TISSUE REGENERATION

A pulpy story

Over-expression of a transcriptional factor, Alx3, has been shown to revitalize the regenerative capacity of adult progenitor cells to promote enhanced stromal vascularization and formation of parenchymal dental pulp tissue in vivo.

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Tissue engineering involves the use of a combination of cells, tissue scaffolds and biologically active factors to replace or regenerate organs for therapeutic purposes. The identification of endogenous stem cell populations in many organs, and the potential for generating tissues from induced pluripotent stem cells, have provided much impetus to this field. However, while tissue engineering approaches have been used successfully to regenerate organs such as skin and cartilage, regeneration of more complex tissues remains challenging and requires a better understanding of the factors that can promote integration of therapeutic cells into a three-dimensional scaffold and direct their differentiation into a functional organ. The tooth provides an accessible and clinically relevant system in which to test new approaches to tissue regeneration. Dental caries that infect dental pulp necessitate endodontic treatment, which currently involves insertion of artificial fillers into the root canal after pulp removal. Almost 20% of these treatments ultimately fail, due to secondary infections or tooth fractures, resulting in tooth loss[1].

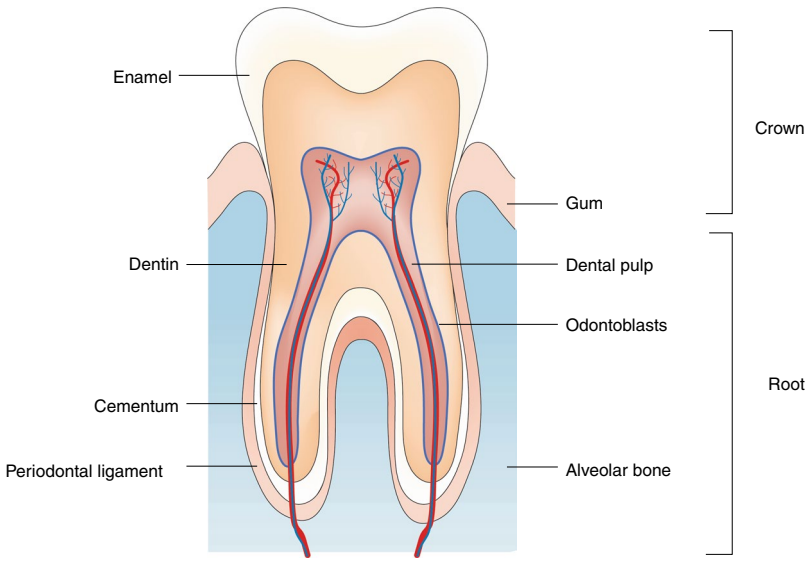

Fig. 1 | Diagram of a mature molar tooth. The schematic indicates the locations of mineralized enamel and dentin. Odontoblasts secrete dentin and line the outer edge of the dental pulp. Cementum is a calcified substance covering the root of the tooth. The cementum attaches the tooth to the alveolar bone by anchoring the periodontal ligament, which is composed of specialized connective tissue fibres. The crown and root regions of the tooth are indicated to the right of the diagram.

Writing in *Nature Materials*, Jeremy Mao and co-workers[2] describe novel approaches to promote regeneration of dentin, the mineralized substance underlying tooth enamel, following removal of dental pulp. They focus on a transcription factor, aristaless-like homeobox 3 (Alx3), a member of the homeobox family, and its interactions with the Wnt/β-catenin signalling pathway, a broadly utilized developmental pathway important for dental morphogenesis and repair. Dentin is produced by specialized mesenchymal cells termed odontoblasts (Fig. 1). Superficial damage to the dentin stimulates existing odontoblasts to secrete reactionary dentin to repair the injury. Depending on the severity and duration of injury, this type of dentin may have a tubular structure, similar to undamaged dentin, or may be atubular. More severe injury that exposes the dental pulp results in activation of mesenchymal stem cells in the pulp. These differentiate into odontoblast-like cells that secrete reparative dentin, which is more similar in structure to bone than to normal dentin. Complete removal of the pulp results in minimal repair of the dentin (Fig. 2). Activation of Wnt/β-catenin signalling is critical for embryonic tooth morphogenesis, formation of molar cusps and odontoblast development[3,4], while postnatal Wnt inhibition causes defective odontoblast maturation and dentin formation in vivo[5,6]. Wnt signalling is also active in dentin regeneration following injury to dentin and pulp, and activation of this pathway promotes differentiation of pulp cells to odontoblast-like cells that secrete reparative dentin[7–9].

Mao and colleagues demonstrate that Alx3 is expressed in dental mesenchyme during embryonic mouse development but disappears in adult molar teeth, suggesting that it may play a specific role in dentinogenesis. They found that forced expression of Alx3 in mesenchymal cells caused them to express higher levels of several Wnt genes including Wnt3a, as well as dentin sialophosphoprotein (DSPP), which encodes a precursor protein required for mineralization. Conversely, knockdown of Alx3 resulted in decreased expression of Wnt genes and DSPP. To test whether Alx3 could promote adult tissue regeneration, the authors seeded decellularized human dentin slices with human dental pulp progenitor cells (DPSCs) and implanted these subcutaneously in immunocompromised mice. They found that control DPSCs produced little interstitial tissue or mineralized dentin. By contrast, Alx3-expressing DPSCs regenerated vascularized pulp-like tissue, odontoblast-like cells, and a dentin-like matrix. Mechanistically, they

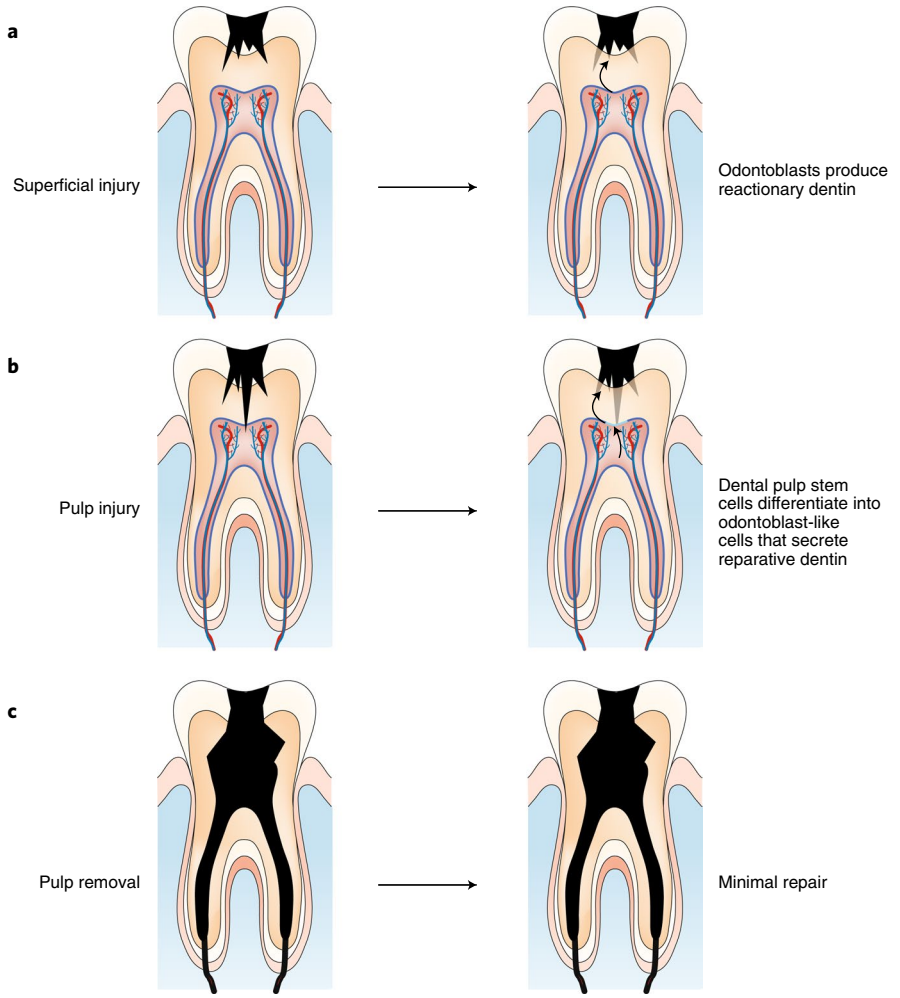

Fig. 2 | Mechanisms of dentin repair. a, Superficial dentin injury stimulates odontoblasts to secrete reactionary dentin. **b**, Injury that exposes the dental pulp causes mesenchymal stem cells in the pulp to differentiate into odontoblast-like cells that secrete reparative dentin. **c**, Complete removal of the pulp results in minimal production of reparative dentin.

demonstrated that Alx3 promotes vascular endothelial growth factor (VEGF)-mediated vascularization as well as Wnt-mediated odontoblast-like differentiation and mineralization.

The authors utilized a porcine model to determine whether recombinant Wnt3a delivered in a collagen gel could promote regeneration following complete removal of the tooth pulp, a procedure that mimics root canal treatment. Modest formation of stroma was observed in teeth injected with control collagen gel, while delivery of recombinant bone morphogenetic protein-7 caused excessive mineralization resembling bone. Interestingly, delivery of Wnt3a resulted in formation of vascularized pulp-like tissue, mineralized tubular dentin-like structures, and neural filament-like structures.

Taken together, the authors' findings indicate that forced expression of the embryonic transcription factor Alx3 in adult cells can enhance their ability to promote regeneration of both dentin and stromal tissue. Alx3 appears to accomplish this feat in part by directly activating expression of Wnt3a, which promotes dentin formation and neural sprouting, as well as VEGF, which enhances vascularization of pulp tissue. The ability of recombinant Wnt3a to increase vascularization following pulp removal suggests that Wnt signalling may also have direct pro-vascular effects. Indeed, activation of Wnt signalling has been previously shown to promote vasculogenic differentiation of dental pulp stem cells in vitro[10]. These findings collectively demonstrate that a cell-based approach involving seeding of Alx3-expressing cells in a decellularized dentin scaffold, as well as delivery of recombinant Wnt3a following pulp removal, were both effective at promoting pulp and dentin formation in vivo. However, they also suggest that transplantation of stem cells may not be necessary for pulp regeneration. This finding is important, as adequate sources of matched healthy donor stem cells are not always available, and differentiation of patient-derived induced pluripotent stem cells to produce cells appropriate for transplantation is time consuming and expensive.

A remarkable aspect of the current study is the ability of Wnt3a to stimulate pulp and tubular dentin regeneration in vivo following complete removal of the dental pulp. It will be interesting in the future to determine the origin of the endogenous cells that respond to Wnt3a in this context. Another open question is the role of Alx3 in normal embryonic tooth development. While the authors nicely show that Alx3 can promote dentin formation in reconstituted embryonic teeth and in multiple in vitro and in vivo models, the effects of loss of Alx3 function in vivo remain to be determined. The authors have now generated Alx3 knockout mice using CRISPR/Cas9 gene-editing approaches; analysis of dental phenotypes in these mutants will allow this question to be resolved. The important findings described in this study pave the way for future work aimed at eventually replacing the use of artificial fillers in root canal procedures with approaches that involve transplantation of reparative stem cells and/or delivery of biologically active factors that can stimulate the regenerative activity of endogenous cells. ❐

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