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Adhesion and biologic behavior of human periodontal fibroblast cells to resin ionomer Geristore: a comparative analysis

Saurabh Kumar Gupta[1], Payal Saxena[1], Vandana Aditya Pant[2], Aditya Bhushan Pant[3,4]

[1]Department of Conservative Dentistry & Endodontics, Government College of Dentistry, Indore; [2]Department of Periodontics, Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow; [3]Indian Institute of Toxicological Research, Lucknow; [4]Council of Scientific & Industrial Research, New Delhi, India

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Correspondence to: Payal Saxena, Department of Conservative Dentistry and Endodontics, Government College of Dentistry, Sardar Patel Marg, Indore, India
Tel.: +91 8103078123
Fax: +91 731 2701608
e-mail: payal.endo@gmail.com

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Abstract – *Aim*: The resin ionomer Geristore, originally designed for restorative procedures, has been used extensively in treating subgingival defects (such as root resorption and perforations) and as a retrofilling material. The purpose of this study was to evaluate the cell adhesion as well as *in vitro* biocompatibility of human periodontal fibroblast cells with resin ionomer Geristore in comparison with mineral trioxide aggregate (MTA) and glass ionomer cement (GIC). *Material and method*: Adhesion, growth, and morphology of human periodontal fibroblasts over test materials were evaluated by scanning electron microscopy (SEM). Biocompatibility was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide salt (MTT) assay. *Results*: Compared to glass coverslips, cells grew and spread qualitatively better over the surface of Geristore in comparison with the other test materials. *In vitro* interpretation indicates that Geristore is significantly less cytotoxic to human periodontal ligament cells. Results of statistical analysis revealed that material extracts had significant effect on cell proliferation at both 24 h ($F = 547.62$, $P < 0.05$) and at 48 h ($F = 6048.18$, $P < 0.05$). *Conclusion*: Our study supports that Geristore has enhanced biologic behavior to human periodontal ligament cells and superior biocompatibility in comparison with MTA and GIC, so it can be suggested as a material of choice in root resorption, perforations, and root-end filling.

A root-end filling material functions as a 'physical seal' to prevent microleakage from the root canal system into adjacent periradicular tissues (1), and because these materials come into direct contact with the periradicular tissues, they are required to be biocompatible. Other desired properties are sealing ability, handling properties, working time, radiopacity, antibacterial activity, and the induction of the periodontal ligament (PDL) cell proliferation (2). Similarly, other materials used adjacent to periodontal tissues (in subgingival defects such as perforations, root caries, root resorption) should also have minimal cytotoxicity toward these cells and should preferentially adhere to them (3).

Geristore is a dual-cure (both self- and light-curing), hydrophilic, nonaqueous polyacid modified composite resin. Advantages of this material include insolubility in oral fluids, increased adhesion to tooth structure, dual-cure capabilities, low-cure shrinkage, low coefficient of thermal expansion, radiopacity, fluoride release, and biocompatibility (4). Relatively, very few studies have addressed biocompatibility and cellular adhesion capabilities of Geristore (3, 5); although several clinical studies have demonstrated that Geristore can repair subgingival and subosseous defects and can be used as a barrier for guided tissue regeneration (4–7).

The purpose of this study was to evaluate the cellular adhesion potential as well as *in vitro* biocompatibility of the resin ionomer Geristore compared to two other materials MTA and GIC that are used commonly as root-end filling material, perforation repair material and in subgingival defects.

Materials and methods

Sample collection

The teeth were collected from healthy individuals having no caries involvement extending beyond the cemento-enamel junction and no periodontal disease. The sex and race of the patients were not considered for this study hence not recorded. Extracted teeth were immediately placed in minimum essential medium (MEM) with low glucose (Gibco BRL) + penicillin 10 000 I.U./100 ml, streptomycin 10,000 I.U./100 ml and fungizone 250 μg/100 ml medium (PSF) to maintain periodontal cell viability during transport and also help to reduce

sample contamination by initiating antibacterial/antifungal activity.

Cell culture

The primary cultures of human PDL fibroblasts were obtained by following the methods of Pant et al. (8). Briefly, the teeth were washed in MEM containing antibiotic–antimycotic; then, adherent soft tissues were removed from the crown and the coronal one-third of the root. These soft tissues were then discarded. The crown and coronal one-third of the root were then placed in 5.25% NaClO for 2 min to reduce bacterial contamination, as well as to kill any remaining gingival epithelial cells. The middle third of the root was then scraped to obtain periodontal ligament tissue specimens. The tissue specimens were placed in a sterile Petri dish containing a thin layer of MEM with 10% fetal bovine serum. The PDL tissues were disaggregated using 0.2% collagenase and 0.125% trypsin for 30 min at 37°C, and the cells were collected by centrifugation at 100 $\times g$ for 5 min. The pellet of packed cells was then resuspended in the six-well culture plates in complete minimal essential medium and allowed to adhere properly after incubating at 37°C with an atmosphere of 95% air and 5% CO_2. Growth was permitted to continue until the cells attained a confluent monolayer, at that time they were trypsinized (trypsin 0.05%–EDTA 0.53 mM) and passaged into T-25 culture flasks to expand the cell population (first cell passage). The cells of third and fourth passages were trypsinized and pooled for experimentation (to control the cell variability). Cell number for experimentation was determined using an Electronic Coulter Counter (Model Zf; Coulter Electronics, Hialeah, FL, USA). The number of viable cells in each batch was measured by the trypan blue dye exclusion test before each experiment, and batches showing cell viability of more than 95% were used for the experiment.

Sample preparation and sterilization

Geristore (DEN-MAT Corporation, Santa Maria, CA, USA), mineral trioxide aggregate (ProRoot MTA; Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) and glass ionomer cement (Fuji II; GC, Tokyo, Japan) were obtained from commercial sources. Disks (6 × 2 mm) from the three materials were fabricated under aseptic conditions by packing the material after mixing in a Teflon washer (internal diameter of 6 × 2 mm) and compressed between two glass slides to generate even thickness of material. Glass coverslips were used as positive controls in all experimental conditions throughout this study. Extracts from all three materials were prepared by preincubating sterilized disks in 50 cm^3 conical tubes with 5 ml of DMEM (Invitrogen, Life Technologies, Grand Island, NY, USA) supplemented with 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37°C in a shaker (Labline; Barnstead Int., Dubuque, IA, USA) at 150 rpm.

Scanning electron microscopy growth assay

Adhesion, morphology, and biologic behavior of human PDL fibroblasts over Geristore were evaluated by scanning electron microscopy (SEM). Disks of Geristore, MTA, and GIC along with control glass coverslips were sterilized as described above and placed in the bottom of a 12-well culture plate. Human PDL fibroblast cells were seeded into the wells at density of 3×10^4 cells per well in DMEM medium containing 10% fetal bovine serum. After incubation, cells were washed three times with phosphate-buffered saline (PBS) and fixed in 2.5% glutaraldehyde in 1 mmol cacodylate buffer (pH 7.4). Samples were then observed using a scanning electron microscope (JEOL JSM 6400; Jeol USA, Peabody, MA, USA). Cell attachment and viability in each experimental condition were assessed by qualitatively comparing morphology of cells over tested materials with that of cells over glass coverslips, which were considered cells with normal morphology.

Cytotoxicity assays

The cytotoxicity of human PDL fibroblasts toward Geristore, MTA, and GIC was assessed using MTT assay. The MTT assay was performed following the method of Siddiqui et al., 2008 (9) with desired modifications. In brief, cells (1×10^4 per well) seeded in 96-well tissue culture plates were exposed to extracts of three test materials as well as control for time intervals of 24 and 48 h. Tetrazolium bromide salt (5 mg ml^{-1} of stock in PBS) was added (10 μl per well in 100 μl of cell suspension) for 4 h. At the end of incubation, the reaction mixture was carefully taken out and 200 μl of culture grade dimethyl sulfoxide (DMSO) was added to each well by pipetting up and down several times until the content gets homogenized. The plates were kept on rocker shaker for 10 min at room temperature and then read at 550 nm using Multiwell Microplate Reader (Synergy HT; Bio-Tek, Winooski, VT, USA). Unexposed sets run under the identical conditions were served as basal control; whereas cells exposed to manganese (10^{-3} and 10^{-4} M) were used as positive control.

Statistical analysis

Normalized percent viability values from multiple experimental samples ($n = 4$) were subjected to statistical analysis. ANOVA was performed to evaluate the effect of material extracts on cell proliferation. *Post hoc* pairwise comparison was carried out using Tukey's multiple comparison tests.

Results

Cellular adhesion and morphology

We employed SEM analysis at different magnification scales to view cell morphology and spreading over 76 h in culture under serum-free conditions. As a control, fibroblast cells attached and spread well over glass cov-

erslips in these studies (Figs 1a and 2a). Compared to glass coverslips, cells spread qualitatively well over the surface of Geristore exhibiting characteristic elongated fibroblastic morphology showing adhesion to the underlying material through their processes (Figs 1b and 2b). However, fibroblasts appear rounded in morphology with blunted extensions on disks of MTA (Figs 1c and 2c). On the surface of GIC, cells were sparse and morphologically altered losing fibroblastic processes (Figs 1d and 2d).

Cytotoxicity assay

Results obtained with the MTT assay indicated that Geristore was less toxic to human PDL fibroblast cells after 24 and 48-h incubation period than MTA or GIC extracts (Table 1). Normalized percent viability values from multiple experimental samples ($n = 4$) were subjected to statistical analysis. ANOVA was performed to assess the effect of material extracts on cell proliferation. Results of analysis revealed that material extracts had significant effect on cell proliferation at both 24 h ($F = 547.62$, $P < 0.05$) and at 48 h ($F = 6048.18$, $P < 0.05$). *Post hoc* pairwise comparison was carried out using Tukey's multiple comparison tests. Results of Tukey's test indicated that Geristore was statistically ($P < 0.05$) significantly better than control, MTA, and GIC, both at 24 h and at 48 h. It also suggested that GIC differs significantly from control and MTA, both at 24 h and at 48 h ($P < 0.05$). However, the difference

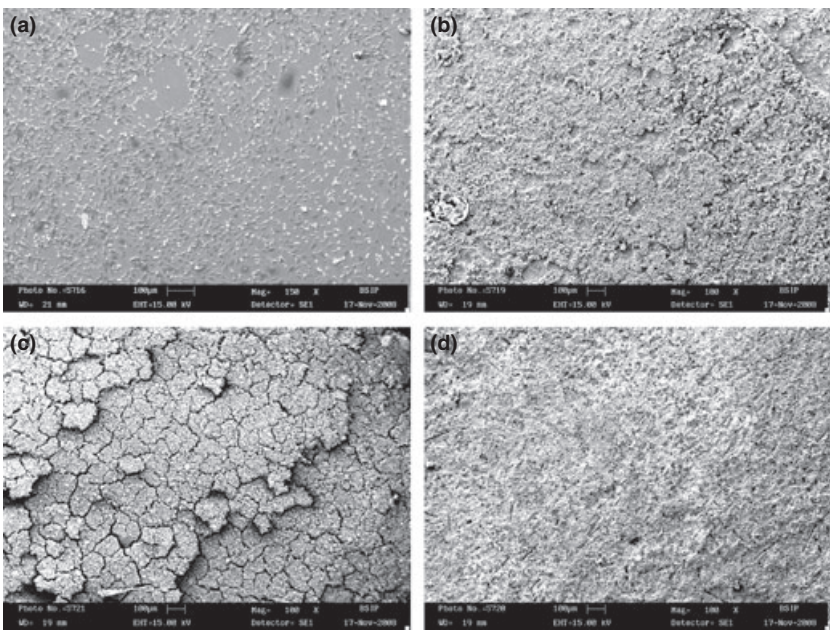

Fig. 1. Scanning electron microscopic analysis of human periodontal fibroblast attachment and growth following 76 h of incubation on (*a*) glass coverslip controls (150×), (*b*) Geristore (100×), (*c*) MTA (100×), and (d) GIC (100×).

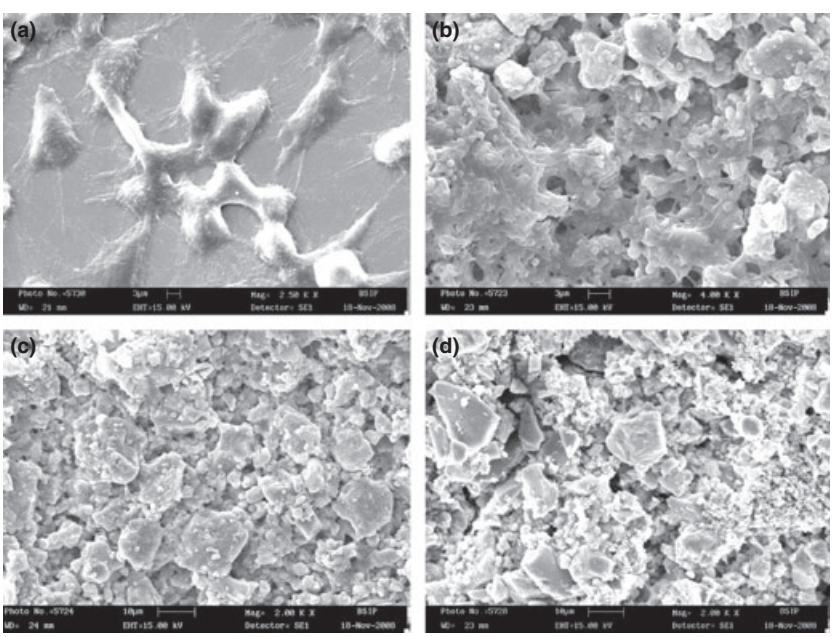

Fig. 2. Scanning electron microscopic analysis of human periodontal fibroblast attachment and growth following 76 h of incubation on (a) glass coverslip controls (2500×), (b) Geristore (4000×), (c) MTA (2000×), and (d) GIC (2000×).

Table 1. Percentage of cell viability after 24 and 48 h for experimental groups and standard deviation of cell viability for each group and each exposure period

Experimental groups	Percent cell viability (standard deviation)	
	After 24 h	After 48 h
Control	96 (3.9)	100 (3.3)
Geristore	130 (2.5)	160 (1.6)
MTA	94 (3.5)	80 (2.3)
GIC	104 (3.8)	60 (3.3)

GIC, glass ionomer cement; MTA, mineral trioxide aggregate.

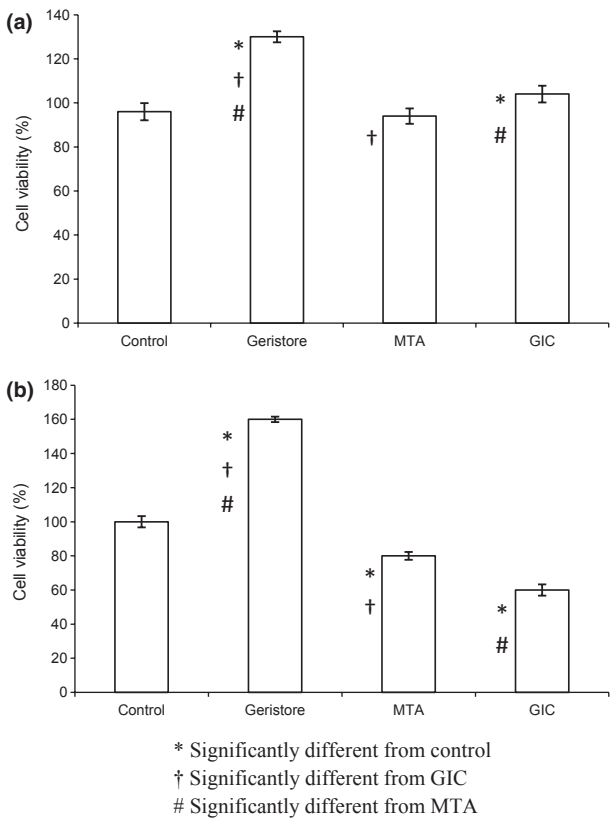

Fig. 3. Graphs show result of human periodontal ligament cell viability (MTT) assay. Geristore, MTA, and GIC material extracts were prepared following 24 (a)- and 48 (b)-hour incubation periods in tissue culture media. Results of statistical analysis revealed that Geristore extracts had significant effect on cell proliferation at both 24 h ($P < 0.05$) and at 48 h ($P < 0.05$). *Post hoc* pairwise comparison was carried out using Tukey's multiple comparison tests.

between MTA and control is insignificant at 24 h ($P > 0.05$) but it is significant at 48 h ($P < 0.05$). Figure 3a,b gives the comparison of Geristore with control, MTA, and GIC at 24 and 48 h, respectively.

Discussion

Evaluation of cellular growth and adhesion has been used to test cytotoxicity of dental materials (3, 10–12). It has been suggested that cell growth on the surface of a material is a more sensitive indicator of cytotoxicity than cell growth in surrounding areas (13). SEM has been used to evaluate adhesion of cells on materials used in a proximity to periodontal tissues as a part of evaluating the cytotoxicity of these materials (14, 15). Geristore did not inhibit growth of PDL fibroblasts as evaluated by scanning electron microscopy and the cells spread well over the disk. However, cells did not grow or spread equivalently well over MTA or GIC. Results of the present study are consistent with earlier studies (3, 16), which also concluded that PDL fibroblasts attached more readily to Geristore. These investigators determined that cellular attachment occurred significantly greater than other endodontic root-end filling materials.

Geristore showed an excellent biocompatibility profile on the first day of exposure, with percent cell proliferation up to 130%. This value also increased significantly ($F = 6048.18$, $P < 0.05$) up to 160% over the next day to show a favorable effect on cells, which is in agreement with previous studies (3, 17). While MTA could maintain the percent cell viability to 94% at 24 h that decreased further to 80% at 48 h. The results of a meta-analysis on biocompatibility of MTA had shown that MTA is more biocompatible than super ethoxy benzoic acid (EBA), intermediate restorative material (IRM), and silver amalgam (18). In the present investigations, GIC was found to decrease the percent cell viability up to 60% at 24 h. In a parallel finding, it has already been reported that GIC is more cytotoxic than MTA (19).

It is unclear why Geristore has demonstrated a more favorable response, but it may be due its surface topography. It showed some granulations with soft edges along with the grooves on the surface, which may facilitate the better scaffolding effects than the other materials used, that is, MTA and GIC. The other possible suggested reason is that Geristore elutes less toxic materials into the medium (3). Our results also support this conclusion; however, the exact component differences are not known at present.

A recent *in vivo* study evaluating root-end filling materials illustrated that Geristore showed the least favorable healing results in the histological evaluation when compared with the other experimental groups, MTA and intermediate restorative material (IRM) (20). However, the study was carried out without using bonding agent recommended by the manufacturer. It has been established through several clinical studies that Geristore can repair subgingival and subosseous tooth defects successfully and can be used as a barrier for guided tissue regeneration and also as a retrofilling material (4–7). Further studies are required before definite conclusions can be made.

References

1. Chong BS, Ford TR, Kariyawasam SP. Tissue response to potential root-end filling materials in infected root canals. Int Endod J 1997;30:102–14.
2. Friedman S. Retrograde approaches in endodontic therapy. Endod Dent Traumatol 1991;7:97–107.

3. Al-Sabek F, Shostad S, Kirkwood KL. Preferential attachment of human gingival fibroblasts to the resin ionomer Geristore. J Endod 2005;31:205–8.
4. Scherer W, Dragoo MR. New subgingival restorative procedures with Geristore resin ionomer. Pract Periodontics Aesthet Dent 1995;7:1–4.
5. Dragoo MR. Resin-ionomer and hybrid-ionomer cements: part II. Human clinical and histologic wound healing responses in specific periodontal lesions. Int J Periodontics Restorative Dent 1997;17:75–87.
6. Shuman IE. Repair of a root perforation with a resin-ionomer using an intentional replantation technique. Gen Dent 1999;47:392–5.
7. Behnia A, Strassler HE, Campbell R. Repairing iatrogenic root perforations. J Am Dent Assoc 2000;131:196–201.
8. Pant V, Dixit J, Agrawal AK, Seth PK, Pant AB. Behavior of human periodontal ligament cells on CO2 laser irradiated dentinal root surfaces: an in vitro study. J Periodontal Res 2004;39:373–9.
9. Siddiqui MA, Singh G, Kashyap MP, Khanna VK, Yadav S, Chandra D et al. Influence of cytotoxic doses of 4-hydroxynonenal on selected neurotransmitter receptors in PC-12 cells. Toxicol In Vitro 2008;22:1681–88.
10. Willershausen B, Schäfer D, Pistorius A, Schulze R, Mann W. Influence of resin-based restoration materials on cytotoxicity in gingival fibroblasts. Eur J Med Res 1999;4:149–55.
11. Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. J Biomed Mater Res 1998;41:474–80.
12. Franz A, König F, Anglmayer M, Rausch-Fan X, Gille G, Rausch WD et al. Cytotoxic effects of packable and nonpackable dental composites. Dent Mater 2003;19:382–92.
13. Hensten-Pettersen A, Helgeland K. Evaluation of biologic effects of dental materials using four different cell culture techniques. Scand J Dent Res 1977;85:291–6.
14. Zhu Q, Haglund R, Safavi KE, Spangberg LS. Adhesion of human osteoblasts on root-end filling materials. J Endod 2000;26:404–6.
15. Zmener O, Cabrini RL. Adhesion of human blood monocytes and lymphocytes to different endodontic cements. A methodological in vitro study. J Endod 1986;12:150–5.
16. Camp MA, Jeansonne BG, Lallier T. Adhesion of human fibroblasts to root-end-filling materials. J Endod 2003;29:602–7.
17. Al-Sa'eed OR, Al-Hiyasat AS, Darmani H. The effects of six root-end filling materials and their leachable components on cell viability. J Endod 2008;34:1410–4.
18. Ferna′ndez-Ya′n˜ ez Sa′nchez A, Leco-Berrocal MI, Martı′ nez-Gonza′ lez JM. Metaanalysis of filler materials in periapical surgery. Med Oral Patol Oral Cir Bucal 2008;13:E180–5.
19. Xia L, Jiang L, Zhu YQ. [Cytotoxicity of MTA, Dycal and GIC on human periodontal ligament cells in vitro]. Shanghai Kou Qiang Yi Xue 2007;16:647–51.
20. Tawil PZ, Trope M, Curran AE, Caplan DJ, Kirakozova A, Duggan DJ et al. Periapical microsurgery: an in vivo evaluation of endodontic root-end filling materials. J Endod 2009;35:357–62.