THE JOURNAL OF PROSTHETIC DENTISTRY

RESEARCH AND EDUCATION

In vitro effects of dental cements on hard and soft tissues associated with dental implants

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ABSTRACT

Statement of problem. Dental cements for cement-retained restorations are often chosen based on clinician preference for the product's material properties, mixing process, delivery mechanism, or viscosity. The composition of dental cement may play a significant role in the proliferation or inhibition of different bacterial strains associated with peri-implant disease, and the effect of dental cements on host cellular proliferation may provide further insight into appropriate cement material selection.

Purpose. The purpose of this in vitro study was to investigate the cellular host response of bone cells (osteoblasts) and soft tissue cells (gingival fibroblasts) to dental cements.

Material and methods. Zinc oxide (eugenol and noneugenol), zinc phosphate, and acrylic resin cements were molded into pellets and directly applied to confluent preosteoblast (cell line MC3T3 E1) or gingival fibroblast cell cultures (cell line HGF) to determine cellular viability after exposure. Controls were defined as confluent cell cultures with no cement exposure. Direct contact cell culture testing was conducted following International Organization for Standardization 10993 methods, and all experiments were performed in triplicate. To compare either the MC3T3 E1 cell line, or the HGF cell line alone, a 1-way ANOVA test with multiple comparisons was used (α=.05). To compare the MC3T3 E1 cell line results and the HGF cell line results, a 2-way ANOVA test with multiple comparisons was used (α=.05).

Results. The results of this study illustrated that while both bone and soft tissue cell lines were vulnerable to the dental cement test materials, the soft tissue cell line (human gingival fibroblasts) was more susceptible to reduced cellular viability after exposure. The HGF cell line was much more sensitive to cement exposure. Here, the acrylic resin, zinc oxide (eugenol), and zinc phosphate cements significantly reduced cellular viability after exposure with respect to HGF cells only.

Conclusions. Within the limitation of this in vitro cellular study, the results indicated that cell response to various implant cements varied significantly, with osteoblast proliferation much less affected than gingival fibroblast cells. Furthermore, the zinc oxide noneugenol dental cement appeared to affect the cell lines significantly less than the other test cements. (J Prosthet Dent 2017;118:31-35)

Cement-retained implant-supported restorations have become the option of choice for many clinicians because of esthetics, occlusal form, straightforward fabrication, economics, and similarity to tooth-supported prostheses.[1-3] However, cement-retained restorations introduce another material into the restoration: dental luting cement. Residual excess cement has been implicated as a risk factor in peri-implant disease.[3-6] Also, complete removal of dental cement when the abutment crown margin is located deeper than 1.0 mm below the gingival margin is nearly impossible.[3-6] The etiology of peri-implant disease is poorly understood and may be due to multiple factors.[7]

Often, implant success and survival are attributed to the integrity of the implant bone interface and quality of osseointegration. Parameters frequently evaluated in assessing implant success include implant mobility, presence of infection, inflammation, and radiographic peri-implant bone loss.[8] Additionally, many studies have indicated that peri-implant soft tissue health can impact implant success and survival.[8-11] Also,

The University of Texas at Dallas, Richardson, Texas, provided startup funds and the needed research facilities and resources to complete the work presented.
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Clinical Implications

Where cell growth is considered clinically important (immediately after implant surgery during the hard and soft tissue formation stages), cement selection should be carefully considered, especially if excess cement may inadvertently remain at or near the healing site.

the organisms associated with periodontal disease are often the same as those found in the microbiota surrounding failing dental implants.[12-14]

The selection of dental cements for cement-retained restorations is often based on clinician preference for material properties, mixing process, delivery mechanism, or viscosity.[15] Raval et al[14] recently investigated the bacterial response of several late-stage Gram-negative colonizers recognized for involvement in peri-implant disease. The authors hypothesized that different cement compositions (zinc oxide with eugenol, zinc oxide with noneugenol, zinc phosphate, and resin materials) would result in different bacterial responses. *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* were all subject to exposure to several commercially available dental cements, and the bacterial viability was tracked to determine bacterial response to the dental cement. The results of their study demonstrated that the composition of the dental cement plays a significant role in the proliferation or inhibition of different bacterial strains associated with peri-implant disease. However, the study only evaluated the effects of cements on microbes. Nevertheless, the effect of dental cements on host cellular proliferation may provide further insight into the selection of appropriate cement material.

Furthermore, bacterial adhesion to the surface of dental implants may hinder host cell adhesion and affect osseointegration. Competition for the surface between bacteria and host cells has been recently discussed.[16] Evaluation of the effects of bacteria and host cells on the surface of dental components is especially important in situations where cement may be introduced in the early healing phase, such as interim restoration, or abutment placement at the time of surgery,[17] or at a later stage in implant restoration, where detrimental bone changes may occur if cement comes into contact with the host tissues.

Bone resorption patterns associated with peri-implant disease generally show marginal bone loss, with implant apical bone loss considered much less frequent.[18] With residual excess cement as a predisposing disease factor, bone loss may be a secondarily related finding to soft tissue cell damage, as soft tissues are considered a protective barrier to the implant-supporting bone.[19,20]

Table 1. Dental cements investigated

Cement Type	Product Name	Manufacturer
Acrylic Resin	Premier Implant Cement	Premier
Zinc Oxide Eugenol	TempoCem	DMG America
Zinc Phosphate	Fleck's	Keystone Industries
Zinc Oxide Noneugenol	Temp Bond NE	Kerr Corp

The purpose of this study was to investigate the response of bone cells (osteoblasts) and soft tissue cells (gingival fibroblasts) to dental cements. Zinc oxide (eugenol and noneugenol), zinc phosphate, and acrylic resin cements were applied to confluent cell cultures to determine cellular viability after exposure. The hypothesis of the study was that host cell lines would not differ in response to contact with the test cements.

MATERIAL AND METHODS

Four dental luting cements were used in this study (Table 1). Specimen cement pellets were molded using polytetrafluoroethylene polymer molds (7×3×3 mm). These dimensions resulted in a surface area in contact with cells of 21 mm^2. Cement materials were obtained from sealed packages, and each cement was mixed in accordance with the manufacturer's instructions under aseptic conditions.

Two commercially available cell lines were investigated to account for the different cell types associated with peri-implant tissues in vivo (soft tissues versus hard tissues). For soft tissues, human gingival fibroblasts (HGF 1 CRL 2014; ATCC) was used.[21,22] For bone, mouse preosteoblasts (MC3T3 E1 subclone 4, CRL 2593; ATCC) were used.[23-28] The MC3T3 E1 cell line has been studied extensively and shown to be a representative model for bone development.[23-25] Its growth has been demonstrated to pass through the 3 stages of proliferation, differentiation, and mineralization while showing defined gene expression patterns within the specific stages.[26] The MC3T3 E1 cell line has been demonstrated to resemble osteoblasts in vitro in a number of ways, including its ability to deposit hydroxyapatite.[27] It is also able to respond to parathyroid hormone in the same manner as osteoblasts in vivo.[28]

Direct-contact cell culture testing was conducted following International Organization for Standardization (ISO) methods 10993-5 and 10993-12.[29,30] Direct contact testing was used to most accurately resemble an in vivo situation and to evaluate the cytotoxicity of the dental cements investigated. Cement specimens were molded to account for >10% the surface area of a well in a 24-well cell culture flask (2 cm^2). All specimens were molded at a thickness of >1 mm per standard specifications. Once prepared, the specimens were plated onto a confluent cell culture (MC3T3 E1, or HGF cell line) plate (Fig. 1).

Figure 1. Experimental arrangement: 24-well cell culture flask with cement pellets, accounting for 10% of well surface area. All cement compositions tested in triplicate. Each color different cement composition.

Control specimens were defined as cell cultures with no dental cement exposure. All specimens were tested in triplicate within each experiment, and all experiments were performed in triplicate to validate the results.

To prepare confluent cell cultures, cell lines were removed from their culture flask using 1× trypsin and seated to 24-well cellular culture plates at a density of 10 000 cells/cm^2. Cells were allowed to adhere and grow to confluence over 48 hours after subcultivation. Before exposure to the investigative material, the culture medium was changed to prevent cell death from over-used medium. The cells remained in direct contact with the luting cements for 24 hours before being trypsinized and counted. Viability was established by comparing test specimens with control specimens (confluent cells with no contact with dental cement pellet). The percentage viability was defined as acceptable when cell viability remained >70% in comparison with the control specimens. All tests were performed in triplicate to demonstrate reproducibility.

A 1-way ANOVA test with multiple comparisons was used to compare either the MC3T3 E1 cell line or the HGF cell line alone (α=.05). To compare the MC3T3 E1 cell line results and the HGF cell line results, a 2-way ANOVA test with multiple comparisons was used (α=.05).

RESULTS

Quantitative analysis of the cytotoxicity testing after exposure to the dental cements investigated is shown in Figures 2-4. Cells/mL (×10^4) are represented in the corresponding bar. As illustrated, the preosteoblast cell line demonstrated a lack of significant reduction (P>.05) in viability after exposure to each of the dental cements investigated with respect to MC3T3 E1 cells only (Fig. 2). In contrast, the HGF cell line was much more sensitive (P<.05) to the cement exposure (Fig. 3). Here, the acrylic resin, zinc oxide (eugenol), and zinc phosphate cements significantly reduced (P<.05) cellular viability after exposure with respect to HGF cells only (Fig. 3). When cell lines were compared, a 2-way ANOVA was used (Fig. 4).

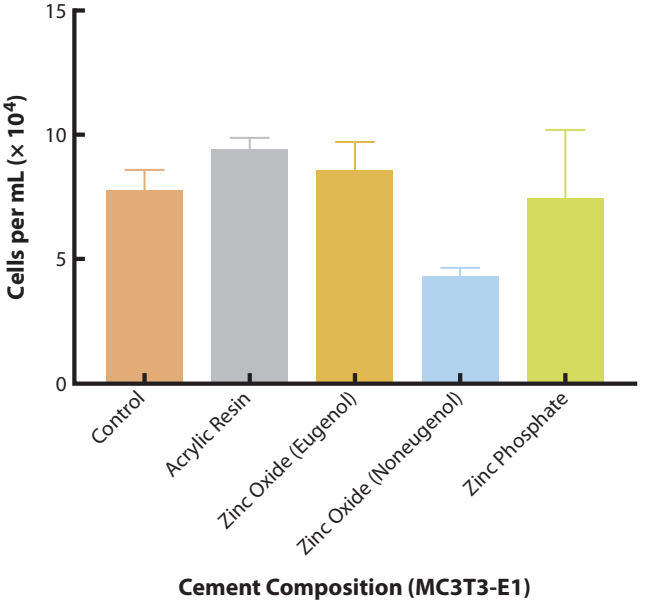

Figure 2. Preosteoblast cell line MC3T3 E1 cell count after 24-hour direct contact exposure to various dental cements.

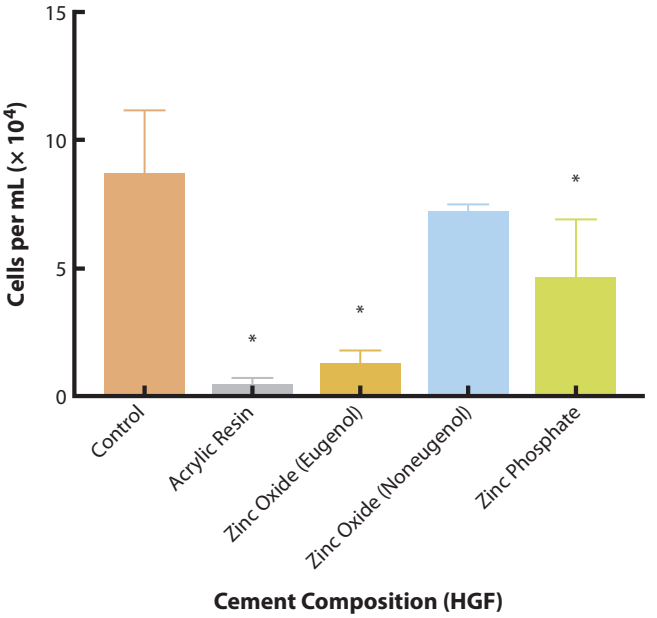

Figure 3. HGF cell count after 24-hour direct contact exposure to various dental cement materials. HGF, fibroblast cell line. *P<.05.

Here, the acrylic resin and zinc oxide (eugenol) cements demonstrated significant reductions (P<.05) in viability in the HGF cell line with respect to MC3T3 E1 cells. Overall, preosteoblast cells were much less sensitive to cement exposure when compared with gingiva fibroblasts.

DISCUSSION

Residual dental cement is an unintended result of cementing an implant-supported crown and may act as

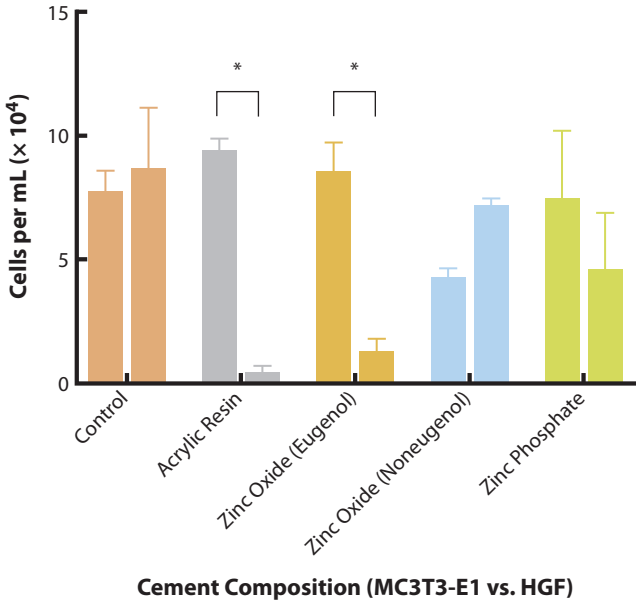

Figure 4. MC3T3 E1 versus HGF cell count after 24-hour direct contact exposure to various dental cement materials. First column represents MC3T3 E1 data, whereas second column represents HGF data. HGF, fibroblast cell line; MC3T3 E1, preosteoblast cell line. *$P<.05$.

dental calculus in the development of periodontal disease.[5] However, periodontal disease does not result in the same bone resorption profile which accompanies residual cement-induced peri-implant disease. Bone resorption surrounding dental implants generally follows a definitive profile from the crestal bone level toward the apex of the implant. This study was designed to investigate how several commercially available dental cements impacted the viability of different cell lines after exposure. This resorption profile may have been caused primarily by the soft tissue interaction with residual cements from cement-retained prostheses.

The results demonstrated that, indeed, human gingival fibroblasts were more susceptible to viability changes after exposure to commercially available dental cements. Gingival fibroblasts demonstrated significant differences in viability compared with osteoblasts, and therefore, the null hypothesis was rejected. Of specific interest was how the acrylic resin, zinc oxide (eugenol), and zinc phosphate cements resulted in greatly reduced viability after exposure to gingival fibroblasts, whereas the zinc oxide (noneugenol) cement resulted in a significant increase ($P<.05$) in viability by more than 135% (Fig. 2). Noneugenol zinc oxide dental cements typically replace eugenol with various types of organic reactants. One advantage of this substitution is the lack of interference with the polymerization of definitive cement, which is characteristic of eugenol-containing cements.[32] For tooth-supported crowns, the lack of sedation in noneugenol cements, which normally accompanies eugenol-containing cements, is considered a drawback.[31] However, upon further investigation of the cements' material safety data sheets, the different components of the cements clarified this phenomenon. Zinc phosphate cements contain phosphoric acid, zinc oxide (eugenol) cements contain eugenol, and acrylic resin cements contain acrylates, and all are well-characterized skin irritants known to cause contact dermatitis.[33] The material safety and data sheets for zinc oxide (noneugenol) cements (TempBond NE), however, indicates that "This product contains no hazardous components as defined in the Occupational Safety and Health Administration Hazard Communication Standard 29 CFR 1910.1200." [33]

Understanding how cements affect the health of peri-implant tissues is important in finding ways to improve the health of peri-implant tissues to prevent unnecessary bone loss surrounding an implant and ultimately to prevent implant failure. Chronic inflammation of almost any cause can be associated with bone loss.[34] Inflammation or inflammatory disease can increase bone resorption and can decrease the rate of bone formation, which results in an excess rate of bone resorption or breakdown.[34] The results of this study illustrated that, although both bone and soft tissue cell lines were vulnerable to the dental cement test materials, the soft tissue cell line (human gingival fibroblasts) was more susceptible to reduced cellular viability after exposure. This phenomenon may account for the patterns associated with peri-implant bone loss seen in in vivo settings despite the contribution of other factors such as physical trauma associated with soft tissue sites, microbial challenge, and the loading effect of forces placed on the implant, all of which may alter blood supplies.

These results support the claim that cellular response should be considered in cement selection, especially at healing sites. Additionally, a subsequent investigation should include an evaluation of osteoclast behavior after exposure to similar test materials. This study could examine the activity of osteoclasts after exposure to dental cements to understand how cement exposure affects osteoclast activity.

CONCLUSIONS

Within the limitations of this in vitro cellular study, the following conclusions were drawn:

1. The cellular response to various implant cements varied significantly, with osteoblast proliferation much less affected than gingival fibroblast cells.
2. The zinc oxide noneugenol dental cement (Temp Bond NE) appeared to affect the cell lines significantly less than the other test cements.

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