Cell Attachment Properties of Portland Cement–based Endodontic Materials: Biological and Methodological Considerations

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Abstract

Introduction: The attachment and spreading of mammalian cells on endodontic biomaterials are an area of active research. The purpose of this review is to discuss the cell attachment properties of Portland cement (PC)–based materials by using scanning electron microscope (SEM). In addition, methodological aspects and technical challenges are discussed. **Methods:** A PubMed electronic search was conducted by using appropriate key words to identify the available investigations on the cell attachment properties of PC-based endodontic materials. After retrieving the full text of related articles, the cross citations were also identified. **Results:** A total of 23 articles published between January 1993 and October 2013 were identified. This review summarizes the cell attachment properties of commercial and experimental PC-based materials on different cell cultures by using SEM. Methodological procedures, technical challenges, and relevance of SEM in determining the biological profile of PC-based materials are discussed. **Conclusions:** SEM observations demonstrate that commercial MTA formulations show favorable cell attachment properties, which is consistent with their successful clinical outcomes. The favorable cell attachment properties of PC and its modified formulations support its potential use as a substitute for mineral trioxide aggregate. However, researchers should carefully select cell types for their SEM investigations that would be in contact with the proposed PC-based combinations in the clinical situation. Despite being a technical challenge, SEM provides useful information on the cell attachment properties of PC-based materials; however, other assays for cell proliferation and viability are essential to come up with an accurate *in vitro* biological profile of any given PC-based formulation. *(J Endod 2014;40:1517–1523)*

Key Words

Cell attachment, mineral trioxide aggregate, Portland cement, scanning electron microscope

In vitro cytotoxicity tests are essential stages of the biocompatibility screening process, with different assays being used to assess the effects of a biomaterial on cell growth, cell membrane integrity, enzyme activity, or genetic effects (1). Cell adhesion is the first step necessary before cells can proliferate, differentiate, and produce an extracellular mineralized matrix on a substrate (2). Thus, cell attachment onto endodontic biomaterials is generally agreed to be a valid criterion for the evaluation of their biological properties (3, 4). The scanning electron microscope (SEM) provides important information in establishing biocompatibility by aiding in the observation of cell morphology and material-cell interactions (5, 6).

Portland cement (PC)–based endodontic materials such as mineral trioxide aggregate (MTA) continue to be the subject of active research since the early 1990s (7). Research has focused on the biosynthetic activity of pulp and periodontal cells when applied in contact with the biologically active MTA and other PC-based formulations (7–9). Cellular interactions and the development of cytoplasmic processes along the crystalline structures of PC-based endodontic materials that are able to regulate osteogenic/dentinogenic events are of particular concern (6, 8, 10). Hence, this review was undertaken to discuss the cell attachment properties of commercial and experimental PC-based materials on different cell cultures by using SEM.

Review Questions

The following questions are addressed:

1. What are the methodological procedures used for examining the cell attachment properties of PC-based formulations *in vitro* by using SEM, and which is the most relevant protocol that would simulate the clinical situation?
2. How can researchers overcome the technical challenges faced while examining samples of PC-based formulations under SEM?
3. Is there any detectable variation in the cell attachment properties of PC-based formulations when applied with different cell cultures?
4. To what extent are SEM observations accurate? Is it necessary to substantiate SEM findings with other examination tools?

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Methods

Literature Search Methodology

A PubMed electronic search (http://www.ncbi.nlm.nih.gov/pubmed) was conducted, spanning the period from January 1993 to October 2013, to identify the available investigations on the cell attachment properties of PC-based endodontic materials written in the English language. The following key words were used in the search: "Portland cement" AND "scanning electron microscope" OR "cell attachment", "Mineral trioxide aggregate" AND "scanning electron microscope" OR "cell attachment". After deleting duplicate articles, the cross citations of the selected articles were identified, and the data were then analyzed. Table 1 (1–5, 9–26) shows the retrieved studies and summarizes the methodological procedures used for SEM analysis.

Preparation of Samples

PC-based samples are usually examined after mixing and letting them set for a given period of time (1–5, 9–11, 13, 15–20, 22–24, 26). Samples on coverslips or disks embedded in well-plates are the most common preparation procedure (Table 1); nevertheless, examining the set samples after application in root slices or sterile acrylic molds is another valid alternative, where cells can be viewed on the top of the material and at the interface (1) (Fig. 1*A–C*). The latter would provide better simulation to the clinical application of PC-based materials, where they are frequently applied in prepared root-end/furcation/side cavities. In addition, coverslips may not withstand the rigors of dehydration, drying, and metal coating, and some samples may become lost (27).

The cell attachment properties of MTA have also been examined in the freshly mixed formulation (12, 14, 16). Such formulation would release a considerable amount of chemical by-products that are toxic to the cells in culture. However, in the clinical situation, these by-products are likely to be diluted in the interstitial tissue fluids and are eliminated through the vasculature, a biological response that is lacking in the tissue culture (28). Therefore, using set samples for SEM evaluation seems to be more suitable.

Sterilizing the samples before application into a given biological examination is preferred to avoid contamination (17, 26, 29). Ultraviolet radiation with or without immersion in 70% ethanol is the most common procedure (2, 5, 15, 17, 23–25). However, other studies have used chemical disinfection such as penicillin/streptomycin followed by washing with phosphate-buffered saline (9, 19), or immersion in ethanol followed by drying (13). The use of gamma irradiation and ethylene oxide gas has also been reported (21, 26). Comparative studies are warranted to determine the most effective yet inert sterilization method.

The repeated immersion of study samples in culture medium or distilled water for a given time before application of the cell culture has been reported (3, 4, 17, 22, 23, 25, 26). This step aims to ensure removal of potentially toxic by-products that may affect the adhesion of cultured cells other than the surface of the material (4, 25). It could be argued that removal of such elutes from the cell culture medium may overestimate the biological profile of the material, especially that such samples usually are immersed after being set (4, 17, 22, 23, 25, 26), which is less toxic than their clinical application in the freshly mixed formulation. Notably, adsorption of serum protein on the surface of study samples immersed in serum containing culture medium may also improve cell adhesion (2). This might explain why this immersion procedure was not performed in many studies (1, 2, 5, 10–16, 18–21, 24). It is worth noting that SEM may provide additional information by examining the effect of such elutes on the attachment and spreading of cells at areas around the material. Toxic by-products, eluted from set samples, may affect the attachment and spreading of cells applied on a biocompatible substrate other than the surface of the test material (Fig. 1*D–G*).

Application of Cell Cultures

Adhesion of mammalian cells to dental biomaterials is an area of active research. The literature shows that human osteosarcoma cell lines (MG-63 and Saos-2) are most commonly used for cell attachment evaluation of PC-based materials (Table 1). Both osteosarcoma cell lines possess several osteoblastic features (30, 31); however, they exhibit abnormal molecular and cellular functions because of their chromosomal alterations (32). As such, these cell lines may not be ideally suited for studying all aspects of osteoblast function (14, 31, 32). Other primary cell cultures and cell lines, such as human fibroblasts from the gingiva and periodontal ligament, murine cementoblasts, and dental pulp stem cells, have also been examined (Table 1).

The application of a given cell culture onto study samples is a relatively simple procedure. Preparing the samples onto sterile coverslips or in transparent acrylic molds would help the researcher to observe the cellular responses at areas around the interface via an inverted microscope before being processed for SEM (Fig. 1*A–G*). Observing the cytotoxic effects of the material at this stage is beneficial because dead cells cannot be detected via SEM because they usually detach and become washed during evacuation of the medium, rinsing, and adding of the fixative agent (Fig. 1*D–G*).

Methods of Processing

Except for 2 studies (14, 24), all investigations listed in Table 1 performed the fixation step via immersion in 2.5% glutaraldehyde solution. Cacodylate buffer has been used as an additive to avoid reaction of the medium with the released calcium hydroxide (2, 3, 15–17). One study used Karnovsky's fixative solution (1% paraformaldehyde, 1.5% glutaraldehyde, and 0.1 mol/L sodium cacodylate buffer, pH 7.2–7.4) (9). Despite being an expensive and severely toxic material (33), the application of osmium tetroxide for further fixation has also been reported (3–5, 12, 13, 19, 21, 25).

Dehydration in a series of increasing concentrations of ethanol is a common procedure before mounting the samples on stubs and coating with gold or gold/palladium. If an acrylic mold is used, immersing the samples at higher concentrations of ethanol (>70%) for a short time (3–5 minutes each) is preferred to prevent cracking of the acrylic mold. Critical point dryness has been used as the final step before mounting the samples (1, 4, 5, 11–16, 20, 23). Hexamethyldisilazane has also been used as a time-saving, inexpensive alternative to critical point drying (2, 3, 17, 21, 34).

Camilleri et al (15) compared the different processing methods of MTA samples and found that critical point drying may affect the material. The calcium hydroxide produced during setting may react on contact with CO_2 to produce a calcium carbonate precipitate on the specimens, which may affect the field viewed under the SEM (15). The use of hexamethyldisilazane is a reasonable substitute for critical point drying that may allow better cell preservation (15). However, this technique can still produce surface carbonation of the materials (15). Drying the samples in dry air may also show satisfactory results (9) (Fig. 1*A–G*).

The formation of different shapes and sizes of calcium compound crystals (mainly composed of calcium carbonate or calcium phosphate) has been reported (4, 35, 36). The same crystals can also be observed when a setting accelerator, such as calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), is added to either white MTA (WMTA) (Fig. 1*H* and *I*) or PC (5), which may show the presence of sulfur in