Research Article

Biocompatibility and Surface Characteristics of Resin-Modified Glass Ionomer Cements with Ammonium Quaternary Compounds or Silver Nanoparticles: An *In Vitro* Study

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Glass ionomer cements are materials with diverse clinical applications. Its use is indicated in patients with special needs, pediatric patients, and the elderly; accordingly, it is important to know its properties. The aim of the present study was to determine the cytotoxicity, surface roughness, microhardness, and surface characteristics of GC Fuji ORTHO LC and GC Fuji PLUS resin-modified glass ionomer cements (RMGICs) with 1 and 2% of benzalkonium chloride, cetylpyridinium chloride, hexadecyltrimethylammonium bromide, or silver nanoparticles. All the experimental groups increase or decrease statistically significantly the VHN ($P < 0.05$) compared with the control group, except for GC Fuji PLUS added with hexadecyltrimethylammonium bromide 1 wt%. In the same way, all groups show a statistically significant ($P < 0.05$) increase or decrease in Ra compared with the control group except for GC Fuji ORTHO added with benzalkonium chloride 2 wt%, GC Fuji PLUS added with benzalkonium chloride 2 wt%, and GC Fuji PLUS added with cetylpyridinium chloride 2 wt%. The SEM micrographs show similar surface images between the control and experimental groups. When a dental material is modified, it is important to reevaluate its biological and mechanical characteristics. In the present study, all the additions modified the cytotoxicity and surface characteristics of RMGICs, by increasing or decreasing these properties.

1. Introduction

In clinical practice, the dentist has a series of materials among which he must choose the best option for each treatment. Regarding the cementation materials, there is a wide variety; currently, the most commonly used are glass ionomer cements (GICs) and resin composites. These two materials are different in their chemical composition; the GICs are formed by calcium fluoroaluminum silicate glass powder and polyacrylic acid, whilst the resin composites consist of resin monomers that in contact with an initiator form chains of resin polymers. Their clinical indications are also different, since the resin composites require a longer operative time to

be placed as they must be placed in thin layers, as well as a specific care so that they are not in contact with the moisture present in the mouth [1].

GICs are materials whose use is indicated for the treatment of patients with special needs, pediatric patients, and elderly patients. This is because these materials provide the clinician advantages such as shorter working time than resins, an important aspect since sometimes these patients suffer anxiety or fear when going to the dental office and they must work in short sessions with them. Furthermore, it has been reported that its use is appropriate to avoid caries secondary to cementation and it has remineralization properties, which is important because some of these patients have

limited access to dental care services and it is necessary to use materials that will have greater operative success [2–6].

Wilson and Kent in the late 1960s introduced the glass ionomer cements (GICs) at the Laboratory of the Government Chemist, London [7]. The resin-modified glass ionomer materials are hybrid materials of traditional glass ionomer cement with a small addition of light-curing resin and hence exhibit intermediate properties of both, with some characteristics superior to conventional glass ionomer materials. These materials have some properties like adhesion to tooth structure and base metals, release of fluoride, thermal compatibility with tooth structure, and biocompatibility [8]. Resin-modified glass ionomer cements (RMGICs) have multiple clinical applications including restorations, lining and base, core build-up, and luting [9].

To improve the antibacterial efficacy of GICs, antibacterial substances such as chlorhexidine [10–12], zinc oxide [13], doxycycline hyclate [14], quaternary ammonium compounds, benzalkonium chloride, cetylpyridinium chloride, hexadecyltrimethylammonium bromide, quaternary ammonium polyethyleneimine nanoparticles [15–18], and silver nanoparticles were used [19]. These studies reported acceptable results in the antibacterial effect. Quaternary ammonium compounds (QAC) are cationic compounds that possess a basic structure (NH_4^+). The QAC penetrate the bacteria cell wall, react with the cytoplasmic membrane, and induce wall lysis caused by autolytic enzymes. These compounds are used as disinfectants, antiseptics, pharmaceutical products, and cosmetics [20]. Some quaternary ammonium compounds in dental materials have been studied and include the incorporation of methacryloxylethylcetyl ammonium chloride in bonding agents [21, 22], the incorporation of methacryloyloxydodecyl pyridinium bromide and quaternary ammonium polyethyleneimine nanoparticles into composite resins [23–25], and the incorporation of poly-quaternary ammonium-dyed salt glass ionomer cements. These studies found that the materials with quaternary ammonium compound have significant antibacterial activity [26]. In the present study, the incorporation of benzalkonium chloride, cetylpyridinium chloride, or hexadecyltrimethylammonium bromide to conventional glass ionomer cements has been found to have significant bactericidal effect against *Streptococcus mutans*, *Lactobacillus casei*, *Streptococcus salivarius*, *Lactobacillus acidophilus*, *Actinomyces odontolyticus*, *Actinomyces naeslundii*, and *Enterococcus faecalis* [27].

The silver nanoparticles are insoluble clusters of silver atoms measuring less than 100 nm. The nanoparticles penetrate in the bacteria and get attached in the bacterial membrane. Studies indicated that silver interacts with sulfhydryl groups of proteins and with DNA, altering hydrogen bonding, respiratory chain, cell division, cell wall synthesis, and unwinding finally leading to cell death [28]. Recent studies have investigated the possibility of incorporating silver nanoparticles into bonding agents to improve their antibacterial properties [29–31]. It has been found that the incorporation of silver nanoparticles has a significant bactericidal effect against *Streptococcus mutans*, *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*, and

Porphyromonas aeruginosa; therefore, it is proposed to incorporate them into glass ionomer cements [32].

The potential toxic effect of antibacterial substances should be investigated. Few studies have evaluated the effect of quaternary ammonium compounds and silver nanoparticles on the cytotoxicity of glass ionomers. The physical properties of RMGICs may be altered by the addition of antibacterial compounds, and it is important to evaluate some main characteristics. Hardness is the resistance of a material to indentation or penetration, and it has been used to predict the wear resistance of a material against applied forces such as occlusal forces [33]. On the other side, the surface roughness of the RMGICs is related to its ability to retain dental plaque [16].

This study evaluated the cytotoxicity, microhardness (VHN), surface roughness (Ra), and surface morphology of RMGICs with the addition of benzalkonium chloride (BC), cetylpyridinium chloride (DP), hexadecyltrimethylammonium bromide (CE), or silver nanoparticles (AgNP).

2. Materials and Methods

2.1. Sampler Preparation. The luting resin-modified glass ionomer (RMGIC) GC Fuji PLUS and GC Fuji ORTHO LC (GC Corporation, Tokyo, Japan) were chosen as controls for the present study. Sixteen experimental groups were made by adding BC, DP, and CE (Sigma-Aldrich, Steinheim Germany) at 1 wt% and 2 wt% into the powder of the RMGIC and AgNP synthesis previously reported [34] at 1 wt% and 2 wt% into the liquid of the RMGIC.

Nine specimens of each group were prepared for cytotoxicity test measuring 5 mm in diameter and 2 mm length, 25 specimens for microhardness and surface roughness measuring 5 mm in diameter and 2 mm length, and one for scanning electronic microscopy measuring 5 mm in diameter and 2 mm length, per the manufacturer's instructions, in standard cylindrical Teflon molds. Specimens were prepared between cellophane strips and glass slabs and light curing if needed with a LED.B (Woodpecker Medical Instrument Co., Ltd., Guilin, China). The specimens for the microhardness test, surface roughness, and scanning electron microscopy were stored at 37°C in distilled water and in dry conditions for 24 hr. The specimens that were stored in dry conditions showed cracks on the surface that prevented them from performing the tests; Figure 1 shows the surface of the control materials observed with scanning electron microscopy to an amplification of $\times 50$. All specimens used in the cytotoxicity test were sterilized with UV radiation for 40 min on each side and immersed in 200 μl of minimum essential medium (MEM) for 24 h at 37°C [35]. The pH of the medium of 3 specimens per group was recorded before being in contact with the samples and after 24 hours of incubation with the samples.

The cytotoxic effect of RMGIC GC Fuji PLUS and GC Fuji Ortho LC was evaluated on epithelial cells.

2.2. Cytotoxicity Testing. African green monkey kidney epithelial cells (strain MA104) were cultured in MEM supplemented with 3% of fetal bovine serum (FBS, Biowest,

FIGURE 1: Scanning electron microscopy micrographs at $\times 50$ of the specimens stored in dry conditions: (a) GC Fuji Ortho LC and (b) GC Fuji PLUS.

TABLE 1: pH variations at 24 hr for GC Fuji ORTHO LC modifications.

Groups	Mean pH
GC Fuji ORTHO LC	7.1
GC Fuji ORTHO LC + hexadecyltrimethylammonium bromide 1%	7.3
GC Fuji ORTHO LC + hexadecyltrimethylammonium bromide 2%	7.2
GC Fuji ORTHO LC + benzalkonium chloride 1%	7.3
GC Fuji ORTHO LC + benzalkonium chloride 2%	7.1
GC Fuji ORTHO LC + cetylpyridinium chloride 1%	7.5
GC Fuji ORTHO LC + cetylpyridinium chloride 2%	7.3
GC Fuji ORTHO LC + silver nanoparticles 1%	7.3
GC Fuji ORTHO LC + silver nanoparticles 2%	7.2

Nuaillé, France) at 37°C with 5% of CO_2 , in 96-well microtiter plates (Thermo Fisher Scientific, MA, USA) for 48 hours until we got a confluent monolayer. After this period, $100\ \mu\text{l}$ extract of experimental and control materials was applied to the previously cultured MA104 cells for 24 hr. Cultures with MEM were used as the negative control of cytotoxicity.

Nine specimens of each group were used for analysis of cell metabolism by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. For the MTT assay, the extracts were aspirated and replaced by $90\ \mu\text{l}$ of MEM plus $10\ \mu\text{l}$ of MTT solution (5 mg/ml sterile PBS) and incubated at 37°C and 5% CO_2 for 4 hr. Then, the culture medium with the MTT solution was aspirated and $100\ \mu\text{l}$ DMSO was added to each well to dissolve the formazan crystal resulting from the cleavage of the MTT salt ring by the SDH enzyme present in the mitochondria of viable cells. Cell viability was evaluated by spectrophotometry as being proportional to the absorbance measured at 570 nm wavelengths with an ELISA microplate reader (model 3550-UV, Bio-Rad Laboratories, Hercules, CA, USA). The values obtained from the experiments were averaged to provide a single value for each material. The means were calculated for the groups

TABLE 2: pH f GC Fuji PLUS modifications.

Groups	Mean pH
GC Fuji PLUS	7.8
GC Fuji PLUS + hexadecyltrimethylammonium bromide 1%	7.6
GC Fuji PLUS + hexadecyltrimethylammonium bromide 2%	7.9
GC Fuji PLUS + benzalkonium chloride 1%	7.7
GC Fuji PLUS + benzalkonium chloride 2%	7.5
GC Fuji PLUS + cetylpyridinium chloride 1%	7.8
GC Fuji PLUS + cetylpyridinium chloride 2%	8.1
GC Fuji PLUS + silver nanoparticles 1%	7.6
GC Fuji PLUS + silver nanoparticles 2%	7.7

and transformed into percentages, which represented the inhibitory effect of the mitochondrial activity of the cells by extracts. The negative control (MEM) was defined as having 100% cell metabolism [36].

2.3. Confocal Laser Scanning Microscopy (CLSM). Three specimens of each group were used for CLSM. The MA104 cells previously cultured with the RMGIC extracts were stained with calcein acetoxymethylester (Calcein AM, Molecular Probes) and diluted in DMSO for a final concentration of $2\ \mu\text{M}$; this fluorescein derivate crosses the cell membrane in an electrically neutral form. The calcein AM is converted by intracellular esterase into de negatively charged fluorescent calcein, and is retained in the intracellular compartment so long as plasma membranes are intact; however, the dye rapidly leaks from the cells with compromised membranes, even in the presence of residual intracellular esterase activity. After 30 min, the images were obtained through a Nikon Plan Apo X60, NA L.4, oil objective [37], and a Nikon camera.

2.4. Microhardness Test. The specimens for the microhardness test were stored at 37°C in distilled water for 24 hr. Vickers hardness measurements were made with a standard microhardness tester (Micro Vickers Hardness Tester HV-

TABLE 3: Cell viability percentages by MTT assay for GC Fuji ORTHO LC modifications.

Groups	Mean	SD	Maximum	Minimum
MEM control	100	0	100	100
GC Fuji ORTHO LC	11.16	1.94	13.75	9.62
GC Fuji ORTHO LC + hexadecyltrimethylammonium bromide 1%	10.95	0.72	11.5	10
GC Fuji ORTHO LC + hexadecyltrimethylammonium bromide 2%	10.37	0.75	11.37	9.75
GC Fuji ORTHO LC + benzalkonium chloride 1%	11.25	0	11.25	11.25
GC Fuji ORTHO LC + benzalkonium chloride 2%	9.41*	0.488	10	8.87
GC Fuji ORTHO LC + cetylpyridinium chloride 1%	9.66	1.19	11.25	8.75
GC Fuji ORTHO LC + cetylpyridinium chloride 2%	10.62	0.49	11.12	10
GC Fuji ORTHO LC + silver nanoparticles 1%	10.83	1.65	12.5	8.75
GC Fuji ORTHO LC + silver nanoparticles 2%	11.95	0.43	12.5	11.5

SD: standard deviation; MTT assay: 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay. *Statistically significant differences between GC Fuji ORTHO LC and the experimental group.

TABLE 4: Cell viability percentages by MTT assay for GC Fuji PLUS modifications.

Groups	Mean	SD	Maximum	Minimum
MEM control	100	0	100	100
GC Fuji PLUS	9.83	0.17	10	9.62
GC Fuji PLUS + hexadecyltrimethylammonium bromide 1%	9.75	0.38	10	9.25
GC Fuji PLUS + hexadecyltrimethylammonium bromide 2%	10.83	0.62	11.25	10
GC Fuji PLUS + benzalkonium chloride 1%	9.46	0.60	10.12	8.75
GC Fuji PLUS + benzalkonium chloride 2%	10.38	0.76	11.25	9.5
GC Fuji PLUS + cetylpyridinium chloride 1%	9.79	0.31	10	9.37
GC Fuji PLUS + cetylpyridinium chloride 2%	11.25*	0.00	11.25	11.25
GC Fuji PLUS LC + silver nanoparticles 1%	10.17	0.82	11.25	9.5
GC Fuji PLUS + silver nanoparticles 2%	12.33*	1.63	14.12	10.37

SD: standard deviation; MTT assay: 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay. *Statistically significant differences between GC Fuji PLUS and the experimental group.

1000 Dongguan Sinowon Precision Instrument Co., Ltd., South District, Dongguan, China). A diamond indenter was used with a load of 300 g for 15 s. Each sample was submitted to three indentations located 200 μm far from each other, and the mean of the VHN was recorded. The diagonal length of the impressions was measured, and the VHN was calculated according to the standard formula $H = 1.854 P/d^2$.

2.5. Surface Roughness. The Ra specimens were stored in distilled water for 24 hr at 37°C before measurements. Atomic force microscopy (AFM) was evaluated in contact mode for measurement of surface roughness. All samples were evaluated at the same scan size ($49.5 \times 49.5 \mu\text{m}^2$) in triplicate in different areas. These were selected at random, and the mean roughness was obtained for each sample. The evaluations of Ra were carried out at a scanning rate of 49.5 $\mu\text{m/s}$ by using an AFM (Nanosurf easyScan 2, SPM Electronics, Liestal, Switzerland) in contact mode with the silicon nitride (SiN) probe. The conditions used for the short cantilever contact mode were the following: spring constant, 0.1 N/m; resonant frequency, 28 kHz; length, 225 μm ; mean width, 28 μm ; thickness, 1 μm ; tip height, 14 μm ; and radius, <10 nm. The Nanosurf easyScan 2 software (Version

1.6) was used to measure the AFM parameters. The feedback gains with a set point of 20 nN were as follows: P-Gain: 10000, I-Gain: 1000, and D-Gain: 0. A calibration grid silicon oxide on silicon material (Nanosurf AG, CH-4410, SPM Electronics, Liestal, Switzerland) with an XY periodicity of 10 μm and a Z height of 119 nm was used for calibration prior to each evaluation session.

2.6. Scanning Electron Microscopy (SEM). The samples were viewed with gold coating under SEM. Observations were made in a scanning electron microscope (SEM) (JSM-6510, JEOL, Tokyo, Japan) at 10 kV at $\times 2000$ and $\times 3500$ of magnification.

3. Statistical Analysis

Statistical analysis was carried out using the program JMP SAS software (SAS Institute Inc.) with one-way analysis of variance (ANOVA). Tests of differences of the modifications were analyzed using the Tukey-Kramer test, and a value of $P < .05$ was considered statistically significant for MTT assay, VHN, and Ra.

FIGURE 2: Calcein AM staining of MA104 cells after 24 hr incubation: (a) control with MEM, (b) GC Fuji PLUS, (c) GC Fuji PLUS and hexadecyltrimethylammonium bromide 1 wt%, (d) GC Fuji PLUS and hexadecyltrimethylammonium bromide 2 wt%, (e) GC Fuji PLUS and benzalkonium chloride 1 wt%, (f) GC Fuji PLUS and benzalkonium chloride 2 wt%, (g) GC Fuji PLUS and cetylpyridinium chloride 1 wt%, (h) GC Fuji PLUS and cetylpyridinium chloride 2 wt%, (i) GC Fuji PLUS and silver nanoparticles 1 wt%, and (j) GC Fuji PLUS and silver nanoparticles 2 wt%. Scale bar: 50 μm .

FIGURE 3: Calcein AM staining of MA104 cells after 24 hr incubation: (a) control with MEM, (b) GC Fuji Ortho LC, (c) GC Fuji Ortho LC and hexadecyltrimethylammonium bromide 1 wt%, (d) GC Fuji Ortho LC and hexadecyltrimethylammonium bromide 2 wt%, (e) GC Fuji Ortho LC and benzalkonium chloride 1 wt%, (f) GC Fuji Ortho LC and benzalkonium chloride 2 wt%, (g) GC Fuji Ortho LC and cetylpyridinium chloride 1 wt%, (h) GC Fuji Ortho LC and cetylpyridinium chloride 2 wt%, (i) GC Fuji Ortho LC and silver nanoparticles 1 wt%, and (j) GC Fuji Ortho LC and silver nanoparticles 2 wt%. Scale bar: 50 μm .

TABLE 5: Mean Vickers microhardness and surface roughness $\pm$ SD for GC Fuji ORTHO LC modifications.

Groups	VHN	Ra (μm)
GC Fuji ORTHO LC	54.48 $\pm$ 1.36	23.45 $\pm$ 2.31
GC Fuji ORTHO LC + hexadecyltrimethylammonium bromide 1%	50.76 $\pm$ 1.31*	29.25 $\pm$ 1.43*
GC Fuji ORTHO LC + hexadecyltrimethylammonium bromide 2%	45.40 $\pm$ 1.44*	15.09 $\pm$ 2.08*
GC Fuji ORTHO LC + benzalkonium chloride 1%	51.65 $\pm$ 1.27*	21.25 $\pm$ 1.69*
GC Fuji ORTHO LC + benzalkonium chloride 2%	44.48 $\pm$ 1.22*	22.34 $\pm$ 1.68
GC Fuji ORTHO LC + cetylpyridinium chloride 1%	41.03 $\pm$ 1.71*	29.32 $\pm$ 2.80*
GC Fuji ORTHO LC + cetylpyridinium chloride 2%	36.10 $\pm$ 1.22*	17.47 $\pm$ 1.37*
GC Fuji ORTHO LC + silver nanoparticles 1%	50.20 $\pm$ 1.78*	14.76 $\pm$ 1.14*
GC Fuji ORTHO LC + silver nanoparticles 2%	33.45 $\pm$ 1.57*	17.19 $\pm$ 0.95*

SD: standard deviation; VHN: Vickers microhardness number; Ra: surface roughness. *Statistically significant differences between GC Fuji ORTHO LC and the experimental group.

TABLE 6: Mean Vickers microhardness and surface roughness $\pm$ SD for GC Fuji PLUS modifications.

Groups	VHN	Ra (μm)
GC Fuji PLUS	31.29 $\pm$ 1.24	24.21 $\pm$ 1.25
GC Fuji PLUS + hexadecyltrimethylammonium bromide 1%	34.11 $\pm$ 1.74*	14.07 $\pm$ 1.50*
GC Fuji PLUS + hexadecyltrimethylammonium bromide 2%	32.51 $\pm$ 1.89	20.49 $\pm$ 1.35*
GC Fuji PLUS + benzalkonium chloride 1%	28.32 $\pm$ 1.72*	28.31 $\pm$ 1.45*
GC Fuji PLUS + benzalkonium chloride 2%	24.25 $\pm$ 1.78*	24.07 $\pm$ 1.37
GC Fuji PLUS + cetylpyridinium chloride 1%	24.10 $\pm$ 1.58*	24.18 $\pm$ 1.59*
GC Fuji PLUS + cetylpyridinium chloride 2%	23.34 $\pm$ 1.73*	22.53 $\pm$ 1.75
GC Fuji PLUS + silver nanoparticles 1%	47.23 $\pm$ 1.84*	20.22 $\pm$ 1.09*
GC Fuji PLUS + silver nanoparticles 2%	36.30 $\pm$ 1.87*	17.58 $\pm$ 1.58*

SD: standard deviation; VHN: Vickers microhardness number; Ra: surface roughness. *Statistically significant differences between GC Fuji PLUS and the experimental group.

4. Results

The pH of the minimum essential medium without being in contact with the samples was 7, after contact with the samples presented the variations observed in Tables 1 and 2.

4.1. Cytotoxicity Testing. Descriptive data of cell survival rates for each group and comparisons of cell viability percentages between the different modifications of GC Fuji PLUS and GC Fuji ORTHO are given in Tables 3 and 4. All the experimental groups showed a significantly decreased cell survival percentage when compared to the control group ($P < 0.05$). The addition of BC at 2% in the GC Fuji ORTHO LC reduces in a meaningful way similar to that of the cell viability percentage ($P < 0.0112$). DP and AgNP at 2% addition to GC Fuji PLUS increase meaningfully the cell viability ($P < 0.0019$ and $P < 0.0001$).

4.2. Confocal Laser Scanning Microscopy (CLSM). Figures 2 and 3 show the appearance of the cell cultures stained with calcein AM and calcein acetoxymethylester, including MEM control, control cements, and experimental groups. After treatment with the control cements and experimental group elutes, the fluorescence calcein level in the cytoplasm

had declined. The control group shows elongated and spindle-shaped cells. The control and experimental groups display rounded and dead fibroblasts and also increased the intercellular space.

4.3. Vickers Microhardness and Surface Roughness. Mean and standard deviations of VHN and Ra are shown in Tables 5 and 6. All the experimental groups increase or decrease statistically significantly the VHN ($P < 0.05$) when they are compared with the control group, except GC Fuji PLUS added with CE 1 wt%. In the same way, all groups show a statistically significant ($P < 0.05$) increase or decrease in surface roughness when compared to the control group except for GC Fuji ORTHO added with BC 2 wt%, GC Fuji PLUS added with BC 2 wt%, and GC Fuji PLUS added with DP 2 wt%.

4.4. Scanning Electron Microscopy. Figures 4, 5, 6, and 7 present the SEM micrographs obtained in this study; control and experimental groups show very similar surface features. The relatively homogeneous and flat surface of glass ionomer is clearly observed with the presence of pores and air voids in the surface.

FIGURE 4: Scanning electron microscopy micrographs at $\times 2000$: (a) GC Fuji Ortho LC, (b) GC Fuji Ortho LC and silver nanoparticles 1 wt%, (c) GC Fuji Ortho LC and silver nanoparticles 2 wt%, (d) GC Fuji Ortho LC and hexadecyltrimethylammonium bromide 1 wt%, (e) GC Fuji Ortho LC and hexadecyltrimethylammonium bromide 2 wt%, (f) GC Fuji Ortho LC and benzalkonium chloride 1 wt%, (g) GC Fuji Ortho LC and benzalkonium chloride 2 wt%, (h) GC Fuji Ortho LC and cetylpyridinium chloride 1 wt%, and (i) GC Fuji Ortho LC and cetylpyridinium chloride 2 wt%. Scale bar: 10 μm .

5. Discussion

The *in vitro* studies can be conducted under controlled conditions and provide a significant amount of information [38]. Different cell lines are used to probe the biocompatibility of dental materials like pulp cells extracted from the maxillary incisors of Sprague Dawley rats, multipotent stromal cells, human foreskin fibroblast, human lung fibroblast, primary human osteoblast, L929 mouse fibroblast, SV40 large T-antigen-transfected bovine dental pulp-derived cells, MDPC-23 odontoblast-like cell line, primary pulp fibroblast, and human alveolar osteoblast, for example [39–46]. In this study, the MA-104 epithelial cell line was used because the glass ionomer cements are in direct contact with the epithelial tissue of the oral mucosa. The use of cell lines permits an accurate evaluation of the changes,

excluding factors such as age and metabolic and hormonal states of the donor that may influence the cell in primary culture [47]. Different formulations are under development to increase the antibacterial properties of GICs. It is important to determine the biocompatibility properties of new formulations. Adding QAC and silver nanoparticles may alter the *in vitro* cytotoxicity of GICs. The present *in vitro* study demonstrated that GC Fuji ORTHO LC and GC Fuji PLUS had higher cytotoxicity in MA104 cells by MTT assay. For the materials with modifications, only the addition of BC at 2% in the GC Fuji ORTHO LC reduces the cell viability compared with the control cement without the modification. In part, this is explained because all materials used in the study, both glass ionomers and antimicrobials, have a reported degree of toxicity [48, 49]. Different *in vitro* studies assessed the cytotoxicity of GICs and RMGICs on cultured cells

FIGURE 5: Scanning electron microscopy micrographs at $\times 3500$: (a) GC Fuji Ortho LC, (b) GC Fuji Ortho LC and silver nanoparticles 1 wt%, (c) GC Fuji Ortho LC and silver nanoparticles 2 wt%, (d) GC Fuji Ortho LC and hexadecyltrimethylammonium bromide 1 wt%, (e) GC Fuji Ortho LC and hexadecyltrimethylammonium bromide 2 wt%, (f) GC Fuji Ortho LC and benzalkonium chloride 1 wt%, (g) GC Fuji Ortho LC and benzalkonium chloride 2 wt%, (h) GC Fuji Ortho LC and cetylpyridinium chloride 1 wt%, and (i) GC Fuji Ortho LC and cetylpyridinium chloride 2 wt%. Scale bar: $5\ \mu\text{m}$.

[42–52]. The studies have supported the concept that leachable components of the dental materials are responsible for the cytotoxicity in cell culture. It has been suggested that GICs, which released fluoride in high quantities, were highly cytotoxic to human dental pulp stem cells [53]. A study conducted in mouse lymphoma cells support the idea that the cell membrane was the main target for the toxic agent of the GICs and the damage occurred quickly [54, 55]. It is in accordance with our findings that show the damage in the first 24 hr and the loss of membrane integrity by calcein AM staining.

When a modification is made in a dental material, it is very important to reevaluate its biological and mechanical characteristics. It has been reported in the literature that the modifications of the powder-liquid ratio of the glass ionomer cements could affect some of the mechanical properties of the

materials as well as their resistance to acid erosion [55], that is why in the present study only antibacterial substances were added at 1 wt% and 2 wt%. In the results, we can see that the addition of antibacterial substances to 1 wt% or 2 wt% gives different results for the Vickers microhardness and the average roughness of the surface, which indicates that the materials do not behave in the same way when modifying this powder-liquid ratio.

Various factors such as the manufacture of specimens, the shape of the specimens, and the storage, among others, can influence the mechanical tests, for which it is important to use the methods most accepted by the literature [56]. The international regulations specify that samples must be left in incubation for 24 hours prior to being subjected to physical or mechanical tests as specified in ISO 9917-2: 2010 [57]. In several studies, the mechanical characteristics

FIGURE 6: Scanning electron microscopy micrographs at $\times 2000$ (a) GC Fuji PLUS, (b) GC Fuji PLUS and silver nanoparticles 1 wt%, (c) GC Fuji PLUS and silver nanoparticles 2 wt%, (d) GC Fuji PLUS and hexadecyltrimethylammonium bromide 1 wt%, (e) GC Fuji PLUS and hexadecyltrimethylammonium bromide 2 wt%, (f) GC Fuji PLUS and benzalkonium chloride 1 wt%, (g) GC Fuji PLUS and benzalkonium chloride 2 wt%, (h) GC Fuji PLUS and cetylpyridinium chloride 1 wt%, and (i) GC Fuji PLUS and cetylpyridinium chloride 2 wt%. Scale bar: 10 μm .

of the glass ionomer evaluated after conserving the samples in dry and wet conditions are reported; these studies have found a difference in the behavior of the materials in various conditions [58]. In the present study, samples were preserved in dry conditions but the formation of cracks in the surface prevented having a sufficient firm area to perform the indentations in the Vickers microhardness test and to have an appropriate scanning area for atomic force microscopy. The surface hardness is a test that allows us to have an idea of how the behavior of the materials will be when exposed to occlusal trauma and its relation with the elastic modulus of the material. In the present study, the majority of the modifications decrease the VHN; this result could be related to the modification of the liquid powder proportion of the materials when adding the antibacterial agents, since it is reported that microhardness can be affected by the ratio of glass particles to polyacid. In the current literature, there is

no information about the surface roughness or hardness of the control materials. In other RMGIC, different values are reported; for example, de Ra for Fuji II varies from 0.41 μm , 0.56 μm , or 1.54 μm and the VHN varies from 42.28 VHN, 583.44 VHN, or 739.38 VHN. In the present study, the experimental groups are evaluated in comparison with the control group [59–61].

The roughness of the surface is related to wear and biological outcomes including periodontitis, periodontal disease, and the development of secondary caries due to dent bacterial plaque accumulation. A roughness of the surface greater than 0.2 μm allows the accumulation of bacteria on the surface of the material [62]. The micrographs obtained in this study show pores and air voids in the surface, similar with other SEM micrographs of the RMGIC and GIC.

The results of this study show that the modifications reduce the roughness of the surface of the material, which

FIGURE 7: Scanning electron microscopy micrographs at $\times 3500$: (a) GC Fuji PLUS, (b) GC Fuji PLUS and silver nanoparticles 1 wt%, (c) GC Fuji PLUS and silver nanoparticles 2 wt%, (d) GC Fuji PLUS and hexadecyltrimethylammonium bromide 1 wt%, (e) GC Fuji PLUS and hexadecyltrimethylammonium bromide 2 wt%, (f) GC Fuji PLUS and benzalkonium chloride 1 wt%, (g) GC Fuji PLUS and benzalkonium chloride 2 wt%, (h) GC Fuji PLUS and cetylpyridinium chloride 1 wt%, and (i) GC Fuji PLUS and cetylpyridinium chloride 2 wt%. Scale bar: $5\ \mu\text{m}$.

can be a positive aspect in its use in the clinic; in materials modified with QAC, this can be explained by the strong chemical affinity of these compounds with the matrix derived from polyacrylic acid of the RMGICs and in the case of the nanoparticles by the particle size used. It is important to understand the limitations of the *in vitro* study but also to take into account that they are important indicators of the behavior that we can expect from the materials in their clinical application.

6. Conclusions

Based on the results obtained in the present *in vitro* study, it was concluded that GC Fuji ORTHO and GC Fuji PLUS have high cytotoxicity compared with the medium control group. According with the methodology employed in the present study, only the addition of BC at 2% in the GC Fuji ORTHO LC reduces the cell viability compared with the control cement without the modification. All the modifications with the exception of one experimental group (GC Fuji PLUS

added with 2% AgNP) decreases the VHN of the RMGICs. The addition of 1% CE and 1% BC to Gc Fuji ORTHO LC and the addition of 1% BC to Gc Fuji PLUS increases the Ra of the RMGICs. The SEM micrographs obtained in this study show that control and experimental groups have very similar surface characteristics. Further *in vivo* studies are necessary to determine whether these results can be extrapolated to clinical situations.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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