

EXTRACELLULAR MOLECULES STIMULATE THE FORMATION OF REPARATIVE DENTIN IN EXPERIMENTALLY EXPOSED ADULT DENTAL PULP OF RATS AND MICE.

[Michel Goldberg^{\(1\)}](#), [Sally Lacerda-Pinheiro^{\(2\)}](#), [Ngampis Six^{\(1\)}](#), [Nadege Jegat^{\(1\)}](#), [Dominique Septier^{\(1\)}](#), [Mireille Bonnefoix^{\(1\)}](#), [Arthur Veis^{\(3\)}](#), [Pamela DenBesten^{\(4\)}](#), [Anne Poliard^{\(2\)}](#).

(1) Faculté de Chirurgie Dentaire EA 2496, Université Paris-Descartes 1 rue Maurice Arnoux 92120 Montrouge France (2) Laboratoire de Différenciation, Cellules Souches et Prions, CNRS-FRE2937 7 rue Guy Môquet 94801 Villejuif Cedex-France (3) Cell & Molecular Biology, Northwestern, Chicago, IL, USA, (4) Department of Orofacial Sciences, University of California at San Francisco, San Francisco, CA, USA.

Pulp exposure may occur during the preparation of deep cavities or be the consequence of carious decay. For decades the only biological option have been to heal the pulp by forming a reparative dentinal bridge using calcium hydroxide. Over the past few years, bioactive molecules have been experimentally implanted, and variable results were obtained (Goldberg & Smith, *Crit Rev Oral Biol Med* 2004). We have developed an experimental model using the first maxillary molar in the rat, first, drilling a cavity after gingival electrosurgery, and then pushing the deeper part of the cavity with a steel probe to expose the pulp. We also used a surgical approach for the incisor, allowing implantation of bioactive molecules in the forming zone of the mandibular incisor in rodents. Agarose beads soaked in a culture medium supplemented with the bioactive molecule have been implanted for 8 to 90 days in the molar, and for 10-20 days in the incisor.

In separate experiments, we implanted in the rat molar structural extracellular molecules, which may be also matricellular proteins, including Bone Sialoprotein (BSP), or signaling molecules such as leucine-rich amelogenin peptides (LRAP) produced by ameloblasts, or the low molecular spliced amelogenins that are synthesized by odontoblasts (A+/-4). These procedures induced a slight inflammation at day 8, and stimulated the recruitment of cells involved in the reparative process. After 15 days, reparative dentin started to be formed in the exposure area, appearing as a dentinal bridge or a diffuse mineralization in the coronal pulp. In addition, with A-4, the root canals were totally occluded by reparative dentin. The recruitment of reparative pulp cells may involve 1- either dormant (or latent) adult committed odonto/osteoblast progenitors, or 2- cells issued from the transdifferentiation of pulp fibroblasts; or 3- selected lineage(s) derived from inflammatory cells that de-differentiate and re-differentiate into odontoblast-like progenitors. As shown by PCNA staining, at day 8 labeled cells were seen in the central part of the coronal pulp, near the agarose beads acting as carrier, and at the periphery of the root pulp beneath the Höehl's (sub-odontoblastic)

layer. At day 15, proliferation was reduced in the coronal pulp and disappeared in the root. We used RP59, a marker for osteoblast progenitors, and positively labeled cells appeared near and around the carrier beads. Osteopontin (OPN)- positive cells increased in number between 1-3 and 8 days. At day 8, cells located around the beads were strongly OPN positive, whereas they were negative for dentin sialoprotein (DSP) staining, suggesting an early differentiation along the osteoblastic lineage. At day 15, a few cells located near the pulp exposure site were DSP positive, whereas the cells in close contact or around the carrier beads formed a ring immunostained for OPN. Mineralization, which started at day 15, was achieved at day 30, and did not vary much at day 90. The reaction created by Dentonin, a peptide from MEPE, differed from other biomolecules in that though cell recruitment was stimulated, with proliferation and commitments toward early stages of differentiation. The process was then apparently stopped at that point. Terminal mineralization was heterogeneous, some teeth forming reparative dentin whereas in other pulps, there was no evidence for such formation (Six et al. *J Dent Res*, 2007). In the rodent incisor, implantation of A+/-4 revealed at day 10 the formation of bone-like dendritic structures in the central part of the pulp, which increased in size and developed into a diffuse mineralization. The pulp exposure site where beads were implanted was filled firstly by osteodentin and later, with thickening in some areas by reactionary orthodentin.

Altogether, these experimental approaches allow a better understanding of the cascade of events occurring between cell commitment and the terminal differentiation. It also allows screening of the individual effects of bioactive molecules. The specificities of each molecule lead to their reclassification as matricellular, structural and bioactive molecules (growth factors), although most structural proteins appear to be involved as signaling and bioactive molecule.

