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Histological evaluation of the osteoinduction capability of human dentine

M. E. L. Machado, A. S. Souza & V. C. Araújo
Discipline of Endodontics, School of Dentistry, Camilo Castelo Branco University, São Paulo, Brazil

Abstract

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Aim To assess whether human dentine has the potential to promote the development of calcified tissues when implanted in the muscle tissue of mice.

Methodology Root canals in extracted human teeth were instrumented to produce dentine fragments. The dentine fragments produced were divided into two. In group 1, fragments were demineralized and sterilized. In group 2, the fragments were not submitted to any additional treatment. The dentine fragments were then implanted in the muscle of mice. In group 3, the muscles were implanted with rehydrated lyophilized human bone powder. Animals were killed following test periods of 7, 15, 30, 60, 120 and 180 days, the fragments were removed together with adjacent muscle and examined under light microscopy to assess calcification.

Results Areas of calcification were observed in groups 1 and 3 after a period of 180 days. In group 2, the surrounding tissues displayed only chronic inflammatory infiltration.

Conclusions On the basis of the experimental model adopted in this study, fibroblast-rich connective tissue formed in groups 1 and 3, which could reflect an osteoinductive process. Further studies are suggested to identify which dentinal factors are capable of inducing the formation of a calcified matrix.

Keywords: cell differentiation, dentine, endodontics, histology, osteoinduction.

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Introduction

One goal of root canal treatment is the development of an apical barrier of calcified tissues, induced by some kind of biomaterial (Machado 1992). This calcification would have the potencial to isolate the body from the external environment, thus promoting more favourable conditions for healing.

The search for a material capable of inducing the formation of a calcified tissue has been ongoing. Solomons & Neuman (1960) studied the capacity of human and bovine demineralized dentine to develop calcium phosphate *in vitro*. This was the first time that the formation of a calcium phosphate precipitate had been observed in an acellular, mineral-free system.

Bessho *et al.* (1990) studied the effects of bone morphogenetic-purified protein (BMP) extracted from bone matrix, dentine matrix and surgical wounds when introduced inside the alveolus after extraction of teeth in a rabbit model. BMP belongs to a large family of growth and differentiation factors that play an active role in cellular proliferation as well as in controlling the development of vertebrate and invertebrate species.

In a review of the related literature, Machado (1992) found that the inductive properties of dentine depends on the origin of the dentine, the implant site, the animal species of the host, the chemical treatment of the implant, and the degree and manner of sterilization. On the basis of this review, guidelines for the most appropriate procedure for the optimization of osteoinduction were established (Machado 1992).

Correspondence: Manoel Eduardo de Lima Machado, Rua Guarará no 350, 12° andar, Jardim Paulista, São Paulo, SP, 01425-000 São Paulo, SP, Brazil (Tel./fax: 55 11 3884 2658; e-mail: professormachado@hotmail.com).

Rutherford *et al.* (1993) assessed the quantity of reparative dentine stimulated by BMP and its relation to the quantity of protein used in experimental direct pulp capping. They concluded that dentine production was significantly higher in the BMP group when compared with the calcium hydroxide group. They also concluded that the quantity of the dentine was proportional to the quantity of BMP applied.

A detailed study of factors related to the induction of calcified tissues is essential, especially those related to dentinal induction during endodontic therapy. Application of a dentine barrier to induce the formation of calcified tissues could play a major role in achieving post-treatment repair (Holland 1984), in addition to avoiding overfilling, which would have negative effects on the healing process (Holland 1984). Clinical applications require further investigation, insofar as the dentine debris produced during canal preparation are likely to be contaminated and are not subject to any decalcification process. Demineralized tissues and even BMP bear the highest calcification inductive potential (Tziafas *et al.* 1992). The present study aimed to assess histologically whether human dentine implanted in the muscle tissue of mice would promote the development of calcified tissue.

Materials and methods

The experimental design was approved by the Ethics in Research Committee, Camilo Castelo Branco University. Dentine fragments were collected from human teeth with apical lesions where extraction was recommended. After preparation of the root canal roots were sectioned transversally with carborundum discs and each section was cut again to obtain dentine fragments of approximately 3 mm × 3 mm × 1 mm, which were then randomly divided into two groups, G1 and G2. Sections of the latter were kept in 0.9 % sterile saline solution (Aster Medical Products, São Paulo, Brazil) until implantation.

G1 sections were demineralized through immersion in 0.6 mol L^{-1} hydrochloric acid for 48 h at 4 °C. After that period, the fragments were washed thoroughly in physiological saline solution, radiographed to insure they had been thoroughly demineralized, and then sterilized in ethylene oxide.

Eighteen laboratory white male mice weighing from 24 to 40 g were selected. They were anaesthetized with a solution of tiletamine hydrochloride/zolazepam hydrochloride (Zoletil 50®, VIRBAC S/A, Cedex, France) via intra-muscular injection. Following a scalpel-made incision, three pouches were created in the muscular tissue of the animals' thighs (*gluteus medius*) to accommodate the fragments, one on the external side of the left thigh, and two on the external side of the right thigh.

G1 dentine fragments were introduced into the pouch of the left thigh. Lyophilized bone previously rehydrated in physiological saline solution for an hour before use was introduced into the upper pouch of the right thigh (G3), and G2 untreated dentine fragments were introduced into the lower pouch of the right thigh. Each fragment was maintained in position through sutures using 4–0 nylon thread. Following implantation, the skin was sutured with 3–0 nylon thread.

The inner suture was not removed, although in some animals it had been completely resorbed. The external suture was removed after 7 days. Animals were fed a balanced diet and water *ad libitum*, for periods of 7, 15, 30, 60, 120 to 180 days (three animals per period), after which they were killed using a large dose of anaesthetic.

Each fragment was removed with the surrounding tissue and stained with haematoxylin and eosin and Masson's technique. The tissue excised after each experimental period was submitted to histological analysis with a light microscope (Axioskop 2 Plus; Zeiss, Gottembourg, Germany).

The appearance of the tissue and the fragments were examined. Records were kept on the state of the dentine fragments and whether they had been resorbed or not. The formation of a connective tissue and the presence of a calcification process inside the muscular tissue was also recorded.

Results

G1 – Demineralized and sterilized dentine fragments

At day 7, the fragments placed inside the muscular tissue were associated with a little or no inflammatory infiltrate (Fig. 1a). At 15 days, the histological sections displayed dentine fragments wrapped and invaded by an inflammatory infiltrate with resorption cells found inside the muscular tissue.

Clear persistence of the inflammatory infiltrate, interposed between muscular tissue and dentine, was observed from 30 to 60 days. After 120 days, dentine fragments were surrounded by inflammatory cells, although their prevalence had decreased in relation to the earlier periods. In some cases, dentine fragments

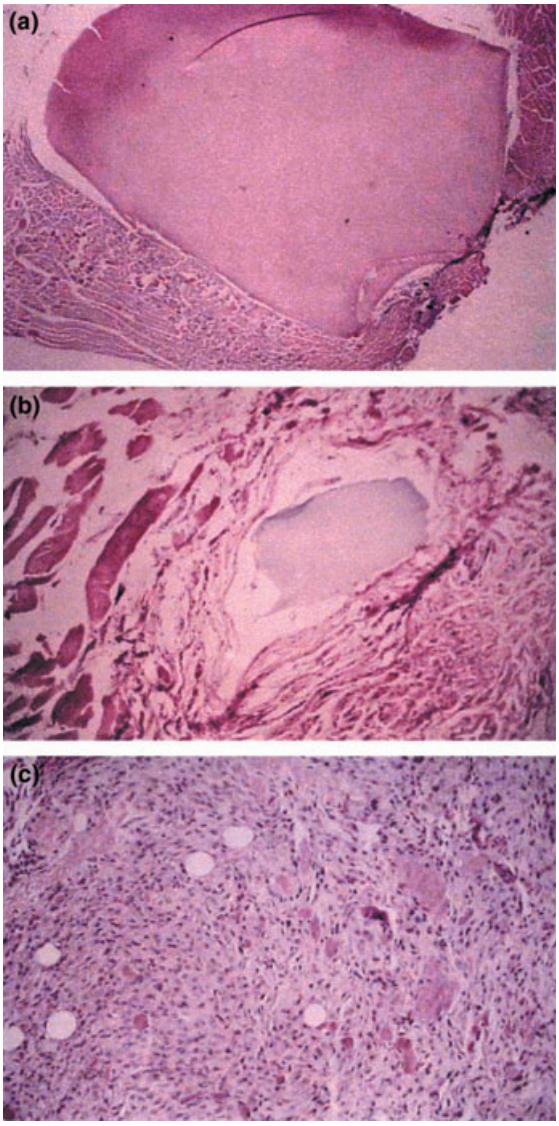

Figure 1 (a) Demineralized and sterilized dentine fragments. (a) 7th day. Dentine implant inside muscular tissue. No significant area of inflammatory infiltrate can be seen. H.E. 25,6×. (b) Decalcified and sterilized dentine fragments. (b) 120th day. Dentine implants surrounded by a connective tissue with a discrete inflammatory infiltrate. Signs of resorption can be observed on the edges of the fragment, H.E. 100×. (c) Decalcified and sterilized dentin fragments. (c) 180th day. Proliferation tissue inside the muscular tissue. Bony matrix is observed amidst the cellular proliferation. H.E. 100×.

had resorbed edges (Fig. 1b). No dentine fragments were observed in the histological section after the 180-day experimental period. However, a connective tissue rich in fibroblasts was observed in the muscular mass (Fig. 1c).

G 2 – Nonsterile, nondemineralized dentine fragments

At the 7-day experimental period mononuclear inflammatory infiltrate surrounded the entire fragment (Fig. 2a). At 15 days, the infiltrate was present, but it was less intense. At the 30, 60 and 120-day periods, there were numerous mononuclear cells and signs of resorption on the surface of the dentine fragments. At 180 days, remnants of the inflammatory process and fragmentation activity were observed (Fig. 2b).

G 3 – Lyophilized bone implants

At 7 days, an inflammatory infiltrate inside the adjacent muscular tissue was observed. After 15 days a decrease in the concentration of inflammatory cells and the beginning of a resorption process in the more peripheral portions of the fragments were observed.

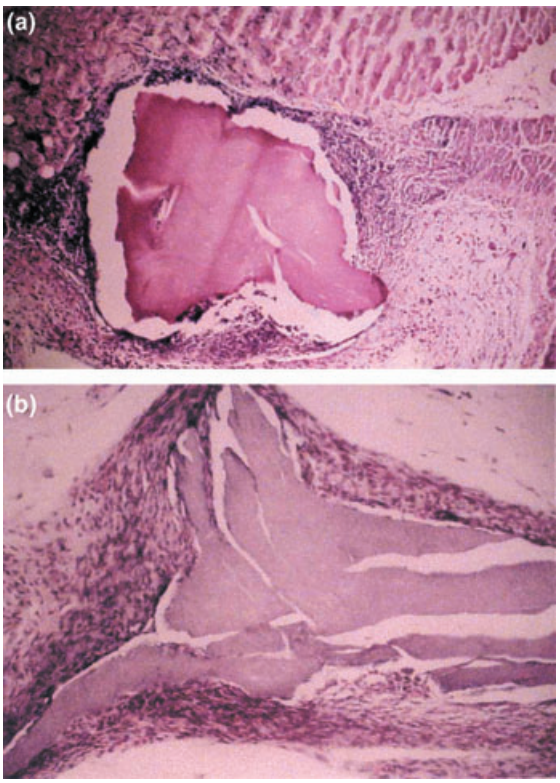

Figure 2 (a) Nonsterile, nondemineralized dentine fragments. (a) 7th day. Fragment surrounded by an intense mononuclear inflammatory infiltrate and muscular fibres in some areas. H.E. 100×; (b) Nonsterile, nondecalcified dentine fragments. (b) 180th day. Fibrous capsule with inflammatory cells surrounding the fragment. H.E. 200×.

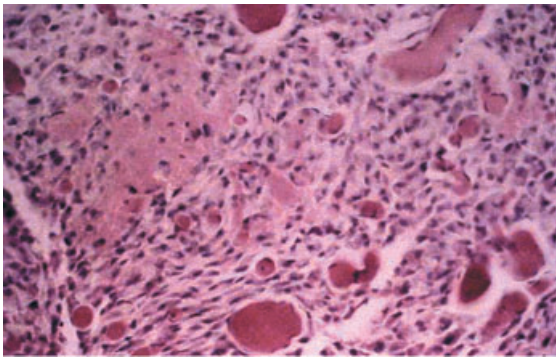

Figure 3 Lyophilized bone fragments – 180th day: Bony matrix between cellular proliferation and some remnant muscular fibres. H.E. 200×.

From 30 to 60 days, it was possible to observe an inflammatory-cell infiltration fragmenting the lyophilized bone particles. Adjacent muscular tissue had a reduced quantity of inflammatory cells. At 120 days, the inflammatory infiltrate was located in the focal areas of bone. Some resorption areas could still be observed. A tissue mobilization process had begun in the connective tissue interposed between muscular fibres, probably where the calcification process was likely to occur. At 180 days, a calcification process in the adjacent muscular tissue had been initiated (Fig. 3).

Discussion

The osteoinductive potential of several materials has been investigated and a consensus on their ideal properties has been achieved (Machado 1992). Allogenic or heterogenic bone and dentine fragments in different forms, including bone or dentine morphogenetic protein, have excellent osteoinductive activity (Conover & Urist 1979, Bessho *et al.* 1990, Machado 1992, Rutherford *et al.* 1993, Nakashima 1994, Hogan 1996, Ripamonti & Reddi 1997).

The formation of a calcified tissue barrier in the apical region after root canal treatment can be attributed to the presence of dentine chips produced during canal preparation. Likewise, after pulpotomy the pulp chamber and, in some cases, the canal entrance can be obliterated by deposition of calcified tissue generated from induction of dentine chips deposited in the pulp tissue (Tronstad 1978, Rossmeisl *et al.* 1982, Holland 1984, Patterson *et al.* 1988, Rutherford *et al.* 1993, Nakashima 1994).

Calcification does not occur in the presence of infection, which could jeopardize the repair process (Holland *et al.* 1980, Brady *et al.* 1985, Safavi *et al.* 1985). That was the reason for adopting two experimental groups in the present study, in which dentine was either subjected or not subjected to a sterilizing process, with the purpose of determining whether contamination would affect its inductive capacity. On the other hand, in G1, dentine was previously decontaminated by the root canal preparation and further sterilized in ethylene oxide, and was also subjected to a decalcification process, as suggested by others (Inoue *et al.* 1986, Bessho *et al.* 1990, Tziafas *et al.* 1992, Katz *et al.* 1993, Nakashima 1994) since it is believed that the organic portion is responsible for the induction of calcified tissues.

The effectiveness of the decontamination and sterilization procedures was evidenced in the analysis of G1 specimens in which, after 7 days, no inflammatory infiltration could be observed. At the same time G2 displayed a different pattern, with an intense inflammatory infiltration that lasted until the end of the experiment, associated with a resorption process of the fragment at the end of the 30th day.

Lyophilized bone was used as a control group since its osteoinductive capability has already been thoroughly reported (Bessho *et al.* 1990, Katz *et al.* 1993, Rutherford *et al.* 1993, Nakashima 1994, Hogan 1996, Ripamonti & Reddi 1997). The present study revealed that, following a period of 180 days, G1 displayed fibroblast-rich connective tissue proliferation inside the muscle mass, with some sites suggesting calcification. Proliferation could also be observed in G3, within the same experimental time frame. On the other hand, G2 did not display this type of tissue at any experimental periods, merely an inflammatory process associated with regular resorption of the fragments. The results infer that absence of microorganisms is a mandatory condition for osteoinductive activity, since the group in which nonsterilized dentine was applied displayed no osteogenic activity, up to 180 days.

To assess the osteoinductive potential of any material, it is necessary to detect bone formation in an area of the system where it does not naturally exist. That is why fragments were placed in the muscle tissue. It has also been established that increased blood supply to the tissue in which the fragment will be placed improves the inductive process (Machado 1992).

There is evidence that fragment resorption may play a major role in the calcification process since resorption, in the absence of contamination, is the first stage of hard tissue formation; this was observed in the present study. This was not the case in G2, where resorption was not followed by bone formation

probably due to the presence of contamination. Even though G1 dentine fragments may not have been ideal in terms of size and shape (Bessho *et al.* 1990), they did prove effective in promoting osteoinduction. A resorption process also occurred in G2, in the absence of connective tissue inside the musculature in which the fragment was applied, and only an inflammatory infiltration remained. This fact was probably due to bacteria prevailing inside the dentine fragments.

Tissue reactions seen in all sections of G1 and G3 were similar, which provides evidence that demineralized dentine (G1) presents an osteoinductive capability similar to that of lyophilized and demineralized bone (G3). This observation is consistent with the findings of others (Solomons & Neuman 1960, Inoue *et al.* 1986, Bessho *et al.* 1990, Tziafas *et al.* 1992, Katz *et al.* 1993, Rutherford *et al.* 1993, Nakashima 1994).

Further studies are suggested to identify which dentine factors are capable of inducing the formation of a calcified matrix since endodontic and surgical healing depends on the calcification of the apical foramen and on the calcification of periradicular lesions.

Conclusions

It can be concluded that:

1. During the experimental periods, nonsterile and nondemineralized dentine was unable to induce calcification processes.

2. Demineralized and sterilized dentine, as well as lyophilized bone, presented similar osteoinductive capacity, as detected by the formation of a fibroblast-rich connective tissue in all specimens, which could be the beginning of an osteoinductive process.

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