dark for 15 min. Finally, the cells were washed twice for 5 min and rinsed with deionized water for 5 min at 25 °C. A mixture of 1 ml Pro-long antifade and 5 μl DAPI (4′,6-diamidino-2-phenylindole, ThermoFisher Scientific, MA) (0.2 μl) were put on each glass slide (76 × 26 mm; Carl Roth, Karlsruhe, Germany), the cover slips were removed from the wells and the sides with cells were placed on the mixture.

2.4. Image acquisition

HGFs on the slides were observed using an imaging fluorescence microscope (Zeiss Axioplan2, Carl Zeiss, Jena, Germany). Images were obtained using a 63× and 100× Plan-Neofluar oil immersion objective (Zeiss) and the ISIS fluorescence imaging system (MetaSystems, Altlussheim, Germany). For quantitative analysis of the γ-H2AX test, foci were visually counted from the images. Cell counting was performed until at least 40 cells containing at least one damage focus were observed. Each experiment was performed three times.

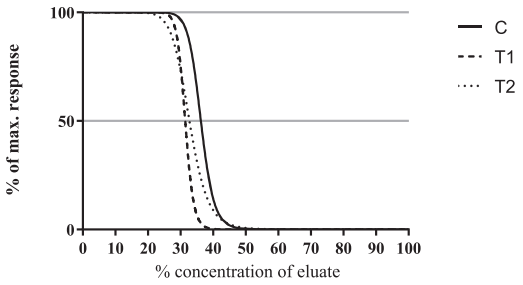

Fig. 1 – The EC50 values calculated for the T1, T2, C eluates were 31.4, 32.7 and 36.2% concentrations of eluates respectively. The curve obtained for each group was similar.

2.5. Statistical analysis

The mean number of foci per cells was counted in each group. The counts were compared using One-way ANOVA with Tamhane's *post hoc* multiple comparison test ($p < 0.05$). The percentage of foci-free cells was compared using the Chi-square test ($p < 0.05$).

3. Results

3.1. XTT cell viability assay (Fig. 1)

The EC50 values for the T1, T2, and C groups were 31.4, 32.7, and 36.2% eluate concentration respectively. The curve obtained for each group was similar.

3.2. γ-H2AX immunofluorescence assay (Fig. 2)

3.2.1. Mean foci per cell (Fig. 3)

The cells exposed to each cement at both concentration demonstrated significantly lower mean foci compared with the cells in the positive control. At 5% eluate concentration, the T1 group had the lowest mean foci per cell and significantly lower mean foci per cell compared with the negative (culture medium) and T2 groups ($p < 0.05$). At 20% eluate concentration, the T2 group had significantly higher mean foci per cell compared with the C and T1 groups ($p < 0.05$). Compared within groups, the cells in the 20% concentration presented higher mean foci compared with those in 5%, however, the difference was not significant.

3.2.2. Percentage of free foci cell (Fig. 4)

The images from all cement groups at both concentrations demonstrated a significantly higher percentage of foci-free

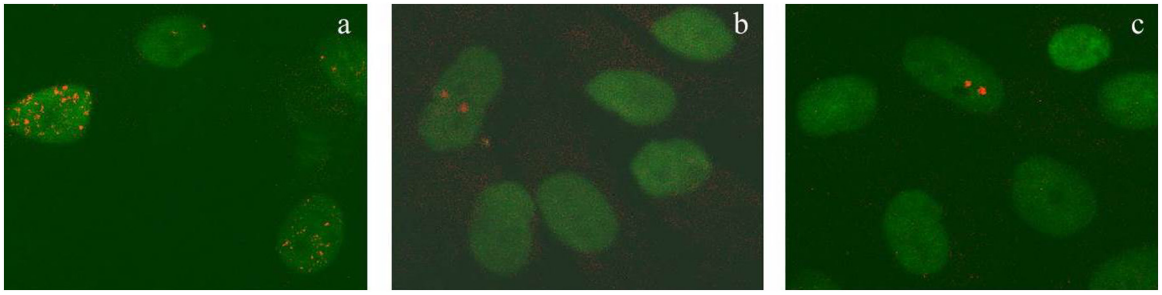

Fig. 2 – Representative images of immunofluorescence staining of γ-H2AX phosphorylation (red) in HGFs after exposure to different eluates. Sybr green (green) is a marker for DNA. (a) Representative image from positive control (H_2O_2), demonstrated multiple foci in all cells. (b) Representative image from GIC (C), demonstrated two foci in six cells. (c) Representative image from ZrO_2NPsGIC(T1), demonstrated two foci in six cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

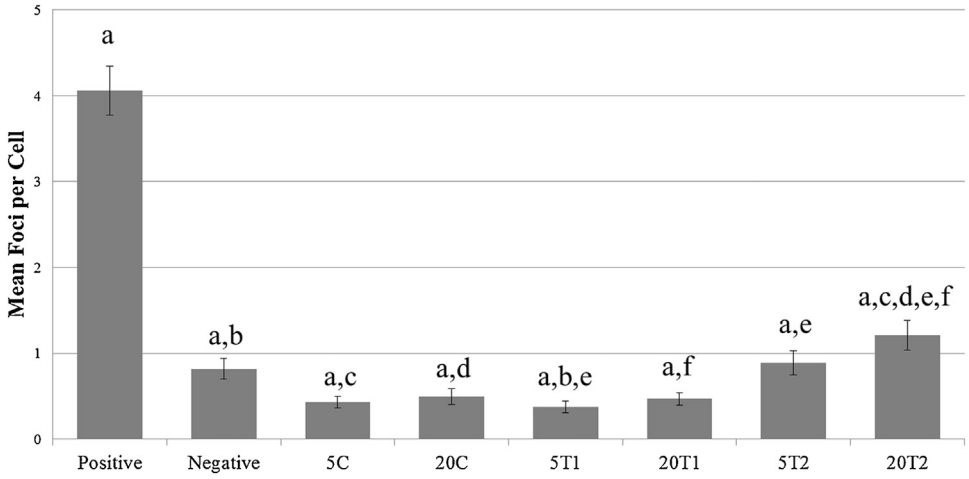

Fig. 3 – Cells in all cements (ZrO_2NPsGIC(T1), ZrO_2MPsGIC(T2) and GIC(C)) at both concentration had lower mean foci per cell compared with positive (H_2O_2) group ($p < 0.05$). At 5% eluate concentration, cells in the T1 group demonstrated the lowest mean foci per cell and significantly lower mean foci per cell compared with the negative (culture medium) and T2 groups ($p < 0.05$). At 20% eluate concentration, the cells in the T2 group had significantly higher mean foci per cell compared with the C and T1 groups ($p < 0.05$).

cells compared with the positive control group. Although the C and T1 groups at both concentrations showed a higher percentage of foci-free cells compared with the negative (medium) group, this was not significant. At 20% eluate concentration, the T2 group had lower percentage of free foci cells compared with the C group.

4. Discussion

GIC has a major drawback of being brittle [4]. Although adding nanoparticles to GIC improves its physical properties [11] the toxicity of nanoparticle used in dentistry is a concern. Several studies have added nanoparticles to GICs and investigated its physical properties [19,20], however there is limited data on its toxicity to human cells.

We formulated these zirconia-modified GICs to be used as restorative materials. The setting time and compressive strength required by the International Standard Dentistry-Water-based cements-Part 1: Powder/liquid acid-base cements (ISO 9917-1: 2007(E)) for restoration is within 6 min and above 100 MPa. In our previous study (unpublished), we found that the highest concentration of zirconia NPs and zirconia MPs which could be mixed to GIC and passed the ISO 9917-1 requirement for setting time and compressive strength was 10% w/w so we chose this concentration for our study.

We used a XTT cell viability assay, which is a well-known method to evaluate cell viability to determine a substance's EC50. The EC50 value of each cement was similar (31–36%). To obtain sufficient human gingival fibroblast cells, we chose the 20% and 5% of the eluate concentration from each cement for the γ-H2AX immunofluorescence assay to determine their effect on inducing DNA double-stranded breaks (DSBs).

In our study, we investigated the DSBs because the DSBs are irreparable and often cause errors in DNA synthesis during replication or repair, which are a major source of mutation. Early senescence, apoptosis, or cancer will be occurred if the rate of DNA damage exceeds repairing capacity of the cell [21]. While single stranded break (SSB), which is defined as when

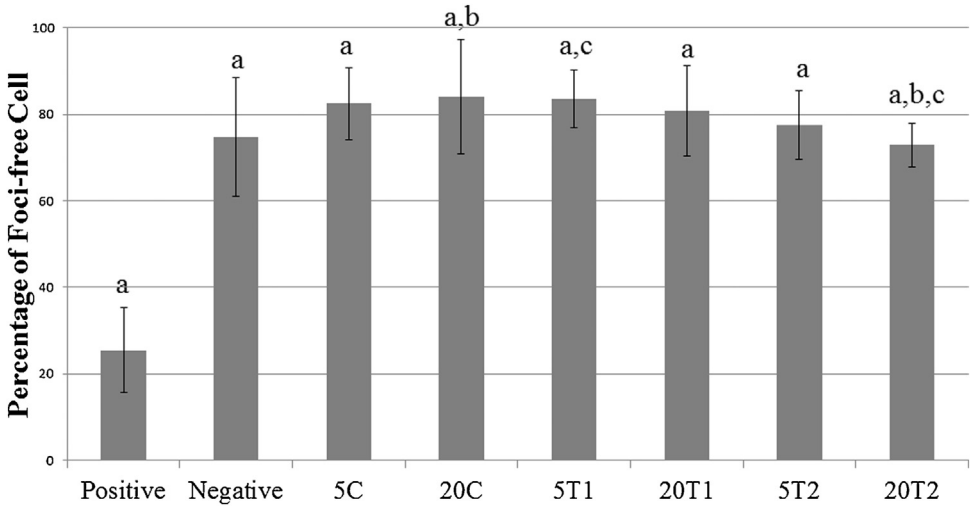

Fig. 4 – Cells in all cements (ZrO_2NPsGIC (T1), ZrO_2MPsGIC(T2) and GIC(C)) groups at both concentration had a higher percentage of foci-free cells compared with the positive (H_2O_2) group ($p < 0.05$). Eluates from C and T1 at both concentration had a higher percentage of foci-free cells compared with the negative (medium) group, however, this was not significant. At the 20% eluate concentration the T2 group had a lower percentage of foci-free cells compared with the C group.

only one of the two strands of a double helix has a defect, is not lethal because when occurs, the other strand can be used as a template to guide the correction of the damaged strand [22]. Measuring DSBs has become more common because of its predictable nature. There are many methods for detecting DSBs, such as neutral elution, pulse field electrophoresis (2-D gel electrophoresis), comet assays and γ-H2AX immunofluorescence assay.

DNA damage is always followed by the phosphorylation of the histone H2AX. The formation of γ-H2AX foci represent the DSBs in a 1:1 manner that is used as a biomarker for damage [23]. The γ-H2AX immunofluorescence assay is a technique used to detect γ-H2AX foci. We used this technique in our study because this technique is very precise, simple, and sensitive to DSBs compared with other techniques. It can detect DSBs in intact cells and has also proved useful for detecting low levels of DNA damage [24].

Our literature search did not reveal any reports on the genotoxic effect of zirconia modified GICs on human cells using the γ-H2AX immunofluorescence assay. Our previous study used this technique, however, that study investigated the genotoxic effect of eluates from GIC and titanium (TiO2) (NPs, MPS) modified GICs on human gingival fibroblast cells [25].

Studies have reported on the toxic effects of zirconia NPs, however, *in vivo*, one study investigated the toxicity of these NPs in 40 rats treated intraperitoneally with 1 ml 0–100 ppm zirconia NPs and found a significant toxic effect at 100 ppm [9]. Another study determined the *in vivo* biocompatibility of zirconia NPs by treating chick embryo with 10, 20, 40, and 80 ppm zirconia NPs in 0.9% NaCl and found that zirconia NPs below 80 ppm was non-toxic and can be used in fabricating dental crowns [26].

This is the first study on the genotoxic effect of zirconia modified GICs on human gingival fibroblasts. We found that higher concentration eluates demonstrated a tendency to have a higher toxic effect on the cells. Nano-zirconia-modified GIC and GIC had no toxic effect on cells and tended to have more positive effects on cells, which were demonstrated by cells with lower mean foci and more foci-free cells compared with culture medium. Our results are similar to those of another study on adding zirconia NPs into GIC that investigated the effect of adding 0%, 5%, 50%, or 100% zirconia NPs on epithelial cell proliferation that found a positive effect at 5% [17]. However, the material used in that study was a resin-modified GIC in which the setting reaction is different from the conventional GIC used in our study. Therefore, further studies should be performed to determine the effect of our nano-zirconia modified GIC on cell proliferation.

In contrast to other studies [9–11,15], we found a higher toxic effect of micro-zirconia-modified GIC compared with nano-zirconia modified GIC. However, in those studies, the particles had been directly injected to animals while in our study, we used the eluates from the modified cements with different particles. The GIC setting chemistry has been determined to be an acid-base reaction in which hardening occurred as a result of ion movement within the cement [16]. To have a better understanding of the role of zirconia NPs and MPs in the setting reaction and ion release profile of the eluate from the cements, further investigations on the chemical analysis and ion release of these modified cements should be performed.

5. Conclusion

Nano-zirconia GIC and micro-zirconia-GIC were successfully formulated. GIC and zirconia-modified GIC with either NPs or MPs did not have a genotoxic effect on HGFs *in vitro*. The GIC and nano-zirconia-GIC tended to have a positive effect to HGFs. Within the limitations of this *in vitro* study, future research should be performed to confirm the improved physical properties of the nano-zirconia-GIC and to investigate the potential of this material for tissue engineering.

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REFERENCES

[1] Crisp S, Ferner AJ, Lewis BG, Wilson AD. Properties of improved glass-ionomer cement formulations. J Dent 1975;3:125–30.
[2] Nicholson JW. Chemistry of glass-ionomer cements: a review. Biomaterials 1998;19:485–94.
[3] Yip HK, Tay FR, Ngo HC, Smales RJ, Pashley DH. Bonding of contemporary glass ionomer cements to dentin. Dent Mater 2001;17:456–70.
[4] Xie D, Brantley WA, Culbertson BM, Wang G. Mechanical properties and microstructures of glass-ionomer cements. Dent Mater 2000;16:129–38.
[5] Wasson EA, Nicholson JW. New aspects of the setting of glass-ionomer cements. J Dent Res 1993;72:481–3.
[6] Priyadarsini S, Mukherjee S, Mishra M. Nanoparticles used in dentistry: a review. J Oral Biol Craniofac Res 2018;8:58–67.
[7] Moshaverinia A, Ansari S, Movasaghi Z, Billington RW, Darr JA, Rehman IU. Modification of conventional glass-ionomer cements with N-vinylpyrrolidone containing polyacids, nano-hydroxy and fluoroapatite to improve mechanical properties. Dent Mater 2008;24:1381–90.
[8] Revell PA. The biological effects of nanoparticles. Nanotechnol Percept 2006:283–98.
[9] Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2005;2:8.
[10] Bruno ME, Tasat DR, Ramos E, Paparella ML, Evelson P, Rebagliati RJ, et al. Impact through time of different sized titanium dioxide particles on biochemical and histopathological parameters. J Biomed Mater Res A 2014;102:1439–48.
[11] Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, et al. The pulmonary toxicology of ultrafine particles. J Aerosol Med 2002;15:213–20.
[12] Denry I, Kelly JR. State of the art of zirconia for dental applications. Dent Mater 2008;24:299–307.
[13] Silva GF, Guerreiro-Tanomaru JM, da Fonseca TS, Bernardi MIB, Sasso-Cerri E, Tanomaru-Filho M, et al. Zirconium oxide and niobium oxide used as radiopacifiers in a calcium silicate-based material stimulate fibroblast proliferation and collagen formation. Int Endod J 2017;50(Suppl. 2):e95–108.
[14] Arefian Z, Pishbin F, Negahdary M, Ajdary M. Potential toxic effects of zirconia oxide nanoparticles on liver and kidney factors. Biomed Res 2015;26:89–97.
[15] Olmedo DG, Tasat DR, Evelson P, Rebagliatti R, Guglielmotti MB, Cabrini RL. In vivo comparative biokinetics and biocompatibility of titanium and zirconium microparticles. J Biomed Mater Res A 2011;98:604–13.
[16] Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. J Funct Biomater 2016;7.
[17] Semyari H, Sattari M, Atai M, Pournasir M. The effect of nanozirconia mixed with glass-ionomer on proliferation of epithelial cells and adhesive molecules. J Periodontol Implant Dent 2011:63–8.
[18] Urcan E, Scherthan H, Styllou M, Haertel U, Hickel R, Reichl FX. Induction of DNA double-strand breaks in primary gingival fibroblasts by exposure to dental resin composites. Biomaterials 2010;31:2010–4.
[19] Elsaka SE, Hamouda IM, Swain MV. Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: influence on physical and antibacterial properties. J Dent 2011;39:589–98.
[20] Garcia-Contreras R, Scougall-Vilchis RJ, Contreras-Bulnes R, Sakagami H, Morales-Luckie RA, Nakajima H. Mechanical, antibacterial and bond strength properties of nano-titanium-enriched glass ionomer cement. J Appl Oral Sci 2015;23:321–8.
[21] Best BP. Nuclear DNA damage as a direct cause of aging. Rejuvenation Res 2009;12:199–208.
[22] Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R. In: Cummings PB, editor. Molecular biology of the gene. 5th ed. CSHL Press; 2004.
[23] Kuo LJ, Yang LX. Gamma-H2AX – a novel biomarker for DNA double-strand breaks. In Vivo 2008;22:305–9.
[24] Kinner A, Wu W, Staudt C, Iliakis G. Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. Nucl Acids Res 2008;36:5678–94.
[25] Laiteerapong A, Reichl FX, Yang Y, Hickel R, C. HOgg. Induction of DNA double-strand breaks in human gingival fibroblasts by eluates from titanium dioxide modified glass ionomer cements. Dent Mater 2018;34:282–7.
[26] Vani C, Jaya DS, Kalidass S. In vivo biocompatibility study of zirconia dioxide nanoparticles for its application in dental treatment. Int J Pharm Bio Sci 2017;8:382–9.