

Study of reparative dentin formation after pulp capping with Mineral Trioxide Aggregate in the transgenic mouse *Msx1* +/-.

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Introduction

Pulpo-dentinal healing requires the recruitment of cells and their differentiation into odontoblasts. To date, the origin of the recruited cells, the process of their differentiation and the molecular reactions involved have not been clearly elucidated. Precise characterisation of the cells at the origin of the differentiation would make it possible a) to define their origin, and b) to elucidate their specific behaviour in the phenomena of repair, and thus to consider suitable therapies.

Proposals for the origin of stem cells include: a local origin (stem cells present in the pulpal mesenchym, pericytes), or remote origin (bone marrow stem cells). The presence of pluripotent cells has been established in dental pulp (Dental Pulp Stem Cells DPSCs)¹ but their eventual implication in the process of repair has not yet been shown. Stem cells are delicate to highlight *in vivo* because there is no reliable means of characterising them. The *Msx1* homeobox gene is one of the transcription factors expressed by the pluripotent cells from the first brachial arch during their development², and confers a high level of phenotypic plasticity on the cells. It seems to characterise the early stages of cellular differentiation. Thus, *Msx1* could be used as a molecular marker of the early stages of cellular recruitment in the healing process.

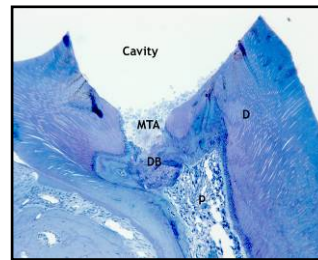
Material and method

45 wild type mice and 25 transgenic mice *Msx1* +/- were used. A standard cavity was drilled on the first upper molar until the pulp was exposed, and the pulp capping was set with white Pro Root MTA[®]. Finally, the cavity was filled with a light cured bonded resin. The animals were sacrificed at 1, 2, 3 and 4 days, 1, 2, 3 4, 5 and 11 weeks with an intra-cardiac perfusion of 4% paraformaldehyde in PBS. The *Msx1* +/- samples were secondarily incubated overnight in XGal solution at 37°C

After decalcification and embedding in paraffin, the samples were cut into 7µ slices. Routine histology was performed.

Results

Formation of the dentin bridge was systematically obtained and presented 3 layers: a first in direct contact with material presenting an increased affinity for the dyes and which seemed to



Histology at 11 weeks post-operative after pulp capping with ProRoot MTA[®] (MTA). The dentin bridge (DB) is in perfect continuity with the dentin wall. Staining: methyl blue/ blue Azur II. (P: pulp, D: Dentin)

correspond to a structural modification of the extracellular matrix; a second layer made of fibrodentin; and a third formed with predentin bordered by cells in full synthesis phase. On all the transgenic samples, we systematically noted the absence of β -Galactosidase revelation (β -Gal) in the pulp.

Discussion:

The mouse as laboratory model

The results obtained reveal the chronological formation of the dentin bridge. Unlike the dentin bridge described after capping with calcium hydroxide, no necrotic layer was observed, nor was any persistent inflammatory zone. The dentin bridge was in perfect continuity with the predentin of the side walls of the cavity. The hiatus often described with the calcium hydroxide between the bridge and the dentinal wall was not seen.

Msx1 Expression

No expression of *Msx1* was highlighted by the histo-enzymology. Two explanations can be given for these negative results (1) *Msx1* is not expressed during the process of pulp healing or (2) a technical problem prevented the revelation of protein. The marking of the buccal bone area of the jawbone proved that the X-Gal staining was effective, excluding a false negative result. The immuno-histochemistry using an anti-LacZ antibody could be used for a more reliable detection of the β -Gal reporter protein.

References: ¹Gronthos, S., J. Brahimi, et al. (2002). "Stem cell properties of human dental pulp stem cells." *J Dent Res* **81**(8): 531-5. ² Houzelstein D, C., A Buckingham, ME Robert, B (1997). "Insertional mutation of the mouse *Msx1* homeobox gene by an *nlacZ* reporter gene." *Mech Dev* **65**: 123-133.

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