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From understanding tooth development to bioengineering of teeth

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Remarkable breakthroughs in the fields of developmental biology and stem cell biology during the last 15 yr have led to a new level of understanding regarding how teeth develop and how stem cells can be programmed. As a result, the possibilities of growing new teeth and of tooth bioengineering have been explored. Currently, a great deal is known about how signaling molecules and genes regulate tooth development, and modern research using transgenic mouse models has demonstrated that it is possible to induce the formation of new teeth by tinkering with the signaling networks that govern early tooth development. A breakthrough in stem cell biology in 2006 opened up the possibility that a patient′s own cells can be programmed to develop into pluripotent stem cells and used for building new tissues and organs. At present, active research in numerous laboratories around the world addresses the question of how to program the stem and progenitor cells to develop into tooth-specific cell types. Taken together, the remarkable progress in developmental and stem cell biology is now feeding hopes of growing new teeth in the dental clinic in the not-too-distant future.

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The tooth is a typical example of an organ in which the ‘program’ of development is written in the genes while environmental factors play practically no role. Moreover, like all organs, teeth develop from different cell types. Teeth form from the epithelium of the oral surface and the underlying tissue, called mesenchyme (Fig. 1). Their development starts from an epithelial thickening, called the placode, and the underlying condensed mesenchyme. The epithelium forms a bud which grows and undergoes morphogenesis, determining the form of the future tooth crown. The production of dentine and enamel starts at the interface of epithelium and mesenchyme, and after completion of the tooth crown, roots develop (1). All instructions for building the tooth are already present in the cells before the epithelial budding starts. This was demonstrated experimentally in the 1960s: when the early tooth germ was dissected from a mouse embryo and was transplanted to an adult mouse in the anterior chamber of the eye, or under the skin or kidney capsule, it developed into a complete tooth (2). Using the same transplantation technique it was also shown that tooth development is regulated by communication between the epithelial and mesenchymal tissues, and that the separated tissues were not able to form tooth structures on their own. Today, the communication between tissues is regarded as the most important mechanism in regulating the development of teeth, as well as all other organs. This communication is reciprocal and continues throughout the morphogenesis of the organ (Fig. 1) (3).

Active research in many groups worldwide, including our group in Helsinki, has led to the discovery of some hundreds of genes that regulate tooth development (http://bite-it.helsinki.fi/) (4). Mutations in dozens of these regulatory genes result in congenital dental defects in mice and/or humans (5, 6). The specific roles and functions of these genes in tooth formation have been actively explored in genetically modified mice; it has become evident that most of the regulatory genes are involved in the mediation of epithelial–mesenchymal interactions, and we have started to understand the molecular details of the complex gene-regulatory networks (7). Of key importance are the signaling molecules that mediate communication between the cells – they constitute a type of cell language (Fig. 1). The most important signaling molecules belong to one of four main families, and it is interesting that these signals have been conserved throughout evolution and that they regulate organ development in all animals. These signaling families are WNT, bone morphogenetic protein (BMP), hedgehog (HH), and fibroblast growth factor (FGF). In addition, a signal called ectodysplasin

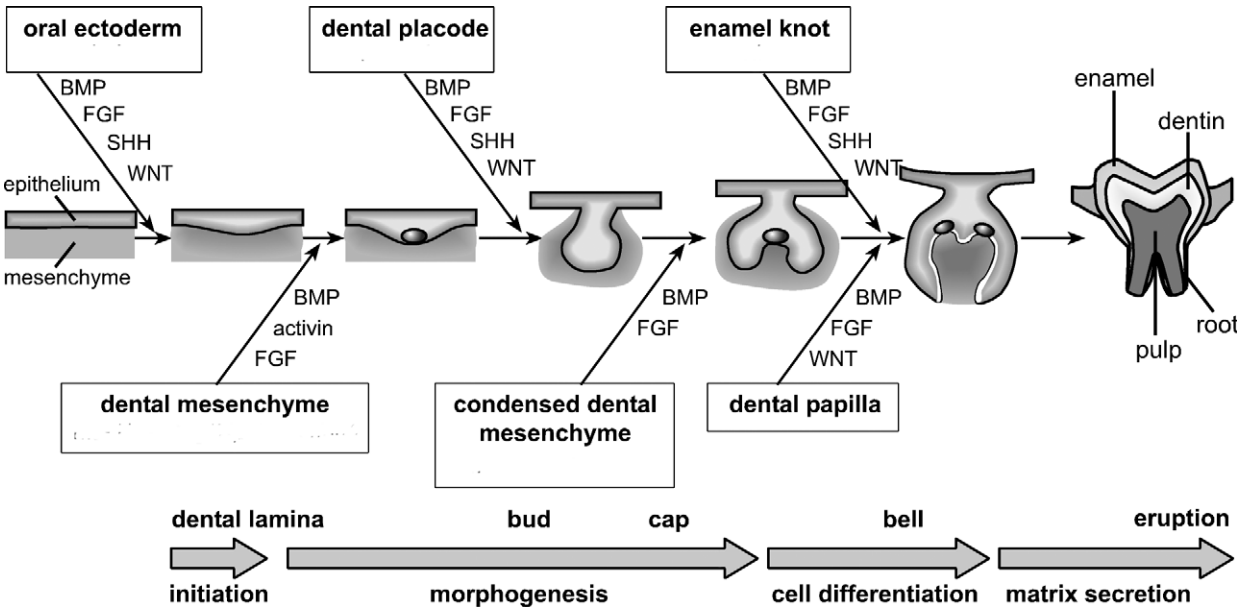

Fig. 1. Regulation of tooth development by a chain of interactions between the epithelial and mesenchymal tissues. The reciprocal interactions are mediated by the signaling molecules bone morphogenetic protein (BMP), fibroblast growth factor (FGF), sonic hedgehog (SHH), WNT, and ectodysplasin (EDA).

(EDA) is required specifically for the development of teeth and other organs developing from the surface of the embryo, such as hairs and several glands. EDA and the four signal families mentioned above are necessary for tooth development: blocking the signaling by any of these families results in arrested or otherwise disturbed tooth development (5, 7). Numerous mutations in the genes of these signaling pathways have been shown to result in congenital absence of teeth or structural changes in dental hard tissues in both mice and humans (5, 8).

Exploring the functions of signaling pathways in genetically modified mice and organ cultures

It is possible to block or activate different signaling pathways in vivo by generating transgenic and mutant mice. On the other hand, ex vivo culture of tooth buds or isolated dental tissues allows the daily follow-up and manipulation of tooth development, for example by adding signals or their inhibitors to the culture medium at specific tooth-developmental stages (9) (Fig. 2). As a result of such studies, a 'program' of tooth development is emerging in which the interactions between the epithelial and mesenchymal tissues play key regulatory roles. These interactions are mediated by signals from the five families mentioned above and regulate the expression of genes important for tooth morphogenesis and differentiation of the dental cells (Fig. 1).

When the goal is to bioengineer teeth from stem cells it is essential to discover the factors that specifically regulate the initiation of tooth formation. Research findings from many laboratories and institutions during the last 10–15 yr indicate that tooth development is initiated by a WNT signal. In our laboratory, we addressed the role of WNT signaling in tooth formation by generating a genetically modified mouse in which the WNT pathway was activated in the embryonic oral epithelium (10). This resulted in massive development of supernumerary teeth (Fig. 3); when a tooth germ was dissected from a mutant embryo and its development was followed in an organ culture set-up, new teeth were continuously formed, indicating that WNT signaling can induce the initiation of new teeth. The interest in the functions of WNT signaling has steadily increased during the last 15 yr, not only in research on tooth formation but generally in developmental and regenerative biology. WNT signaling is now considered as the single most important pathway in the initiation of teeth as well as other organs. For example, WNT signaling also initiates hair development (11). Interestingly, WNT signals specifically regulate stem cells during the initiation of many tissues and organs (12) and they have also been pinpointed as regulators of dental stem cells (7).

The causes and possible prevention of hypodontia in humans

Interestingly, during the last decade, WNT signaling has been linked with missing teeth (hypodontia) in humans. Now, we know that mutations in the *WNT10A* gene constitute the most common cause of hypodontia, and together with mutations in other WNT pathway genes they cause more than half of

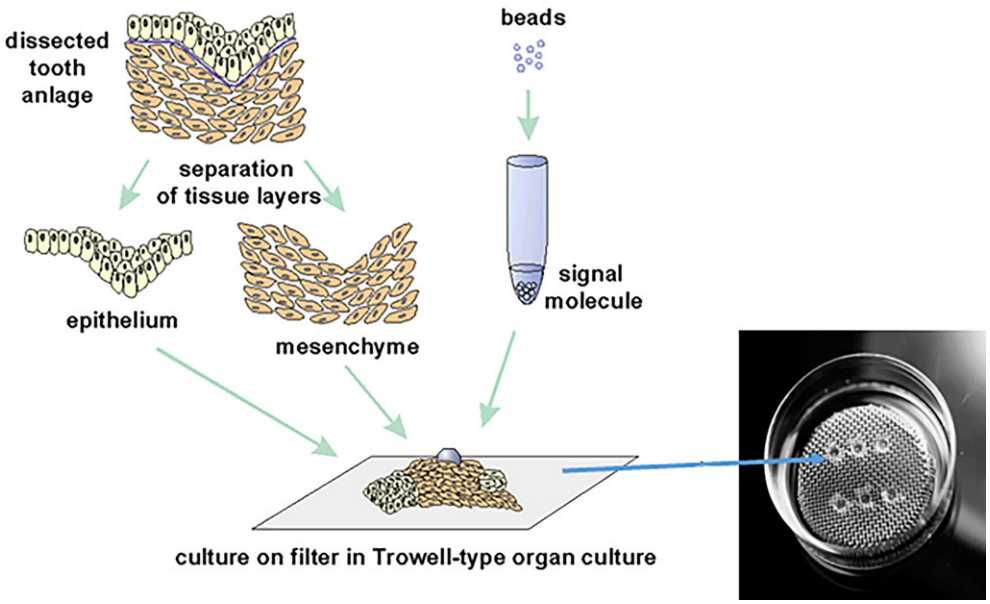

Fig. 2. In vitro model system for examining the effects of signaling molecules on dental tissues. An agarose bead is soaked in signal and placed on top of the dissected dental tissue. The tissue is placed on a thin filter supported by a metal grid in a dish containing culture medium.

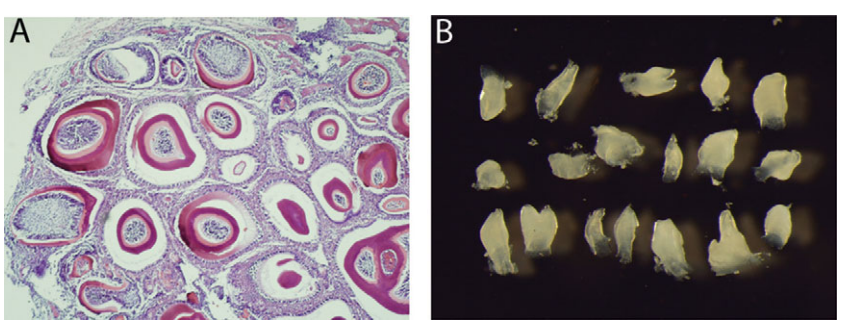

Fig. 3. Increased WNT signaling stimulates the initiation of teeth. Forced expression of WNT in a transgenic mouse induces massive numbers of teeth. (A) Histological section of teeth that developed from one transgenic tooth bud. (B) Teeth that developed from one transgenic tooth bud.

human hypodontia cases, including mild and severe tooth agenesis as well as syndromic hypodontia (13–15).

Another important signaling pathway that has been specifically linked with hypodontia, both in humans and mice, is the pathway mediated by the EDA signal. The *EDA* gene was originally discovered as the gene behind the rare human syndrome, hypohidrotic ectodermal dysplasia (HED) (16). The *EDA* mutations in patients with HED block the function of the gene and the patients therefore have no EDA signal. Patients with HED typically present severe oligodontia and may even lack most teeth (Fig. 4). In addition, other organs developing from the ectodermal epithelium covering the embryo, such as hairs and several glands, are severely reduced in number and are hypoplastic. The *EDA* mutant mice have a similar phenotype and we and others have used these mice for research (17). Research in our laboratory has demonstrated that EDA is required for the development of several ectodermal organs during their initiation from placodes, and in the *EDA* mutant mice the dental placodes, as well as the teeth forming from them, are small or completely absent (18). On the other hand, when the EDA pathway was activated throughout the oral epithelium in transgenic mice, extra placodes formed in front of the molars and these gave rise to supernumerary teeth (18).

Because EDA is a soluble signaling molecule, it was possible to study whether its absence in the *EDA* mutant mouse could be compensated by injecting EDA protein. This experiment was conducted by scientists in Switzerland and they reported a dramatic effect: when injected after birth, the protein rescued the tooth phenotype *EDA* mutant mice and hairs also grew normally (19). The same experiment was repeated in *EDA* mutant dogs, and their severe oligodontia was almost completely prevented (20). These results have prompted scientists to start clinical trials in humans to rescue the

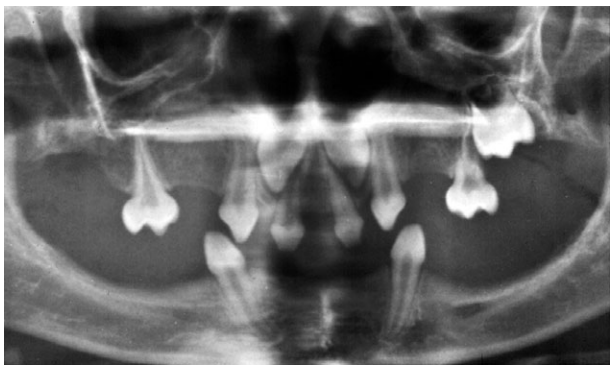

Fig. 4. Severe hypodontia (oligodontia) in hypohidrotic ectodermal dysplasia (HED) syndrome. The phenotype is caused by a mutation in the ectodysplasin (*EDA*) gene, resulting in a lack of the signaling molecule, EDA.

HED phenotype and, interestingly, promising results were reported recently from one trial (21). EDA research is an excellent demonstration that the results of basic biology research may be applied in the future for the prevention of congenital malformations.

Growing teeth from stem cells

Humans have been dreaming for decades, probably for hundreds of years, about replacing their lost teeth by growing new ones. During the last 20 yr, the realization of these dreams has come closer, as the process of tooth development has started to be understood at the level of genes and molecules and as the stem-cell technologies have advanced at an unbelievable speed.

For many decades, developmental biologists have searched for answers to the question of how stem cells differentiate toward various directions and how different tissues and organs form. The research methods created by developmental biologists and the accumulated knowledge now form the basis for the extremely active research aiming at discovering the precise instructions and programs for building tissues and organs from stem cells.

As a result of a groundbreaking discovery by the Japanese scientist, SHINYA YAMANAKA, in 2006, it became possible to program the cells of adult humans to pluripotent stem cells (22). YAMANAKA called them induced pluripotent stem (iPS) cells and their huge potential in medicine was instantly realized in the scientific community. YAMANAKA won the Nobel prize in 2012, exceptionally soon after his discovery. Now patients′ own cells can be used for generating a variety of tissues and organs by first reprogramming them to iPS cells, and then reprogramming the iPS cells to the desired cell types (23).

The obvious applications of iPS cells are in tissue-replacement therapies in which a damaged tissue or a congenitally missing organ is developed from a patient′s own stem cells. Nowadays, the reprogramming of differentiated human cells to iPS cells is a routinely used method in laboratories all over the world. However, the second step of tissue-replacement therapies (i.e. the actual building of the tissue or organ in demand) still requires research and development of technologies. For this work, developmental biologists are needed.

In principle, it is possible to program the iPS cells to dental cells. However, so far, the 'recipe' for this programming is not known. However, as the research in the field is extremely active in many countries all over the world, it can be expected that it will not take long to figure out the recipes for differentiating stem cells to epithelial and mesenchymal cells of the tooth. Then, a 'bioengineered' tooth can be built using the tissue-recombination techniques applied by developmental biologists over the years for studying the mechanisms of tooth development (Fig. 5). Those experiments indicate that the early embryonic dental epithelium and mesenchyme can be separated, and thereafter recombined and cultured in vitro or as transplants, and that the transplant can develop to a complete tooth (2). Japanese scientists demonstrated, in 2007, that this method works, even if the epithelium and mesenchyme are disaggregated to single cells and reaggregated before recombination (24). When such reaggregates were transplanted to the jaws of adult mice at the site of an extracted tooth, a complete tooth with a crown and root formed. Importantly, using this technique the regenerated tooth becomes vascularized and innervated and the surrounding alveolar bone also forms (25).

In conclusion, the method for building teeth from the patient′s own cells already exists in principle (Fig. 5). Based on current research it should be possible to grow new teeth for humans using their own cells,

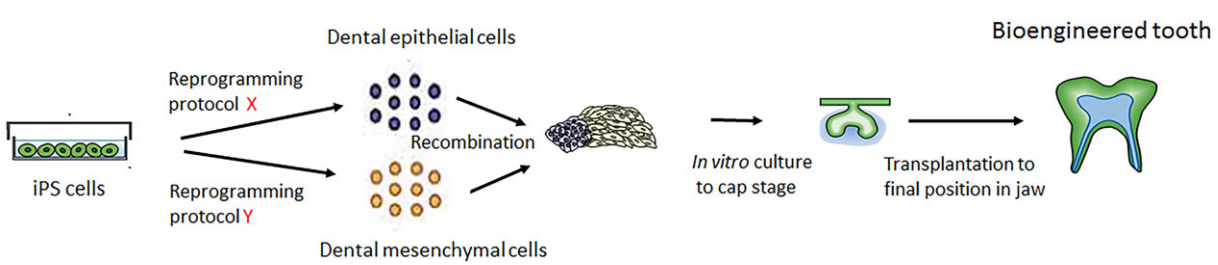

Fig. 5. A potential method for bioengineering teeth from stem cells. Induced pluripotent stem (iPS) cells are programmed to develop into epithelial and mesenchymal dental cells. These are combined and cultured in vitro (Fig. 2) to reach the cap stage, and then transplanted to their final position in the jaw bone.

and it is probably just a question of time before the missing pieces of the method are worked out. A different question is how usable such a method will be in the dental clinics in the future. Technically, this method is (at least so far) demanding, requiring several problems to be solved and risks to be addressed. In addition to the problem of the genetic programming of dental cells from stem cells, an obvious challenge is the slow speed of tooth development. In vivo, the formation of a tooth is a long process, which in children takes from one to several years. This problem could perhaps be solved by growth-stimulatory signaling molecules. Other remaining challenges include control of the shape, size and color of the tooth crown, but these may be fixed by existing methods in clinical dentistry. Finally, a serious risk is that cells grown outside the human body may transform to cancer cells. This risk concerns most stem-cell treatments and research aiming at the inhibition of the cancer risk is underway at present.

Conflict of interest – The author declares no conflicts of interest.

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