# Biocompatibility of Two Novel Root Repair Materials

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## Abstract

**Introduction:** The purpose of the present study was to evaluate the biocompatibility of 2 root-end filling materials, Endosequence Root Repair Material Putty (ERRM Putty) and Paste (ERRM Paste) and compare them with gray mineral trioxide aggregate (MTA). **Methods:** ERRM Putty, ERRM Paste, MTA, intermediate restorative material (IRM), and Cavit G were tested. For cytotoxicity assay, human gingival fibroblasts were incubated for 1, 3, and 7 days with extracts of varying concentrations from materials set for 2 days or 7 days. Cell viability was evaluated by methyl-thiazol-tetrazolium (MTT) assay. For cell adhesion assay, materials set for 7 days were examined under scanning electron microscope directly after setting, after incubation in cell culture medium for 7 days, and after incubation in gingival fibroblast suspension at a density of $5 \times 10^4$ cells/well for 2 and 7 days. The constituents of crystals formed on surface of materials were determined by energy dispersive analysis by x-ray. **Results:** Cell viability was significantly correlated with the type of material, setting time, and incubation time ($P < .001$ for all parameters). ERRM Putty and ERRM Paste displayed similar cell viabilities to MTA at all experimental conditions, except that fresh samples of ERRM Paste showed slightly lower cell viabilities than MTA. Cell viabilities with IRM and Cavit G were significantly lower than with the other 3 materials ($P < .001$). Similar surface crystallographic features and cell adhesion were observed on ERRM Paste, ERRM Putty, and MTA. **Conclusions:** ERRM Putty and ERRM Paste displayed similar *in vitro* biocompatibility to MTA. *(J Endod 2011;■:1–6)*

## Key Words

Bioceramic, biocompatibility, cell adhesion, cytotoxicity, human gingival fibroblast, mineral trioxide aggregate, root repair material

Root-end filling materials are commonly used in endodontic surgical procedures. An ideal endodontic root-end repair material should be biocompatible, radiopaque, antibacterial, dimensionally stable, easy to manipulate, and unaffected by blood contamination. It is also desirable for the selected material to induct or conduct bone deposition, provide a good seal against bacteria and fluids, set in a wet environment, and have sufficient compressive strength and hardness (1). Numerous materials have been advocated as root-end filling materials including amalgam, zinc oxide–eugenol cements, polycarboxylate cements, glass ionomer cement, composite resin, epoxy resin, gutta-percha, and Portland-based cements. Among these, mineral trioxide aggregate (MTA) has been recognized as a bioactive material (2) that is hard tissue conductive (3), hard tissue inductive, and biocompatible (4). It is composed of 53.1% tricalcium silicate, 22.5% dicalcium silicate, 21.6% bismuth oxide, and small proportions of tricalcium aluminate and calcium sulfate (5). MTA is currently marketed in 2 forms, gray (GMTA) and white (WMTA). Lower amounts of iron, aluminum, and magnesium are present in WMTA than in GMTA (6). Although MTA is a commonly used material for retrograde filling, apexification, and perforation repair, its handling characteristics are less than ideal as a result of long setting time and difficulty in maintaining consistency of mixture. Efforts have been made to overcome these shortcomings. However, introducing new compositions of MTA (7–9) or using various additives (10, 11) can affect MTA's ideal characteristics and should await comprehensive investigations.

Recently, Endosequence Root Repair Material Putty (ERRM Putty; Brasseler USA, Savannah, GA) and Endosequence Root Repair Material Paste (ERRM Paste; Brasseler USA) have been developed as ready-to-use, premixed bioceramic materials recommended for perforation repair, apical surgery, apical plug, and pulp capping (12). According to the manufacturer, both materials are of broadly similar chemical composition (calcium silicates, zirconium oxide, tantalum oxide, calcium phosphate monobasic). They have excellent physical and biological properties and are easy to work with. They are hydrophilic, insoluble, radiopaque, aluminum-free, and of high pH (12). Presence of moisture is required for the materials to set and harden. The working time is more than 30 minutes, and the setting time is 4 hours in normal conditions. Recently, only one study (13) has evaluated the cytoxicity of ERRM Paste, demonstrating equally low cytotoxicity for ERRM Paste and MTA. The aim of the present study was to assess *in vitro* the biocompatibility of ERRM Putty and ERRM Paste during the first 7 days of setting by using cytotoxicity and cell adhesion assays and compare them with MTA. The reference materials chosen were intermediate restorative material (IRM) and Cavit G. IRM has been evaluated several times for root-end filling together with MTA, whereas chemical (non-eugenol) and biological properties of Cavit G, earlier used also as a root-end filling material, justified its use as a control material (14).

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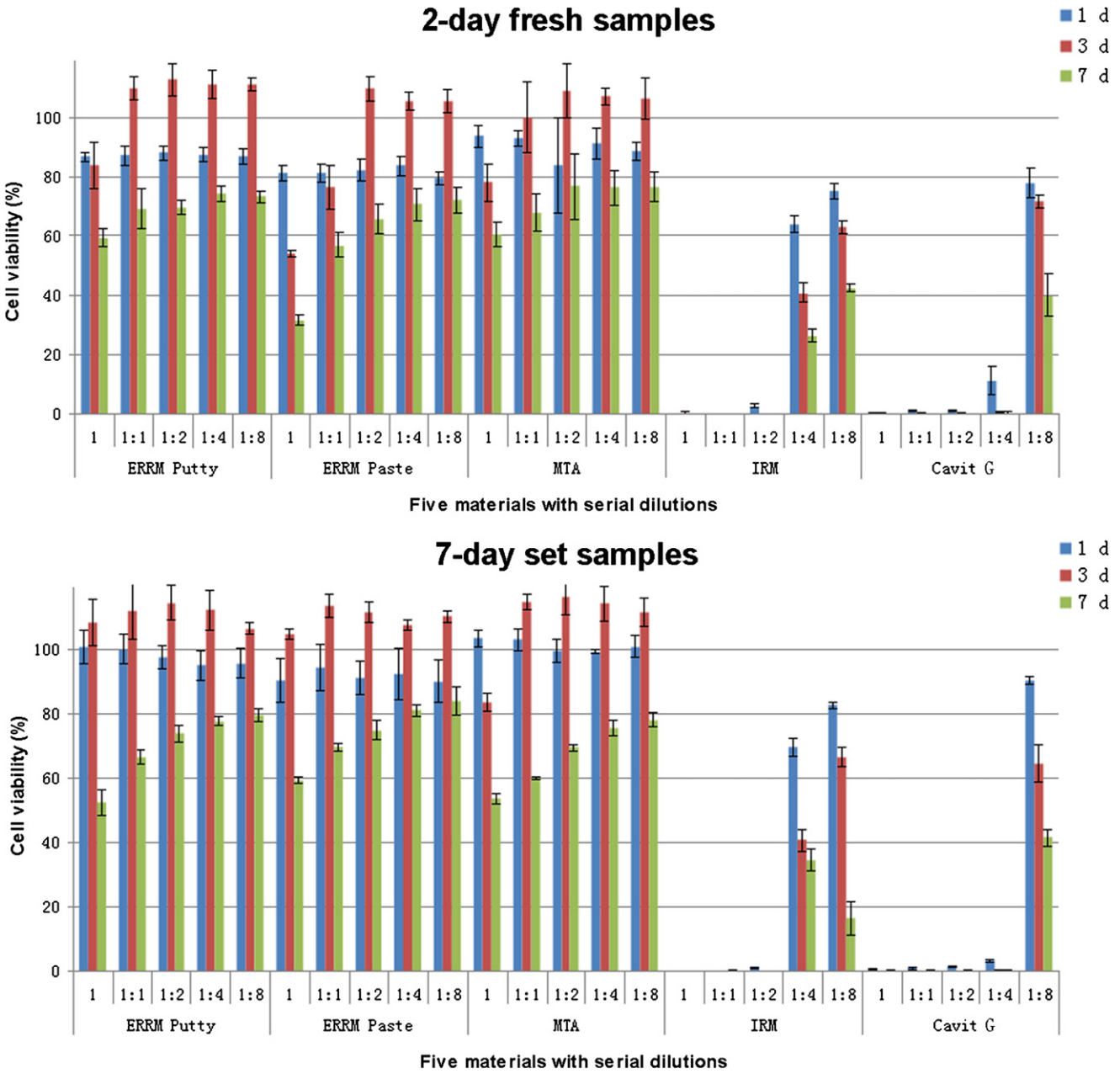


**Figure 1.** Cell viabilities of human gingival fibroblasts after incubation for 1, 3, and 7 days with serial dilutions of 24-hour extract from ERRM Putty, ERRM Paste, MTA, IRM, and Cavit G set for 2 and 7 days.

## Materials and Methods

### Cell Culture Preparation

Human gingival fibroblasts were obtained from previously established stocks cultured from healthy patients who underwent oral surgery (15). Fibroblasts of the seventh to eighth passage were used in this study. Standard protocols were followed in establishing and maintaining the cultures. The cell culture medium Dulbecco modified Eagle medium (DMEM) (Gibco, Grand Island, NY) was supplemented with 100 $\mu$g/mL penicillin G, 50 $\mu$g/mL streptomycin, 0.25 $\mu$g/mL Fungizone, and 10% fetal bovine serum (Gibco).

### Cytotoxicity Assay

ERRM Putty, ERRM Paste, GMTA (ProRoot; Dentsply Tulsa Dental, Johnson City, TN), IRM (Dentsply Caulk, Milford, DE), and Cavit G (3M ESPE AG, Seefeld, Germany) were prepared in accordance to the manufacturers' instructions under aseptic conditions. Two samples of each material were allowed to set at 37°C in 100% relative humidity for 2 and 7 days, respectively. Rubber molds were used to shape samples into disks of 10 mm in diameter and 3 mm thick. After setting, the disks were exposed to ultraviolet light for 20 minutes on each surface to ensure sterility and transferred into 24-well tissue culture plates (Sarstedt, Inc, Newton, NC) containing 1 mL of DMEM per well. One milliliter of extract was drawn from each well after incubation at 37°C and 100% relative humidity for 24 hours. Each extract was divided into 6 aliquots to obtain 5 parallel experimental groups and 1 background group. The extracts were serially diluted 1:1 with DMEM to achieve a total of 5 concentrations of each extract. DMEM incubated for 24 hours served as a negative control.

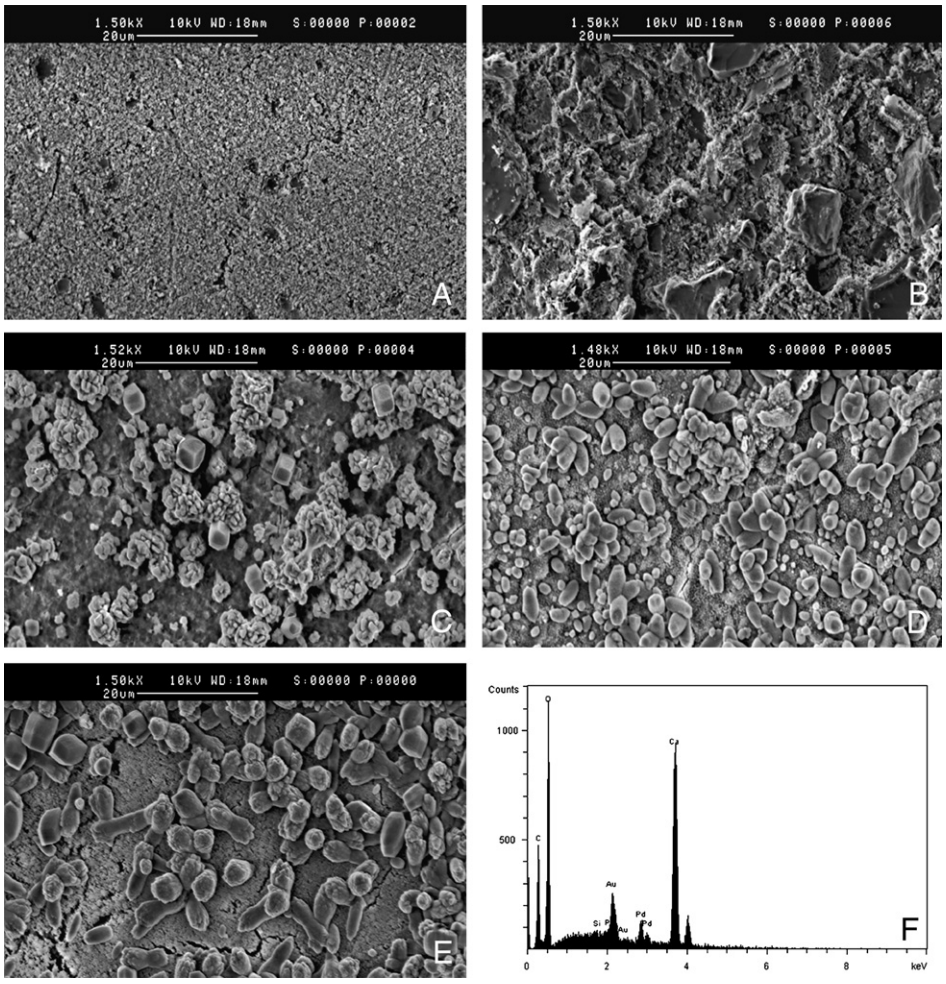


**Figure 2.** SEM micrographs of material surfaces. (*A, B*) Both IRM (*A*) and Cavit G (*B*) displayed poorly crystallized superficial structure. (*C–E*) ERRM Putty (*C*), ERRM Paste (*D*), and MTA (*E*) showed crystallographic features similar to each other. The hexagonal-shaped crystals varying in size present on the surfaces appeared both as discrete and in aggregates. (*F*) EDAX analysis indicated that crystals on the surface of ERRM Putty were mainly composed of calcium, carbon, and oxygen. Gold was detected as a result of the process of gold-palladium coating. A low level of phosphorus was also detected.

Human gingival fibroblasts suspension (100 $\mu$L/well) was seeded to 96-well flat-bottomed tissue culture plates (Nunc, Roskilde, Denmark) at a density of $5 \times 10^3$ cells/100 $\mu$L and incubated for 24 hours to achieve attachment of the cells before adding the extracts. After incubation for 1, 3, and 7 days, cell viability was determined by methyl-thiazol-tetrazolium (MTT) assay by using a CellTiter 96Assay kit (Promega, Madison, WI) according to the manufacturer's instructions. Cell viability was calculated by using the following formula: 100(a-b)/c, where a and b were optical density (OD) values from test wells and background wells, respectively, and c was the mean OD value from control wells. The background absorbance was subtracted from the values. The results were subjected to univariate analysis of variance or *t* test, when necessary, at a significance level of $P < .05$.

## Cell Adhesion Assay

ERRM Putty, ERRM Paste, MTA, IRM, and Cavit G disks were prepared and set under the same conditions as for the cytotoxicity assay. Disks were shaped to be 5 mm in diameter and 1.6 mm thick, 20 disks of each material. After 2 days of setting, the disks were extricated from the molds and incubated at 37°C in the wells of 24-well tissue culture plates (Sarstedt, Inc) containing 1 mL of distilled water that was changed daily for 5 days to allow for removal of toxic by-products. The disks were then examined under scanning electron microscope (SEM) directly (group 1), after incubation in DMEM for 7 days (group 2), and after incubation in gingival fibroblast suspension at a density of $5 \times 10^4$ cells/well for 2 days (group 3) and 7 days (group 4), each group with 5 parallel samples. In group 2, 1 mL of DMEM was added into each well with a disk and incubated for 7 days. In groups 3 and 4, gingival fibroblasts were seeded into the wells at a density of $5 \times 10^4$ cells/well with 1 mL DMEM and cultured overnight and for 7 days.

Specimens for SEM examination were prefixed with phosphate-buffered 2.5% glutaraldehyde for 10 minutes before further fixation in 1% osmium tetroxide for 1 hour. The specimens were then subjected to increasing concentrations of ethanol for dehydration. The dehydrated specimens were dried by using a critical point drier (Samdri-795; Tousimis Research Corporation, Rockville, MD), sputter-coated with gold-palladium (Hummer VI; Technic Inc, Anaheim, CA), and examined by SEM (Stereoscan 260; Cambridge Instruments, Cambridge, UK) at an accelerating voltage of 9–10 kV.

The constituents of crystals formed on the surface of ERRM Putty, ERRM Paste, and MTA were examined by energy dispersive analysis by x-ray (EDAX) (Hitachi S-3000N; Electronic System Ltd, Tokyo, Japan).

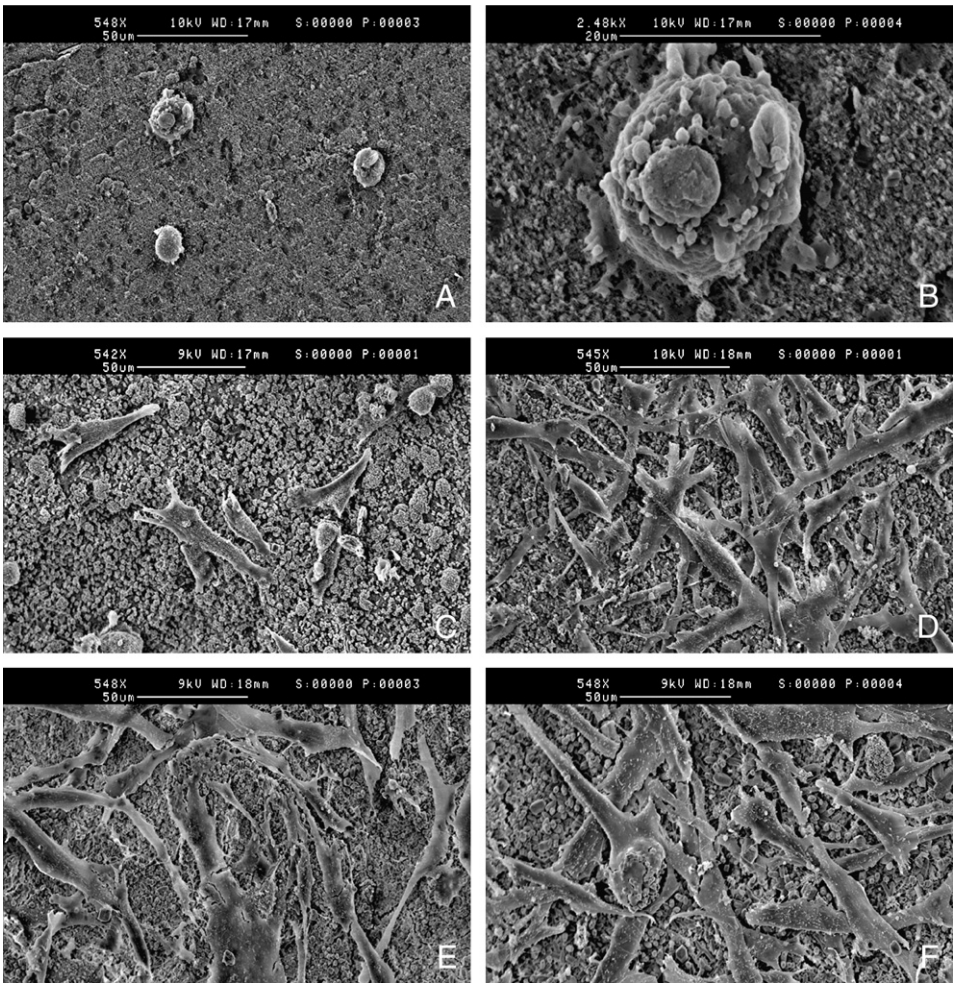


**Figure 3.** SEM micrographs of human gingival fibroblast adhesion. (*A*) Only a few small and round cells without spreading were attached to the surface of IRM after overnight incubation. (*B*) Higher magnification showed a few vacuoles and many blebs on the cell surface. (*C–E*) Gingival fibroblasts seeded to ERRM Putty (*C*), ERRM Paste (*D*), and MTA (*E*) attached to and spread out over the material surface overnight. (*F*) After 7 days of incubation, there were increased numbers of attached cells that contacted with each other by their processes and formed a matrix-like overlay on the surface of ERRM Putty.

## Results

### Cytotoxicity Assay

Fibroblast cell viability was significantly correlated with the type of material, setting time, and incubation time ($P < 0.001$ for all parameters) (Fig. 1). In 2-day setting samples, fibroblast viabilities at high concentration extracts from ERRM Putty (undiluted), ERRM Paste (undiluted and 1:1 dilution), and MTA (undiluted) and all concentrations of IRM and Cavit G decreased over increased incubation time of the cells. However, fibroblast viabilities at all the other (lower) concentrations of ERRM Putty, ERRM Paste, and MTA extracts from both fresh (2 days) and set (7 days) materials were higher than controls after 3 days of incubation but lower than controls after 7 days of incubation. Cells exposed to extracts from ERRM Putty and MTA showed the highest viabilities at all extract concentrations, whereas cells exposed to IRM and Cavit G extracts displayed the lowest viabilities ($P < .001$). Cell viabilities of ERRM Paste were slightly lower at high extract concentrations (undiluted and 1:1 dilution) than ERRM Putty and MTA but similar at lower extract concentrations.

Viabilities of fibroblasts incubated with extracts from ERRM Putty, ERRM Paste, and MTA samples set for 7 days were slightly higher or equal to viabilities of cells incubated with extracts from samples set for 2 days. No difference was detected in viability of cells incubated with extracts from IRM and Cavit G set for 2 or 7 days.

### Material Surface Morphology

For each material, no difference in surface morphology was observed under SEM when incubated in water, DMEM, or DMEM with cells. Both IRM (Fig. 2*A*) and Cavit G (Fig. 2*B*) displayed poorly crystallized superficial structure. ERRM Putty (Fig. 2*C*), ERRM Paste (Fig. 2*D*), and MTA (Fig. 2*E*) showed similar crystallographic surface structures. The hexagonal-shaped crystals varying in size present on the surfaces appeared both as discrete and in aggregates. EDAX indicated that crystals on the surface of ERRM Putty (Fig. 2*F*), ERRM Paste, and MTA were similar and composed mainly of calcium, carbon, and oxygen. Gold was detected as a result of the process of gold-palladium coating. A low level of phosphorus was also detected.

### Cell Adhesion Assay

Only a few small and round fibroblasts without spreading were attached to the surface of IRM (Fig. 3*A*) and Cavit G after overnight incubation. Higher magnification showed vacuoles and blebs on the cell surface (Fig. 3*B*). No increase in cell numbers was observed on these

2 materials after 7 days of incubation. Gingival fibroblasts seeded to ERRM Putty (Fig. 3*C*), ERRM Paste, and MTA attached to and spread out over the material surface overnight. After 7 days of incubation, there were increased numbers of attached cells that contacted with each other by their processes and formed a matrix-like overlay on the surface of ERRM Putty (Fig. 3*D*), ERRM Paste (Fig. 3*E*), and MTA (Fig. 3*F*).

## Discussion

A retrograde root-filling material designed to be placed in permanent and close contact with periradicular tissue should possess the highest possible biocompatibility. *In vitro* cytotoxicity assays have the advantages of being simple, reproducible, cost-effective, and suitable for the evaluation of basic biological aspects relative to biocompatibility (16). However, other factors such as the material's physical structure and surface characteristics, known to influence the tissue response to the materials, should also be considered (17). Many researchers have shown that the quality and quantity of cell attachment to the retrofilling materials could be used as a criterion for the evaluation of the biocompatibility of the materials (18, 19). Therefore, in the present study cytotoxicity, surface morphology, cell adhesion, and proliferation on the materials were examined. Because primary cell strains derived from living tissues are necessary for specific sensitivity testing (20), we chose human gingival fibroblasts to simulate the clinical environment. Because both the Endosequence root repair materials and MTA are hydrophilic and likely to release ionic components that would be more apt to interfere with intracellular enzyme activity, MTT assay was used in our study to measure mitochondrial dehydrogenase activity in living, metabolically active cells (13).

The use of extract in the present investigation simulated the postsurgical root-end environment in which toxic elements of the retrofilling material leach into the surrounding fluids in the bony crypt (21). The surface area to volume ratio used for extract preparation was about 250 $mm^2$/mL, which conformed to ISO standard 10993-5:4.2.3.5 (22). A series of extracts of different concentrations were made to observe a possible dose-response relationship. In our study, viabilities of cells exposed to extracts from IRM and Cavit G set for 2 and 7 days and to a lesser degree ERRM Paste set for 2 days were highly dependent on extract concentration (dilution), whereas the cell viabilities with extracts from ERRM Putty and MTA (set for 2 and 7 days) and ERRM Paste (7 days) remained at high level, irrespective of extract concentration. In pilot studies, we found that ERRM Putty and ERRM Paste should be allowed to set for 48 hours or longer to achieve initial optimum setting. Therefore, we considered 2-day sample as relatively fresh and 7-day sample as completely set. The lower cytotoxicity of set samples in ERRM Putty, ERRM Paste, and MTA than fresh samples could be explained by less toxicant leaching out from the set materials. For the same reason, fresh samples of ERRM Paste demonstrated slightly higher cytotoxicity than ERRM Putty because ERRM Putty sets faster than ERRM Paste, although they have similar chemical composition.

In the present study, we also examined possible changes in cell viability when incubated an extended time with extracts from the materials. Human gingival fibroblasts reached 100% confluence by day 3, and at 7 days of incubation all cells were still vital. However, when exposed to extracts from more toxic materials, IRM and Cavit G, cell proliferation was inhibited from the beginning.

Heavy crystallization was discovered on the surface of ERRM Putty, ERRM Paste, and GMTA. Hence, 7-day samples were used to examine possible influence of storage media on cement surface. Crystal development seemed to be unaffected by the type of incubation media, water, DMEM, or DMEM with fibroblasts. Our findings of EDAX (Fig. 2*F*) indicated calcium carbonate was the main component of the precipitate. This is in accordance with a previous report of MTA (23). Calcium hydroxide, which is a by-product of calcium silicate hydration during setting, might carbonate on contact with $CO_2$ present in air to produce a precipitate of calcium carbonate on the specimen. The carbonation could be accentuated by critical-point drying, which uses carbon dioxide in the drying of the specimens. Both ERRM Putty and ERRM Paste are described by the manufacturer as bioceramic materials. One of the characteristics of a bioactive material is its ability to form a hydroxyapatite (24, 25) or apatite-like layer (26) on its surface when it comes in contact with phosphate-containing fluids, a phenomenon called biomineralization. It can be speculated that these 3 materials could display good biomineralization ability in phosphorus-enriched media.

Examination of cell-seeded cements by SEM provided a visual confirmation of the positive interaction between the cells and the 2 new cements (27). It is known that both surface chemistry and surface topography regulate diverse cell behavior including cell adhesion, spreading, proliferation, and differentiation (28). By our SEM evaluation, the presence of crystals on the surface of ERRM Putty, ERRM Paste, and MTA did not prevent cell adhesion. The cells appeared well-spread on the material surface overnight and proliferated to form a matrix-like overlay after 7 days, demonstrating a favorable cell response to the materials. Some researchers have reported that cells attached to MTA are less likely to express various inflammatory cytokines (29). Such down-regulation of the major mediators of periapical bone resorption is likely to limit periradicular destruction and promote healing. The persistence of rounded cells with little or no spreading on surfaces of IRM and Cavit G suggested leaching of toxic components from these materials. The cytotoxic effect of IRM is possibly caused by the release of free eugenol, which is strongly cytotoxic, from the cement surface as a result of progressive hydrolysis (30). In Cavit G, zinc oxide can be the irritating chemical (31). Such toxic products affect both the morphology and the attachment of the behavior of the cells. The alteration of fibroblast from spindle shape to round and the presence of blebs on the surface are results of cytoplasmic shrinkage. Vacuolization of the cytoplasm is a common finding in injured cells and could be caused by the uptake and storage of early toxic products by the fibroblast (32).

In conclusion, ERRM Putty and ERRM Paste displayed similar *in vitro* biocompatibility to GMTA. Although clear correlation between *in vitro* and *in vivo* biocompatibility was already described for MTA, further investigations for ERRM Putty and ERRM Paste by using genotoxicity, sensitization, and tissue implantation tests are needed to establish a more general outlook on the clinical application of these materials.

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