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LETTER TO THE EDITOR

The role of matrix metalloproteinases and their inhibitors in root fracture resistance remains unknown

Dear Editor,

A recent article in *Dental Traumatology* described the fracture resistance and histological findings of immature teeth treated with mineral trioxide aggregate (MTA) (1). In the study, pulp tissues were removed from extracted sheep incisors and root canals were filled with either calcium hydroxide or MTA, the control teeth being left untreated. Basically, the article has two main findings: MTA treatment was able to significantly improve the long-term fracture resistance; and differential expression of type I collagen, matrix metalloproteinase (MMP)-14 and MMP-2, and tissue inhibitor of metalloproteinase-2 (TIMP-2) in dentin between the groups. The authors conclude that increased TIMP-2/MMP expression ratio in MTA-treated teeth was responsible for the better long-term fracture resistance in MTA-treated teeth.

I read the article with interest. Unfortunately, the findings regarding the TIMP-2 and MMP protein expression in samples cannot be trusted due to several significant problems related to the methodology and interpretation of the finding.

The most apparent error is the clear discrepancy between the methods and the results. In the Material and Methods, the authors describe the use of 3,3′-diaminobenzidine tetrahydrochloride and hematoxylin counterstaining in the detection of proteins in immunohistochemical stainings, in the Results the authors present immunofluorescence images. An even more disturbing flaw in the methodology is that the authors do not present any negative control stainings (leaving out the primary antibody to confirm that the observed staining does not represent non-specific staining). Negative controls are not even mentioned in the Material and Methods or in the Results, indicating that negative control sections were not included at all. This leaves the specificity of staining unproved.

It is unfortunate to note that apparently the authors' knowledge of MMPs is not up to the level required for reliable analysis of the presence and function of these proteolytic enzymes and their inhibitors. In the Introduction, the authors write 'Matrix metalloproteinase (MMP)-2, 14 and membrane-type 1 (MT1) are found to play an important role during the degradation of collagen matrix on dentin', and they base this claim on three basic research articles (2–4). First, MT1-MMP is another name for MMP-14, given originally to describe the protein location in cell membrane. Secondly, none of the references given deals with dentin. Even if the authors later refer to some of the basic articles describing the presence and function of MMPs in dentin, the careless use of enzyme acronyms and references does not improve the credibility of the article.

Based on the immunofluorescence (or immunohistochemical staining?) the authors state that type I collagen, MMP-14 and MMP-2 were observed in all the samples. In the immunofluorescence image, there are marked differences in type I collagen immunofluorescence between the groups, with MTA showing weakest staining. The authors fail, however, to discuss the finding. As it is difficult to believe that MTA would have destroyed type I collagen in mineralized dentin, the problems related to the handling of the samples, staining protocol, specificity of antibodies used, and/or non-specific staining in one, two or all of the samples are more plausible explanations.

The authors also claim that with MTA, reduced expression was observed with MMP-2 staining. As type I collagen staining is clearly unreliable, MMP-2 staining results cannot be trusted either. The possibility of false-positive finding becomes even more apparent when the immunofluorescence is compared with the results of Boushell et al. (5), in which consistent but extremely low staining intensity for MMP-2 was observed in human coronal dentin. MMP-14 expression has been demonstrated in porcine (6) and human (7) odontoblasts, but to the best of my knowledge never in dentin. As MMP-14 (MT1-MMP) is mostly expressed as membrane-anchored MMP [reviewed in Itoh and Seiki (8)], it is perhaps possible that MT1-MMP could be present in the odontoblast cell membrane. However, as the staining results with other antibodies used in this study are not reliable, the true presence of MT1-MMP in mineralized dentin cannot be concluded from the present results.

Perhaps the most disturbing conclusion is that the increased TIMP-2 expression in MTA-treated teeth, resulting in inhibition of MMP-2 and -14, is at least partially responsible for the absence of decrease in fracture resistance in MTA-treated teeth. For any protein expression to be induced, cells need to be present to synthesize the protein. In this study, pulp tissue was removed and teeth were stored in saline at 4°C for 2 weeks after the treatments. Therefore, the increase in TIMP-2 expression is virtually impossible, as there were no living cells left in the root canals. The authors' claim in the Discussion that MTA-induced expression of TIMP-2 would be caused by 'MTA stimulated release of production of interleukin and cytokines' is, therefore, also unjustified. Finally, the authors' claim that using MTA as root canal filling material would prevent the teeth from becoming brittle over time – due to inhibition of MMPs via increased TIMP expression in pulpless teeth! – is both absurd and impossible.

The presence and role of different MMPs in dentin has recently been under increasing interest. Dentin MMPs have been suggested to be involved in caries progression (9, 10), degradation of hybrid layer under composite fillings (11–13) and in pulpal and periapical inflammation (14–16). The physiological role(s) of dentin-embedded MMPs remain unknown, but they have been speculated to be involved, e.g. in peritubular dentin formation, mineralized dentin maturation and liberation of growth factors from dentin during wear or caries (17, 18). The role and importance of dentin MMPs in time-related changes in fracture resistance is an interesting topic. Unfortunately, the study by Hatibović-Kofman et al. (1) does not provide any reliable evidence that would allow conclusions to be drawn in this matter.

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