Biological Behavior of Mineral Trioxide Aggregate and a Calcium Phosphate-Based Compound

(A Comparative *in vitro* Study)

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Dedication

To my great mum and dad who gave me great love, support and care.

To all my friends, who helped me and gave me the power to finish this work.

To my sister and her husband, who stood by my side in the hard times.

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Abstract:

The aim of the present study was to compare the *in vitro* biological behavior of MTA and a calcium-phosphate-based compound (Bonitmatrix) on human pulp cells regarding cell viability, proliferation and morphology. The cells were exposed to the extracts of the materials of 1, 5 and 7 days to measure cell viability using MTT assay, and to the extracts of the materials of 5 days to measure cell number using crystal violet assay. The cells were put in direct contact with discs of the materials to assess morphology at 5, 9 and 11 days. Results showed that Bonitmatrix can be considered a potential pulp-capping material.

Keywords: MTA, Bonitmatrix, pulp-capping, MTT, crystal violet assay.

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Introduction

Since its introduction to endodontics in 1993, mineral trioxide aggregate (MTA) has gained acceptance as the material of choice for several clinical procedures. MTA has been used to seal and repair root perforations, and to create an apical barrier in teeth with open apices. Success as a root-end filling and pulp capping material has also been reported. MTA seems to be an adequate material for these procedures because of its biocompatibility, sealing ability, its capacity to induce hard-tissue formation and its ability to function under blood contamination. Furthermore, MTA has been found to possess antibacterial activity.

Mineral trioxide aggregate proved to be an effective pulp capping material. It has shown to induce hard-tissue repair of exposed pulps in experimental animals and to generate a greater frequency of dentin bridge formation than other materials. It was recently reported that the dentinogenic process in human pulp capping is induced more effectively by MTA than by calcium hydroxide.

Calcium phosphate materials, e.g. tricalcium phosphate ceramic (TCP), hydroxyappatite, and combinations of both, have been used *in vivo* as pulp-capping agents, apexification and bone graft materials. Tricalcium phosphate is a porous bioceramic material with favorable biological properties including biocompatibility and its ability to be used as a scaffold for hard-tissue regeneration. Reparative dentin bridge formation was always observed without initial necrosis except when bacterial infection occurred; new hard tissue was deposited directly on the calcium phosphate biomaterial. Theoretically, the biocompatibility of TCP and its ability to release calcium

ions may allow TCP to promote the formation of dentin bridges. It also provides a better barrier than $Ca(OH)_2$ in the obturation of open apexes, providing equivalent repair .

Recently, a combination of hydroxyappatite and β-TCP ceramic condensed on a silicon dioxide matrix material was introduced in the market. Studies on this material showed that it is biocompatible and enhance hard tissue regeneration when used as a bone grafting material; however, it has not been, yet, examined as a pulp capping material.

Many studies assessed the *in vitro* biological effects of mineral trioxide aggregate. Others studied the *in vitro* biocompatibility effects of calcium phosphate-based compounds. Only very few studies, however, compared the biological effects of these materials. Therefore, it was of interest to shed a light on the biological behavior of both MTA and a calcium phosphate-based material on human, pulp-derived, fibroblast-like cells.

Review of Literature

- **Mineral trioxide aggregate (MTA) :**

Mineral Trioxide Aggregate (MTA) was first developed and reported in the year 1993 by Lee, Monsef and Torabinejad. It is essentially a modified form of Portland cement composed of calcium silicate, bismuth oxide, calcium carbonate and calcium aluminate. It is a powder that sets in the presence of moisture and has a pH of 12.5. It has a setting time of 4 hours and a compressive strength of 70 Mpa, which is comparable to IRM ***(Koh et al. 1995).***

An alternative formulation to gray ProRoot MTA (ProRoot® MTA) was introduced by Dentsply, Tulsa dental, namely tooth-colored (white) ProRoot MTA. A significant difference between the two materials is that the gray MTA contains tetracalcium aluminoferrite (an iron-based chemical), while the tooth-colored MTA does not contain this element. The removal of tetracalcium aluminoferrite from the formulation of the tooth-colored Pro-Root MTA has apparently the objective of decreasing the risk for tooth discoloration observed when the gray MTA is used in anterior teeth ***(Ferris et al. 2004).***

Although MTA was developed with the purpose of serving as a root-end filling material, it has also proven to be successful in vital pulp therapy procedures both in animal ***(Abedi et al. 1996)*** and human trials ***(Torabinejad and Chivian 1999)***. Its applications also include repair of root and furcation perforations ***(De-Deus et al. 2005)*** and apexification***(Torabinejad and Chivian 1999)***.

Both *in vitro* and *in vivo* studies have shown that MTA fulfills certain requirements e.g. good sealing ability, biocompatibility and hard tissue formation induction, quite satisfactorily. Its sealing ability is better than that of amalgam, zinc oxide eugenol, IRM or SuperEBA ***(Torabinejad et al. 1993 and Koh et al. 1995)***. Moreover, both types of MTA seem to have similar ability to seal furcal perforations and prevent leakage of *Fusobacterium nucleatum* ***(Ferris et al. 2004).***

Holland and colleagues 2002 have investigated the biological differences between the gray and the tooth-colored MTA. These authors found, that the reaction of rat subcutaneous connective tissue to the implantation of dentin tubes filled with tooth-colored ProRoot MTA was similar to those reported for gray MTA. They concluded that the biocompatibility of the tooth-colored and gray formulations is similar. Mineral trioxide aggregate also has the ability to stimulate cytokine release from bone cells indicating that it actively promotes hard tissue formation. It was used experimentally for a number of years before it was approved for human usage by the U.S. Food and Drug Administration in the year 1998 ***(Schwartz et al. 1999).***

- **Calcium phosphate compounds :**

The synthetic calcium phosphate biomaterials, e.g. hydroxyapatite and tricalcium phosphate ceramic, are extensively employed in bone repair because of their biocompatibility and their ability to promote new bone formation. Tricalcium phosphate ceramic, hydroxyapatite and compounds of these have been evaluated *in vivo* as pulp-capping agents. Reparative dentin bridge formation was always observed without initial necrosis except when bacterial infection occurred. These *in vivo* investigations showed that new

hard tissue was deposited directly on the calcium phosphate biomaterial, in contrast with the characteristic necrotic area formed under the $Ca(OH)_2$ ***(Furusawa et al. 1991)***. Thus, the tissue responses to these capping agents were different suggesting variable cellular sensitivities to them. The effects of such synthetic materials on pulpal cell metabolism have not been extensively investigated. Because of the complexity and multiplicity of interfering factors *in vivo*, *in vitro* methods have recently been developed to gain understanding of the cellular effects of hydroxyapatite ***(Alliot-Licht et al. 1994).***

Bonitmatrix® consists of a mixture of the two calcium phosphates, hydroxyaptite (HA) and β-tricalciumphosphate (β -TCP), in the clinically proven ratio of 60/40. In contrast to conventional HA- and β-TCP-based ceramics and bioglasses, Bonitmatrix® is manufactured in a sol-gel procedure. In this process, nanocrystalline calcium phosphates are embedded in a biologically-active silicon dioxide matrix.

It is well-known that silicon has regulating and stimulating influence on the natural mineralization process ***(Carlisle et al. 1988 and Hidaka et al. 1993).***

Cell culture studies has demonstrated that Bonitmatrix® is particularly effective in supporting the growth and the functional performance of developing bone cells (osteoblasts and osteoclasts) ***(Lüthen et al. 2003)***.

- **Biological behaviour of MTA:**

Koh et al (1997) examined cultured osteoblasts (MG-63) in the presence of MTA and studied the behavior of these cells as to cytokine and osteocalcin production and alkaline phosphatase activity. Assessment of interleukin-1α (IL-1α), IL-2β, IL-6, macrophage colony-stimulating factor (M-CSF) and

osteocalcin was done using enzyme-linked immunosorbent assay (ELISA). Cells were seen adhering to MTA at 6h and had increased to confluence at 144h. Results revealed that IL-1α, IL-2β and IL-6 had increased levels when the cells were grown in the presence of MTA at 144 h with raised values at all time intervals. This was in contrast to the cells grown without MTA where the cytokines showed negligible levels. The level of M-CSF was unaffected.

Zhu et al. (2000) studied the adhesion of human osteoblast-like Soas-2 on root-end filling materials (MTA, intermediate restorative material (IRM), composite and amalgam) placed at the bottom of 96-well flat-bottomed plates using scanning electron microscope (SEM). The cells were seeded on the discs at a density of $1.5x10^5$cells per well before incubation for 1 day. Results showed that osteoblasts attached and spread on MTA and composite forming a monolayer which indicated favorable response to MTA and composite compared with IRM and amalgam.

Tziafas et al. (2002) studied the early pulpal cell response and the onset of reparative dentin formation after capping application of MTA in mechanically-exposed pulps. Thirty-three teeth from three dogs, 12–18 months of age, were mechanically exposed via class V cavities. ProRoot MTA was placed at the exposure site. The cavities were restored with amalgam and the pulpal tissue reactions were assessed by light and electron microscopy (transmission and scanning) after healing intervals of 1, 2 or 3 weeks. A homogeneous zone of crystalline structures was initially found along the pulp–MTA interface, whilst pulpal cells showing changes in their cytological and functional state were arranged in close proximity to the crystals. Deposition of hard tissue of osteotypic form was found in all teeth in