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Reaction of Rat Connective Tissue to Implanted Dentin Tubes Filled with a White Mineral Trioxide Aggregate

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The purpose of this paper was to study the reaction of rat subcutaneous connective tissue to the implantation of dentin tubes filled with white mineral trioxide aggregate (MTA), a material that will be marketed. The tubes were implanted into rat subcutaneous tissue and the animals were sacrificed after 7 and 30 days. The undecalcified pieces were prepared for histological analysis with polarized light and von Kossa technique for mineralized tissues. Granulations birefringent to polarized light and an irregular structure like a bridge were observed next to the material; both were von Kossa positive. Also, in the dentin wall tubules a layer of birefringent granulations was observed. The results were similar to those reported for gray MTA, indicating that the mechanisms of action of the white and gray MTA are similar.

Key Words: mineral trioxide aggregate, subcutaneous tissue implantation.

INTRODUCTION

A mineral trioxide aggregate (MTA) was developed at Loma Linda University for the sealing between the tooth and the external surfaces (1). Various *in vivo* and *in vitro* investigations have shown good biological properties of this material (2,3). Deposition of neoformed hard tissue in direct contact with MTA was observed in cases of pulp capping (4), pulpotomy (5), repair of furcal perforations (6), root canal fillings (7) and root-end filling (8).

According Holland et al. (9), the mechanism of action of MTA, encouraging hard tissue deposition, is similar to that of calcium hydroxide. Implanting both materials in subcutaneous connective tissue, they observed granules birefringent to polarized light and next to these granulations irregular tissue, like a bridge, that is von Kossa-positive. Torabinejad et al. (10) reported that MTA has calcium oxide and calcium phosphate in its composition. In contact with water, calcium oxide could form calcium hydroxide (9). The calcium ions from calcium hydroxide react with carbon dioxide from tissue to form calcite crystals that are birefringent to polarized light (11,12). Next to these granulations there is deposition of a hard tissue that is von Kossa-positive and resembles a bridge (9).

There is a commercially available MTA called ProRoot MTA (Dentsply, Tulsa, OK, USA) that has a gray powder. However, Glickman and Koch (13) reported that the manufacturer will place a white ProRoot MTA on the market. The biological difference between the gray and the white MTA, when implanted in subcu-

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taneous tissues is unknown. Thus, the purpose of this study was to analyze the reaction of rat subcutaneous connective tissue to the implantation of dentin tubes filled with white MTA.

MATERIAL AND METHODS

The white MTA (Loma Linda University, Loma Linda, CA) was prepared in distilled water and introduced into the root canal of human dentin tubes before being implanted in the subcutaneous connective tissue. The dentin tube preparation and the implantation technique were previously described by Holland et al. (9). The tubes were implanted in 10 rats and the animals were sacrificed after 7 and 30 days. The pieces were removed and fixed in 10% buffered formalin solution at pH 7.0. The undecalcified samples were embedded in paraffin-carnauba wax and serial sections were made at 10 µm intervals using a hard-tissue microtome, according to Holland et al. (14). Some sections were stained by the von Kossa technique for mineralized tissues and other sections, without staining, were examined with a polarized light microscope to locate the birefringent material.

RESULTS

The results observed at 7 and 30 days were the same. For this reason they will be described together. Numerous large granulations birefringent to polarized light and von Kossa positive were observed near the tube opening and generally in contact with the filling material (Figure 1). Next to these granulations, there were extensive areas of irregular tissue positive to the von Kossa technique, like a bridge (Figure 2). A highly birefringent structure was also observed in the interior of the dentin walls tubules (Figure 3). This structure formed a layer that was observed at different depths.

DISCUSSION

It may appear that in this paper we studied white MTA in subcutaneous tissue without a comparative analysis with a control gray MTA group. In reality the data presented here are an experimental group of another paper developed in rat subcutaneous connective tissue (15). That investigation studied the two kinds of MTA before the material was available commercially. When we observed that only the gray MTA was on the market, without any explanation about the matter, we believed white MTA disappeared for unknown reasons.

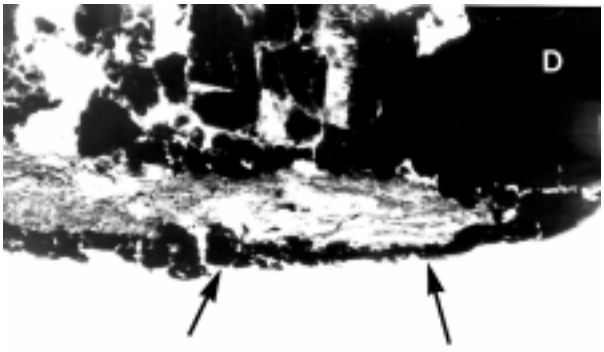

Figure 2. White MTA, 30 days. Note dentine (D) and an irregular tissue like a bridge (arrows) highly positive to the von Kossa technique (100X).

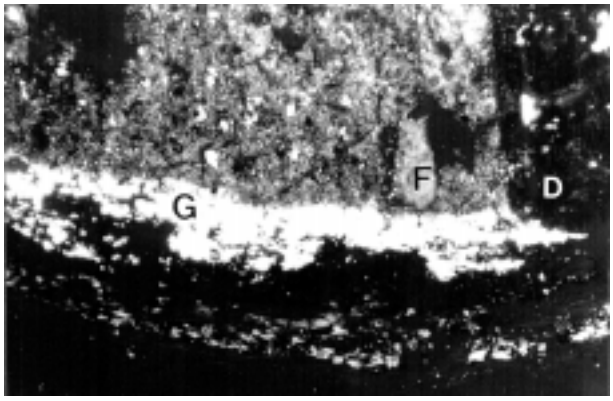

Figure 1. White MTA, 30 days. Note white MTA (F), dentin (D) and birefringent granulations (G) in direct contact with MTA (polarized light, 100X).

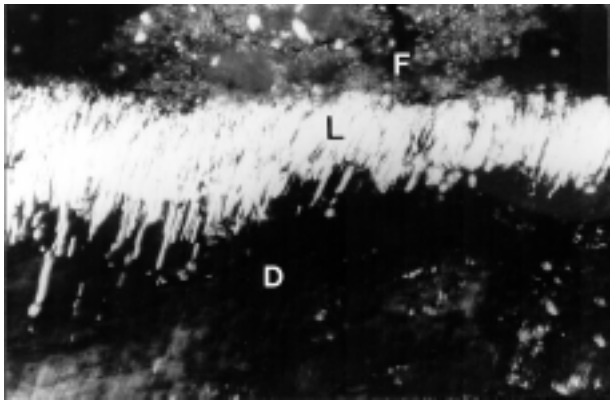

Figure 3. White MTA, 7 days. Observe MTA (F), dentin wall (D) and a layer (L) of a highly birefringent structure localized in the dentin tubules (polarized light, 80X).

In view of this, we decided not to publish the results with white MTA. However, the paper of Glickman and Koch (13) gives new life to the white MTA, at least for us. Thus, the control group of this study is in the previously published paper (15).

The same results reported by Holland et al. (9,15) for gray MTA were observed in this experimentation. This fact suggests that some differences in the materials did not influence the results when they are implanted in rat subcutaneous connective tissue. The MTA employed by Holland et al. (9,15) and the one studied in this experimentation were provided by Dr. Torabinejad (Loma Linda University). We clinically observed two differences with these materials: the color and the setting time, which was greater in the white one.

A large number of papers about MTA were published in the literature before the material was put on the market (1-10); however, the color of the material was not described. Only Faraco (16) compared the two materials in dog dental pulp. He reported slightly better results with gray MTA than with white MTA. The white MTA we studied in this paper was 3 years old and we do not know how much time the material can be kept before its properties change.

The deposition of calcite crystals seems to be very important to the mechanism of action of calcium hydroxide formed after mixing MTA with water. Seux et al. (17) reported a rich extracellular network of fibronectin in close contact with these crystals on incubation in a culture medium without cells. They reported that fibronectin first came from the culture medium and later from the cells. The authors concluded that their findings strongly supported the role of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue barrier. In our paper we observed a von Kossa-positive tissue barrier next to these crystals, remembering the hard tissue deposition in direct contact with MTA as described in a series of papers (6,8). This hard tissue is very irregular in subcutaneous tissue (9,15) but is similar to dentin in pulp tissue (4,5) and looks like cementum in furcal perforations (6) and periapical tissues (7).

In conclusion, the observed results and the mechanism of action of white MTA is very similar to those reported for gray MTA (9,15).

RESUMO

Holland R, Souza V, Nery MJ, Faraco Júnior IM, Bernabé PFE, Otoboni Filho JA, Dezan Junior E. Reação do tecido conjuntivo do rato ao implante de tubos de dentina obturados com um agregado de trióxido mineral branco. Braz Dent J 2002;13(1):23-26.

Foi objetivo deste trabalho estudar a reação do tecido conjuntivo subcutâneo do rato ao implante de tubos de dentina obturados com MTA branco, material a ser introduzido no mercado. Os tubos foram implantados no tecido conjuntivo subcutâneo e os animais sacrificados com 7 e 30 dias de períodos pós-operatório. As peças não descalcificadas foram preparadas para análise histológica com luz polarizada e ténica de von Kossa para tecidos mineralizados. Foi observado junto ao material estudado granulações birrefringentes à luz polarizada seguida de uma estrutura irregular, na forma de ponte, ambos von Kossa positivos. Foi observado também no interior dos túbulos dentinários uma camada de granulações birrefringentes à luz polarizada. Os resultados observados são similares aos descritos para o MTA cinza. Conclui-se que os mecanismos de ação do MTA branco e cinza são similares.

Unitermos: agregado de trióxido mineral, implante em tecido subcutâneo.

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