P43-IMPLANTATION OF ODONTOBLAST PROGENITORS IN THE RAT MOLAR PULP LEADS TO THE FORMATION OF REPARATIVE OSTEODENTIN.

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Key words

Dental pulp stem cells, reparative dentin formation, tissue engineering.

Introduction

The presence of odontogenic stem cells in the human dental pulp has been first reported by gronthos and co-workers in 2000 (1). Stem cell based therapies may provide treatments for tooth injury since ectopic implantation of these cells have been shown to induce the formation of a dentin-like mineralized tissue (1,2). Up to now, their *in vivo* capacity to readily contribute to the formation of reparative dentin after implantation in a pulp exposure has never been directly explored. The primary aim of our study was to evaluate the feasibility of a “cellular therapy” to heal a pulp injury. A preliminary study carried out on the continuously growing mouse incisor paves the way to such treatments (3). To study the effect of stem cell implantation into a tooth of limited growth, after a surgical exposure we introduce pulpal progenitors into the pulp of a rat first maxillary molar. Afterward, the teeth were demineralized and processed for histological observations.

On a trial basis, alginate beads were used as carriers allowing to manipulate easily the cell implant and visualize the implantation site on histological sections. This biomaterial is suitable since it is biocompatible, probably bioactive and ultimately biodegradable. Our secondary aim was to assess the benefit of this scaffold.

Material and Methods

The clone A4 was established from first molar dental pulp cultures of transgenic embryos (ED18)(4). This cell line displays progenitor properties *in vitro*. The A4 clone was used to investigate *in vivo* the potential effects of a cell therapy. Using an institutionally approved research protocol, the rats were anaesthetized, the gingival papilla covering the cervical zone of the mesial aspect of the first maxillary molar of Sprague-Dawley rats was removed by electro-surgery and a cavity was drilled near the enamel-cementum junction in the tooth mesial aspect (5). A pulp exposure was obtained by pushing the deepest part of the cavity with a steel probe. In the first experimental group a cell pellet of 10^5 A4 cells were implanted in the tooth (group 1). The second experimental group received an alginate bead (1mm diameter) loaded with 3.10^3 A4 cells (group 2) whereas the alginate control group was implanted with an alginate bead alone (group 3). The sham group was surgically treated, but without any further implantation (group 4). Then, to avoid bacteria contamination, the cavities were filled with a glass-ionomer cement (Fuji IX, GC). Animals were anesthetized and killed 2 days, 2 or 4 weeks after implantation. Block sections including the three molars were dissected out, demineralized in EDTA and treated by conventional histological staining procedures (Masson’s trichrome).

Results

Neodentin formation after implantation of pulpal progenitors into a rat molar

Two days after the surgery, a discrete inflammation was observed in the pulp of the sham group, both in the mesial and central parts of the pulp chamber, whereas the distal coronal pulp was unaffected (fig.1A). In the cell-implanted molars (group 1), inflammatory cells were recruited near the implantation site, and the mesial pulpal tissue started to become fibrous (fig.2A). After 2 weeks, in the sham group, reorganization of the pulpal tissue and the onset of a diffuse mineralization process can be visualized in the mesial part of the pulp chamber. In the group 1, an osteodentin-like matrix, i.e. an atubular mineralized tissue with some cell inclusions, began to fill the mesial part of the pulp chamber. Moreover, a dentin barrier was formed in the isthmus separating the mesial part of the pulp chamber from the central coronal pulp. After 4 weeks, in the sham group (fig.1B), a diffuse mineralization was observed within the mesial pulp chamber. A dentin barrier was formed between the mesial and the central parts of the pulp chambers. In the group 1 (fig.2B), the mesial pulp chamber was massively filled by osteodentin, a structure appearing to be fully mineralized. The distal and central residual pulps were unmodified, beneath a robust dentin barrier.

Alginate beads as a potential carrier for cell implantation

After 4 weeks, rat molars implanted with an alginate bead alone (group 3) showed the same results as the sham group (not shown). The implantation of A4 cells with an alginate bead (group 2) induced in 4 weeks the formation of a mineralized tissue comparable to the experimental group without carrier (group 1). An osteodentin matrix filled the mesial chamber and a dentin plug protected the residual pulp located in the central and distal pulpal chambers (not shown).

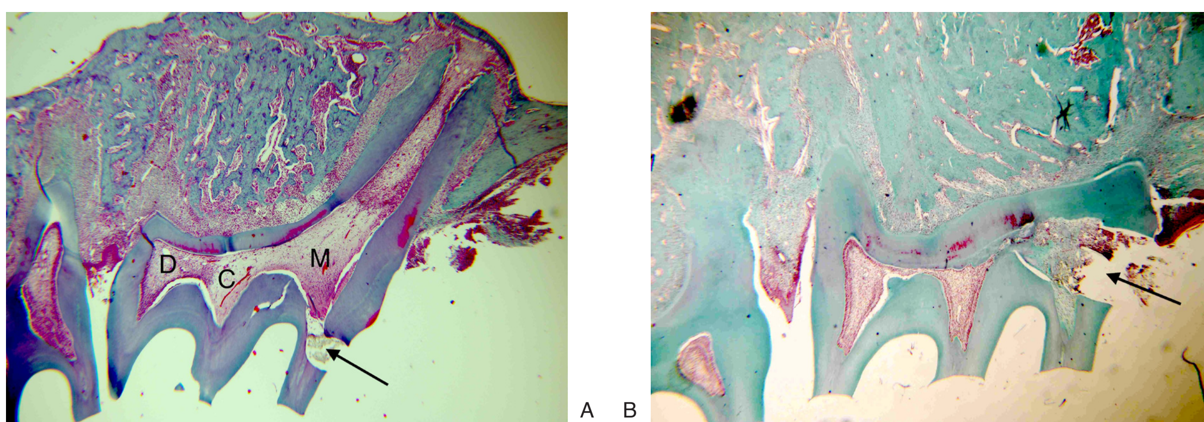

Figure 1: Sham group. Perforation of the rat molar pulp provokes a fibrosis of the mesial pulp tissue (A) 2 days and (B) 28 days after implantation. (M: Mesial chamber, C: Central chamber, D: Distal chamber, black arrow: access cavity)

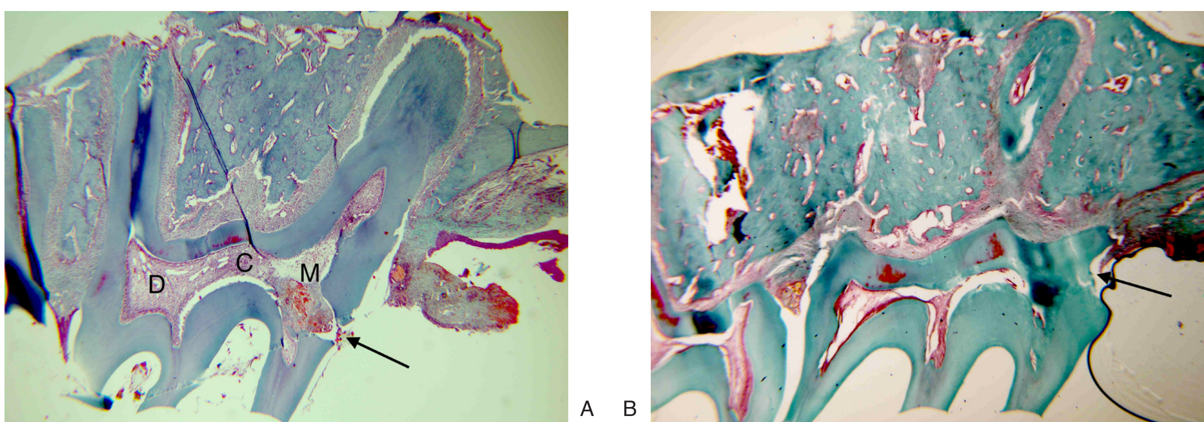

Figure 2: Group 1. Implantation of pulpal progenitors into the rat molar pulp induces the formation of an osteodentin-like mineralized matrix filling the mesial pulp chamber (A) 2 days and (B) 28 days after implantation. (M: Mesial chamber, C: Central chamber, D: Distal chamber, black arrow: access cavity)

Discussion

Our results show that between 2 and 4 weeks, the implantation of pulpal progenitors promotes the formation of an osteodentin-like structure that totally fills the mesial pulp chamber. A robust dentin barrier protects the residual pulp. Pulp vitality is kept after cell implantation in the pulp, the inflammatory process was resolved at 4 weeks in all groups. Interestingly, implantation of mouse cells into a rat's living tissue may not elicit immune responses. To answer our primary question, this investigation provides evidence that pulp progenitors may contribute to heal a wounded pulp, even if the mechanisms that are involved in neodentin formation still need clarifications. To answer our secondary question, this preliminary trial using cell-loaded alginate beads suggests that this carrier is a suitable tool for cell implantation since it could permit to reduce the number of implanted cells leading to efficient dentin formation.

Conclusions

In the near future, we will focus on (i) determining whether the implanted pulpal stem cells are directly involved in the formation of the reparative dentin or whether they provide biomolecules/microenvironment favoring the recruitment and differentiation of host progenitors; (ii) unravelling the putative role of the initial inflammation in the osteodentin formation; (iii) improving the implantation protocol by defining the number of cells and the signals necessary to promote efficient dentinal repair. Challenge in the long-term is to provide odontologists with new therapeutical strategies by using dental pulp stem cells.

Acknowledgements

The authors acknowledge the *Institut Français pour la Recherche Odontologique* (IFRO).

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