Clin Oral Invest (2008) 12:109–112
DOI 10.1007/s00784-007-0172-6

REVIEW

Matricellular molecules and odontoblast progenitors as tools for dentin repair and regeneration

M. Goldberg · S. Lacerda-Pinheiro · F. Priam · N. Jegat · N. Six · M. Bonnefoix · D. Septier · C. Chaussain-Miller · A. Veis · P. Denbesten · A. Poliard

Received: 12 September 2007 / Accepted: 30 November 2007 / Published online: 22 December 2007

Abstract This review summarizes the in vivo experiments carried out by our group after implantation of bioactive molecules (matricellular molecules) into the exposed pulp of the first maxillary molar of the rat or the mandibular incisor of rats and mice. We describe the cascade of recruitment, proliferation and terminal differentiation of cells involved in the formation of reparative dentin. Cloned immortalized odontoblast progenitors were also implanted in the incisors and in vitro studies aimed at revealing the signaling pathways leading from undifferentiated progenitors to fully differentiated polarized cells. Together, these experimental approaches pave the way for controlled dentin regenerative processes and repair.

Keywords Dentin · Pulp · Matricellular molecules · Odontoblast progenitors · Regeneration

M. Goldberg (✉) · S. Lacerda-Pinheiro · F. Priam · N. Jegat · N. Six · M. Bonnefoix · D. Septier · C. Chaussain-Miller
Laboratoire de Réparation et de Remodelages Oro-Faciaux (EA 2496), Université Paris Descartes,
92120 Montrouge, France
e-mail: mgoldod@aol.com

S. Lacerda-Pinheiro · A. Poliard
Laboratoire de Différentiation Cellulaire et Prions-UPR1983 CNRS,
Villejuif, France

A. Veis
Cell & Molecular Biology,
Northwestern, Chicago, USA

P. Denbesten
Department of Orofacial Sciences,
University of California at San Francisco,
San Francisco, USA

Introduction

Tissue engineering involves three partners: (1) specialized cells producing a specific tissue, (2) extracellular matrix (ECM) molecules that display bioactive properties, and (3) scaffolds used as carriers, which also bear some biological properties. Cells are producing complex ECM, with functional redundancies and self-aggregating properties. ECM or some specific domains of the molecules act as intracellular signaling molecules and therefore are matricellular proteins. They stimulate the recruitment, proliferation and differentiation of regenerative cells. For some years, we have investigated this cascade of events leading to reparative dentin formation using pulp capping as a short-term model [3, 4, 6] with the aim of dentin regeneration, even in an ectopic situation.

In vivo models

The rat first maxillary molar

The presence of a large diastema between the incisor and the three molars provides good access to the mesial aspect of the first maxillary molar. After gingival electrosurgery, a half-moon cavity is prepared by drilling with a tungsten round burr in less than 2 s. Pulp exposure is obtained by pushing with a steel probe the deepest part of the cavity. The resulting pulp exposure allows the insertion of agarose beads loaded with bioactive molecules into the pulp. The pulp is protected from bacterial contamination with a glass ionomer cement and the rats killed after 1 to 90 days [1, 14].

The rat and mouse mandibular incisor

We used also another model. After a surgical approach that removes first the skin and muscles, we expose the outer

cortical bone of the mandible. A hole is drilled with a burr through the bone and the outer dentin/cement lateral surface of the incisor. Agarose beads loaded with ECM molecules or cells are implanted into the pulp. This model can be used for shorter-term experiments up to 20 days.

These two in vivo models allow to study the effects of bioactive molecules or cell implantation either in the coronal part of the molar or in the forming part of the incisor.

Effects of ECM molecules

Bone sialoprotein

It is well documented that (1)-collagen pellets loaded with Bone SialoProtein (BSP) implanted in the calvaria after the preparation of a minimal critical-size defect that cannot heal spontaneously recruit osteoblast progenitors that proliferate and differentiate into cells that contribute to the healing of the defect. (2) BSP induces in vitro the nucleation and growth of crystallites. (3) BSP has been shown to enhance collagen fibrillation [5]. One week after implantation of BSP in the pulp, an inflammatory reaction was seen which was resolved at 15 days. Two weeks after implantation in the first maxillary molars, reparative dentin starts to form around the dentin fragments pushed into the pulp during the exposure step. A homogeneous reparative dentin filled the mesial pulp chamber after 1 month. The reparative dentin formed was atubular [1].

Bone morphogenetic protein 7-OP-1

Investigations on the potential role of Bone Morphogenetic Protein 7 (BMP7), also called Osteogenic protein-1 (OP-1) [7, 8, 11–13] were carried out to analyze the effects of OP-1 in the rat molar model compared with BSP. In the same experimental conditions, in the crown part of the pulp, a porous reparative dentin of the osteodentin type was produced with major fissures due to pulp remnants. In contrast, the lumen of the pulp located in the root was totally closed by a homogeneous reparative orthodentin. This pointed to the physio-pathologic differences between the root and the crown pulps [4–6, 15, 16].

Dentonin

The central portion of MEPE (residues 242–264) is a domain that includes an integrin-binding triplet (RGD sequence—residues 247–249), a glycosaminoglycan attachment (SGDG—residues 256–259) and a putative calcium-binding motif. This domain from MEPE, known as dentonin or AC-100, was implanted into the pulp. Dentonin stimulates the recruitment, proliferation and differentiation of reparative cells. The initial formation of a mineralized phase was accelerated, and as early as 8–15 days, dendritic bone-like structures started to form. However, afterward, the mineralization process was slower. This suggested that the initial phases of reparative dentin formation were stimulated by the peptide but not the terminal step of reparative dentin formation [19].

Small-molecular-weight amelogenins: A+4, A−4, LRAP+ exons 8 and 9

Amelogenins are the major components of the forming enamel. For years, the general belief was that they were specific molecules, implicated in enamel formation, characteristic of the ameloblast phenotype. The discovery that odontoblasts are also implicated in the synthesis of some low molecular spliced forms of amelogenins [20] opens new insights on the signaling functions of these molecules. In addition, many other cells have been now recognized to synthesize this group of molecules [2].

Seven exons are implicated in the synthesis of the whole length amelogenins. The last 8 and 9 exons are considered as non-coding, except in rodents. One amelogenin spliced product is expressed by exons 2, 3, 4, 5, 6d, 7 ([A+4], 8.1 kDa, 73aa), and the other by exons 2, 3, 5, 6d, 7 ([A−4], 6.9 kDa, 59aa). The peptide sequence of A−4 is identical to that of the Leucine-Rich Amelogenin Peptide (LRAP), but some differences in the final folding of the molecule cannot be excluded. Although differing only by the 14 amino acid sequence encoded by exon 4, the two peptides display different effects on cultured fibroblast. [A+4] induces the rapid expression of transcription factor Sox9, whereas on a different time course, [A−4] elevates the expression of transcription factor Runx2 [20, 21].

We have shown that the implantation of A+4 or A−4 into the pulp stimulates the recruitment of pulp cells that may be osteo/odontoblast progenitors. They further proliferate, as shown by PCNA labeling at day 3 after implantation. The first row of cells located at the surface of the beads does not display any PCNA signal, whereas the second row of cells is PCNA-positive. We suggest that the cells that are recruited in the central part of the pulp migrate and come in close contact with the beads where they undergo differentiation, as shown by anti-osteopontin (OPN) immunolabeling (undetectable before day 7). Using dentin sialoprotein (DSP) antibody after 15 days, none of the cells in contact with the beads were labeled, but at the surface of the pulp, beneath the exposure site, some cells were positively labeled [17, 18]. Two diverging hypotheses can be proposed: (1) either two groups of cells are recruited simultaneously, osteoblast-like progenitors that are located in the central part of the coronal

pulp and odontoblast-like cells differentiating at the periphery near the pulp exposure, or (2) cells of the osteoblast-like phenotype differentiate firstly near the beads and during the second step of the cascade, they further transdifferentiate into odontoblast-like cells. These two alternative hypotheses are currently under study.

Implantation of LRAP (see above explanation of the acronym) stimulates cell proliferation after 8 days, but the reaction is weaker in the presence of the amino acids expressed by the non-coding exons 8 and 9. Proliferation is strongly decreasing in day 15 molars. Between days 1 and 8, there is a transitory expression of RP59, which has been shown to be expressed by differentiating mesenchymal progenitors. Afterward the labeling decreases. Using LRAP instead of A +/−4, DSP labeling is weak or nil at any of the time periods studied in vivo. OPN labeling increases between days 8 and 15. Its comes out from these preliminary results that in the rat model, the non-coding 8 and 9 amelogenin exons regulates or even represses the formation of reparative dentin.

Immortalized progenitors

Clones

Immortalized pulp cell colonies were obtained from tooth germs of day 18-mouse embryos transgenic for an adenovirus SV40 recombinant plasmid [9]. All the cells isolated from these mice express the large T antigen of SV40. From three initial populations that express DSP, type I collagen and nestin, a cytoskeletal marker of neural-crest-derived cells, sixty clones were obtained and characterized by immunocytochemical and RT PCR analyses. Five of these clones were further analyzed. They express nestin, RP59, Pax9, Msx 1 and 2, Dlx 2 and 9, Lhx6 and 7, DSP, DPP, DMP-1, amelogenin, enamelysin (MMP-20) and alkaline phosphatase, and therefore display the characteristics expected for odontoblast progenitors [9, 10].

Terminal differentiation

When the clones of odontoblast progenitors were cultured together with pure hydroxyapatite blocks or day 18 embryonic mandibular incisors, the cells did not undergo terminal differentiation. In contrast, when some of our clones were cultured with day 19 embryonic incisors or teeth dissected from newborns, odontoblast progenitors differentiate into cells that form long processes at right angles to the tooth surface. This did not occur with mesenchymal progenitors embryonic cells or cells derived from adult bone marrow. This terminal differentiation was observed only on the labial part of the incisor where enamel starts to form. Cell bodies were grouped in a palisade-like appearance. Therefore the proteins of the ECM present in the forming enamel appear as good candidates in the regulation of this process. To test this hypothesis, the embryonic incisor was treated with sodium hypochlorite for 15 min in order to remove the proteins present in the tooth. After this treatment, the tooth keeps the capacity to promote the terminal differentiation of odontoblast progenitor. Western blot analysis of the deproteinized incisors showed that some remnants, or fragmented molecules, of amelogenin and osteocalcin were still present, whereas all the other ECM molecules (DSP, DMP1, BSP and OPN) were undetectable after the deproteinization procedure. Therefore amelogenin and osteocalcin, or some derived peptides, are candidates for inducing the final differentiation.

Ectopic implantation

In the above model, the cells differentiate in front of a forming enamel layer instead of being located at the surface of the pulp beneath the dentin/predentin. Despite this ectopic location, we have observed that some mineral nodules formed between the processes, merging to create a dentin-like layer. Therefore, our project is not restricted to reparative dentin formation but expands to dentin generation or regeneration.

Cell implantation in the incisor

Finally, we have also investigated the usefulness of implanting odontoblast progenitor cells in the pulp to achieve reparative dentin formation. The cells were implanted in the forming part of the incisor as described above for the matrix molecules and the mice were euthanized 10 and 20 days later.

Four clones were implanted into the pulp. The two clones, 17IA4 and 705IH8, induced the formation of dendritic bone-like structures of the osteodentin type, whereas 705IC5, and surprisingly C1, an embryonic mesenchymal precursor, induced the formation of orthodentin (tubular dentin), appearing mostly as a thickening of the dentin walls.

The understanding of phenomena leading to dentin regeneration is growing, at least using experimental models and either matricellular proteins or progenitor cells. The next step will be to evaluate the effects of these new technologies in the clinical situation.

Acknowledgements These investigations were carried out with the support of a CNRS/INSERM grant (Ingénierie tissulaire 2000), INSERM grant Cellules souches adultes 2003 and Fondation de l'Avenir ETS-407). This article is dedicated to more than 25 years of friendship between the first author and Prof. Dr. Gottfried Schmalz.

References

1. Decup F, Six N, Palmier B, Buch D, Lasfargues J-J, Salih E, Goldberg M (2000) Bone sialoprotein-induced reparative dentinogenesis in the pulp of rat's molar. Clin Oral Investig 4:110–119
2. Deutch D, Haze-Filderman A, Blumenfeld A, Dafni L, Leiser Y, Shay B, Gruenbaum-Cohen Y, Rosenfeld E, Fermon E, Zimmermann B, Haegewald S, Bernimoulin J-P, Taylor AL (2006) Amelogenin, a major structural protein in mineralizing enamel, is also expressed in soft tissues: brain and cells of the hematopoietic system. Eur J Oral Sci 114(Suppl 1):189–189
3. Goldberg M, Six N, Decup F, Buch D, Soheili Majd E, Lasfargues J-J, Salih E, Stanislawski L (2001) Application of bioactive molecules in pulp capping situations. Adv Dent Research 15:91–95
4. Goldberg M, Six N, Decup F, Lasfargues J-J, Salih E, Tompkins K, Veis A (2003) Bioactive molecules and the future of pulp therapy. Am J Dent 16:66–76
5. Goldberg M, Smith A (2004) Cells and extracellular matrices of dentin and pulp. A biological basis for repair and tissue engineering. Crit Rev Oral Biol Med 15:13–27
6. Goldberg M, Lacerda-Pinheiro S, Jegat N, Six N, Septier D, Priam F, Bonnefoix M, Tompkins K, Chardin H, Denbesten P, Veis A, Poliard A (2006) The impact of bioactive molecules to stimulate tooth repair and regeneration as part of restorative dentistry. Dent Clin North Am—(Special Issue on Tissue Engineering) 50:277–298
7. Nakashima M (1990) The induction of reparative dentine in the amputated dental pulp of the dog by bone morphogenetic protein. Arch Oral Biol 35:493–497
8. Nakashima M (1994) Induction of dentine in amputated pulp of dogs by recombinant human bone morphogenetic proteins-2 and -4 with collagen matrix. Arch Oral Biol 39:1085–1089
9. Priam F, Ronco V, Locker M, Bourd K, Bonnefoix M, Duchêne T, Bitard J, Wurtz T, Kellermann O, Goldberg M, Poliard A (2005) New cellular models for tracking the odontoblast phenotype. Arch Oral Biol 50:271–277
10. Priam F, Lacerda S, Bonnefoix M, Veis A, Goldberg M, Poliard A (2008) The phenotype of immortalized odontoblast progenitors. Adv Dent Res in press
11. Rutherford B, Fitzgerald M (1995) A new biological approach to vital pulp therapy. Crit Rev Oral Biol Med 6:218–229
12. Rutherford RB, Wahle J, Tucker M, Rueger D, Charette M (1993) Induction of reparative dentine formation in monkey by recombinant human osteogenic protein-1. Arch Oral Biol 38:571–576
13. Rutherford RB, Spängberg L, Tucker M, Rueger D, Charette M (1994) The time-course of the induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. Arch Oral Biol 39:833–838
14. Six N, Lasfargues J-J, Goldberg M (2000) In vivo study of the pulp reaction to Fuji IX, a glass ionomer cement. J Dent 28:413–422
15. Six N, Decup F, Lasfargues J-J, Salih E, Goldberg M (2002) Osteogenic proteins (bone sialoprotéin and bone morphogenetic protein-7) and dental pulp mineralization. J Material Sciences-Material in Medicine 13:225–232
16. Six N, Lasfargues J-J, Goldberg M (2002) Recombinant human bone morphogenetic protein-7 (osteogenic protein-1) induces differential repair responses in the coronal and radicular areas of the exposed rat molar pulp. Arch Oral Biol 47:177–187
17. Six N, Tompkins K, Lasfargues J-J, Veis A, Goldberg M (2002) Bioengineered reparative dentin and pulp mineralization. Proceedings Dentin–Pulp Complex. Chiba 2001, Tokyo, Quintessence pp 52–59
18. Six N, Tompkins K, Septier D, Veis A, Goldberg M (2004) Recruitment and characterization of the cells involved in reparative dentin formation in the exposed rat molar pulp after implantation of amelogenin gene splice products A+4 and A−4. Oral Biosciences & Medicine 1:35–44
19. Six N, Septier D, Chaussain-Miller C, Blacher R, DenBesten P, Goldberg M (2007) Dentonin, a MEPE fragment, initiates pulp healing response to injury. J Dent Res 86:780–785
20. Veis A, Tompkins K, Alvares K, Wei K, Wang L, Wang XS (2000) Specific amelogenin gene splice products have signaling effects on cells in culture and in implants in vivo. J Biol Chem 275:41263–41272
21. Veis A, Tompkins K, Goldberg M (2002) Amelogenin peptides have unique milieu-dependent roles in morphogenic path determination. In: Hascall VC, Kuettner KE (eds) The many faces of osteoarthritis. Birkhäuser, Basel, Switzerland, pp 101–105