

Cellular Response to Mineral Trioxide Aggregate Root-End Filling Materials

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ABSTRACT

Endodontic surgical procedures involve the use of a root-end filling material to provide an apical seal and to facilitate the repair and regeneration of periradicular tissues. Whereas earlier studies typically evaluated the cytotoxicity of these materials, contemporary research has focused on their capacity to support a favourable cellular response. In vitro tissue culture techniques have demonstrated interactions at the cell surface that may be conducive to periapical healing. Recent research with osteoblasts has confirmed their affinity for novel endodontic mineral trioxide aggregate (MTA) materials, with evidence of cell attachment and synthesis of bone matrix. This article reviews the current state of knowledge about MTA as a root-end filling material, with particular emphasis on cellular response to MTA materials.

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Traditional efforts to repair tooth roots used materials that were originally designed for the restoration of tooth crowns, but these materials were barely tolerated by the surrounding ligament and bone. Recent studies have indicated that mineral trioxide aggregates (MTAs) are highly effective for filling root ends, repairing root perforations¹ and restoring damaged root structure. Under physiological conditions, MTAs set gradually (over a period of several hours) through hydration and subsequent interlocking of crystals within the tissue fluid. The hardened material has little cytotoxicity and good biocompatibility and appears to induce a favourable tissue response.²⁻⁴

The purpose of these retrograde fillings is to create an apical seal or protective barrier between the contents of the root canal system and the periradicular tissues in the alveolar bone (Fig. 1).⁵ Therefore, the materials must possess physical properties conducive to creating an apical seal, and they must be nontoxic

and biocompatible with the periradicular tissues. The ability to promote periapical healing could be particularly beneficial.

Periapical healing involves the repair and regeneration of alveolar bone and the periodontal ligament.⁶ Bone regeneration depends on differentiation of osteoblasts and synthesis and mineralization of extracellular bone matrix. However, little is known about the effects of root-end filling materials on these processes in alveolar bone. Therefore, contemporary studies have begun to examine the influence of the root-end filling materials on osteoblasts and other cells in the periradicular tissues.

Limitations of Research and Study Design to Date

The ideal study to examine the effects of endodontic root-end filling materials on the periradicular tissues would be a clinical trial involving humans. However, clinical trials on patients requiring endodontic treatment are



Figure 1: Surgical endodontics and root-end fillings. This patient had traumatic crown fractures of the maxillary incisors, which were treated with root canal therapy, prefabricated posts, core build-ups and crowns. Unsatisfied with the esthetic results, he sought retreatment several years later. Specialists in endodontics, periodontics and prosthodontics performed nonsurgical retreatment of the root canals, surgical lengthening of the crowns, and placement of cast post, cores and crowns. Despite the nonsurgical retreatment of the root canal of tooth 22, a periradicular radiolucency persisted (a). Endodontic surgery revealed a radicular cyst (b). Root-end resection and filling were followed by periapical healing and near-complete resolution of the lesion (c).

limited by obvious ethical constraints. For example, patients should not be exposed to potentially hazardous materials, should not receive suboptimal treatment and should not be left untreated as experimental controls. Furthermore, the information that could be obtained from such a clinical trial would be limited unless the surgical site was periodically resampled.

Given these constraints on clinical studies, several investigators have used animal models to study the effects of endodontic materials on tissues.⁴ These animal studies have provided valuable information, but extrapolation of their findings to the human condition should be undertaken with caution. Accordingly, such studies have been supplemented by *in vitro* tissue culture analyses. *In vitro* studies offer a more rigorous and carefully controlled environment in which to investigate the underlying cellular and molecular mechanisms of tissue responses to endodontic materials.

Tissue Culture Studies

Most tissue culture studies have focused on the cytotoxicity of endodontic materials, particularly in the freshly mixed state, before setting.⁷⁻¹⁰ When freshly mixed, these materials release a host of chemical by-products that are cytotoxic to the cells in culture. However, under clinical (*in vivo*) conditions, these by-products are diluted in the interstitial tissue fluids and are eliminated through the vasculature. Therefore, recent studies have examined the effect of preset, washed materials on cells in culture.¹¹

In one of these studies, Koh and colleagues¹² used scanning electron microscopy to examine the effects of 2 root-end filling materials, MTA and intermediate restorative material (IRM), on MG-63 osteosarcoma cells.

They found that the cells were in contact with the MTA after 1 and 3 days, but remained small and rounded on IRM (i.e., not in contact with the substrate). Zhu and colleagues¹³ also used scanning electron microscopy to look at the effects of amalgam, IRM, resin and MTA on Saos-2 osteosarcoma cells. They found that the cells attached to and spread very well over the resin material, forming a monolayer within 24 hours. Similarly, they had good attachment and spreading on MTA. In contrast, most of the cells applied on amalgam or IRM were small and rounded, with little evidence of attachment and spreading.

The authors of these studies used carefully controlled *in vitro* conditions to examine the microscopic interactions between cells and root-end filling materials. However, their

choice of osteosarcoma cell lines is a cause for concern. Although osteosarcoma cells resemble osteoblasts in some respects, their underlying biological regulatory mechanisms may differ fundamentally from those of primary osteoblasts. For example, when Perez and colleagues¹⁴ compared the responses of MG-63 osteosarcoma cells and primary rat calvarial osteoblasts to MTAs, there were noticeable differences. Whereas the osteosarcoma cells readily made contact with both ProRoot MTA (Dentsply Tulsa Dental, Tulsa, Okla.) and white (tooth-coloured) MTA (Dentsply Tulsa Dental), the primary rat osteoblasts could not be maintained on the white MTA for the study period of 13 days. Furthermore, the primary rat cells formed mineral nodules and expressed an osteogenic phenotype, whereas the osteosarcoma cell cultures failed to mineralize. Accordingly, these investigators concluded that primary cell cultures may be a more sensitive and appropriate model for studying cellular interactions with endodontic materials.

These 3 studies with osteosarcoma cells¹²⁻¹⁴ and the single study that used primary cells from the calvaria of rats¹⁴ were limited to microscopic examinations for cell contact and a few other analyses of limited scope. In contrast, a more recent study used more clinically relevant cells and undertook more complex and rigorous analyses to determine the specific effects of root-end filling materials on cells in the periradicular tissues.¹¹ Bonson and colleagues¹¹ studied the effects of amalgam (Tytin, Kerr Corporation, Orange, Calif.), MTA (ProRoot, Dentsply Tulsa Dental), zinc-oxide eugenol cement (SuperEBA, Bosworth Corporation, Chicago, Ill.) and hybrid ionomer composite resin (Geristore, DenMat, Santa Monica, Calif.) on clinically derived human gingival fibroblasts

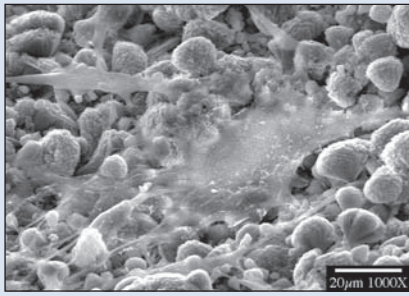


Figure 2: Human bone cell interactions with mineral trioxide aggregates (MTAs). Human alveolar bone cells were grown on tooth-coloured MTA (Dentsply Tulsa Dental) over 24 hours in vitro. Scanning electron microscopy showed that the cells attached to and spread onto the material. Numerous cellular projections were intimately associated with the MTA surface.

and periodontal ligament fibroblasts. As expected, the freshly mixed materials were cytotoxic, but this effect was reduced by washing. Through molecular techniques involving reverse transcription polymerase chain reaction, these authors were able to observe the effects of these materials on the cellular expression of genes. There were some differences in the responses of the gingival and periodontal ligament fibroblasts to each material, as well as differences in their responses to different materials. For example, one of the materials appeared to promote an osteogenic phenotype, whereas another had the opposite effect.

In addition to the periodontal ligament fibroblasts, cells from the surrounding alveolar bone¹⁵ probably play an important role in repair and regeneration at the endodontic surgical site. Accordingly, we have examined the effects of root-end filling materials on primary cell cultures derived from human alveolar bone, as well as mouse pre-osteoblasts that are capable of differentiating into mature cells that synthesize bone matrix. These studies will help to ensure that the choice of root-end filling materials is based on a thorough understanding of their effects on the repair and regeneration of periradicular tissues. This research will also aid in the development of novel endodontic materials that can actively promote the healing process.

Cellular Response to MTA Materials

In our research, we found that human alveolar bone cells¹⁵ attached and spread out on the surface of ProRoot MTA (Dentsply Tulsa Dental) (Fig. 2).¹⁶ In particular, when the cells were seeded onto MTA disks, they attached and spread over the surface within 24 hours. Scanning electron microscopy revealed cellular extensions and processes in intimate contact with the under-

lying MTA surface and with adjacent cells (Fig. 2). These cellular interactions with the surface of MTA appeared to be at least as extensive as those seen when cells were grown on serum-coated plastic surfaces. Similarly, other researchers have recently found that human periodontal ligament fibroblasts attached to MTAs.^{11,17} Bonson and colleagues¹¹ found that clinically derived human gingival fibroblasts and periodontal ligament fibroblasts survived and proliferated in the presence of MTA and made direct contact with MTA particles. Balto¹⁷ found that human periodontal ligament fibroblasts attached to MTA within 4 hours and then spread out over the surface during the subsequent 20 hours. Likewise, earlier studies^{12,13,18} with osteosarcoma cells showed that they readily attached to MTA. One of the studies¹² found that MG-63 osteosarcoma cells were in contact with MTA within 24 hours. Cells from another osteosarcoma cell line, Saos-2, spread out to form a monolayer on MTA within 24 hours.¹³ Saos-2 cells attached within 12 hours and then proliferated over MTA-coated cover slips during a 72-hour period.¹⁸ Additionally, Thomson and colleagues¹⁹ found that an immortalized mouse cementoblast cell line attached to and spread out over the MTA surface in a fashion similar to that observed on glass cover slips.

In our research, cell attachment to and propagation on ProRoot (grey) MTA were similar to what was observed on tooth-coloured (white) MTA.¹⁶ Scanning electron microscopy showed that comparable numbers of alveolar bone cells attached to the grey and to the white MTA surfaces and that elaboration of cellular extensions and processes on the 2 surfaces was similar. Furthermore, in extended culture the cells remained attached to the white MTA surface and formed an extensive matrix-like layer that appeared similar to that observed on grey MTA. This cellular matrix remained intimately associated with the white MTA surface for the full 2-week duration of this experiment. In contrast, in an earlier study, primary rat osteoblasts did not remain attached to the white MTA in extended culture.¹⁴ In that study, fetal rat calvarial osteoblasts and MG-63 osteosarcoma cells were grown in the presence of grey and white MTA particles for 6, 9 and 13 days. Both the primary rat osteoblasts and the osteosarcoma cells proliferated and attached to the grey and to the white MTA particles after 6 and 9 days of incubation. After 13 days, the osteosarcoma cells remained attached to the grey and to the white MTA particles, but the primary rat cells were seen only on the grey MTA. The failure of the primary rat cells to remain attached to the white MTA for 13 days was a cause for concern. Therefore, demonstration of sustained attachment and propagation of human cells on white MTA in our study was a valuable finding. It reassures us that both the grey and the white formulations of MTA are capable of supporting the cell attachment and proliferation that culminate in the synthesis of a matrix-like layer.

Molecular Analysis of the Cellular Response

In addition to this research on interactions at the cell surface, several studies have examined the molecular responses of osteoblasts to MTA materials *in vitro*.^{11,20,21} In their comprehensive 2004 study, Bonson and colleagues¹¹ used human gingival fibroblasts and periodontal ligament fibroblasts, the latter of which possess some osteogenic potential. The periodontal ligament fibroblasts that were exposed to MTA expressed osteoblast-associated proteins such as alkaline phosphatase, bone sialoprotein and periostin. Similarly, Nakayama and colleagues²⁰ found expression of alkaline phosphatase and osteopontin by rat femoral bone marrow cells exposed to MTA. However, their impression was that MTA had an inhibitory effect on osteoblast differentiation. More recently, Tani-Ishii and colleagues²¹ detected the expression of bone sialoprotein and osteocalcin from mouse MC3T3-E1 pre-osteoblasts exposed to MTA.

Likewise, we found that human alveolar bone cells grown on MTA disks *in vitro* expressed type I collagen and synthesized an extensive collagenous matrix during 2 weeks of growth.²² Concomitantly, the osteoblast-specific transcription factor Runx2 was expressed within a week of culture on MTA. Furthermore, this pattern of gene expression was largely unaltered by interactions with alternative formulations of MTA.

Summary of Research and Future Directions

Contemporary researchers have shifted the direction of investigations on endodontic materials, from basic measurements of cytotoxicity to more complex analyses of tissue response. These studies have demonstrated favourable and extensive cell-surface interactions with MTA materials, including cell attachment, cell proliferation and gene expression. The recent utilization of surgically derived human cells has extended these findings with clinically relevant material. Ongoing research into the molecular events involved in cellular responses to these materials are expected to unravel the underlying mechanisms and will facilitate the design and synthesis of novel biomaterials that fully exploit these exemplary properties. ✦

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