Evidence Based Practice in Dentistry
Kuwait, October 2–4, 2001

Medical Principles and Practice

Med Princ Pract 2003;12(suppl 1):43–50
DOI: 10.1159/000069840

Stem Cells and Tissue Engineering: Prospects for Regenerating Tissues in Dental Practice

Irma Thesleff Mark Tummers

Developmental Biology Research Program, Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Helsinki, Finland

Key Words
Stem cells · Bioengineering · Tooth · Bone · Periodontal ligament · Dentin · Enamel

Abstract
In general, human tissues have a very limited potential to regenerate. However, recent progress in stem cell research and in tissue engineering promises novel prospects for tissue regeneration in dental practice in the future. Stem cells have been discovered in many adult tissues, including teeth, and stem cells from embryos have the potential to form all adult tissues. Embryonic stem cells can now be cultured and even produced from adult cells by the nuclear transfer method. Due to the rapid progress of research in molecular biology, particularly in the field of developmental biology, we are now starting to understand at the level of genes and molecules how the development of dental tissues is regulated. For instance, specific signal molecules have been identified which regulate the development of teeth and bones from progenitor cells. This information is already being used for the generation of dentoalveolar tissues in vitro and in vivo. Could we perhaps grow new enamel, dentine, periodontal ligament, bone, or even whole new teeth for our patients in the future?

Introduction

The possibilities to grow new tissue for patients are presently being actively discussed in professional medical and biological journals as well as in the media. Many scientific breakthroughs during recent years have raised expectations that adult tissues could be replaced by biological means ('regenerative medicine') rather than by using artificial spare parts and prostheses. It is hoped that it will be possible to regenerate tissues destroyed by diseases such as cancer, diabetes or periodontal disease, and that tissues or perhaps even whole organs that are congenitally missing could be regenerated. In dentistry the hopes are to regenerate dentoalveolar tissues including alveolar bone, periodontal ligament, dentin and enamel, and perhaps even to grow whole new teeth.

Like all new therapies, the practice of tissue engineering is based on previous fundamental research dating back decades. Knowledge of the molecular mechanisms of normal embryonic development is essential when tissues and organs are to be regenerated in patients and derives from a long research tradition in the field of developmental biology. For example, the concept that cell differentiation in all multicellular organisms is regulated by signals transmitted between embryonic cells was already introduced in the beginning of the 20th century. The molecular identities of these signals, so-called growth and

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1011–7571/03/0125–0043$19.50/0

Accessible online at:
www.karger.com/mpp

Irma Thesleff
Institute of Biotechnology
University of Helsinki, PO Box 56
FIN–00014 Helsinki (Finland)
Tel. +358 9 19159401, Fax +358 9 19159366, E-Mail irma.thesleff@helsinki.fi

differentiation factors, have been revealed only during the last two decades.

The present immense interest in the prospects of tissue engineering is, however, due to very recent advances in the field of stem cell biology. In this review, we shall first focus on the most important recent discoveries in this field, and then discuss the possibilities to regenerate dental tissues by stimulating the differentiation of stem cells by bioactive signal molecules, the growth and differentiation factors.

What Are Stem Cells?

Stem cells are defined as cells that have the capacity to self-renew as well as to give rise to differentiated progeny. In early embryos all cells are totipotent stem cells, as they have the ability to form all tissues of the organism. There are also stem cells in adult tissues that contribute to the renewal and regeneration of specific tissues. In humans, stem cells are definitely present in all continuously renewing tissues, such as hematopoietic tissue, skin, bone and intestinal epithelium. In addition, stem cells must be present in tissues which regenerate after injury such as liver and muscle. Interestingly, recent evidence indicates that stem cells are much more widely distributed than previously believed. In particular, the identification of stem cells in the adult brain has led to dramatically increased research activity in the field of neuroscience, and to hopes that stem cell therapies may be used to cure brain damage, for instance in Alzheimer's and Parkinson's diseases.

In the tooth, stem cells were recently identified in adult dental pulp [1]. The researchers transplanted clones of cultured dental pulp cells to muscle and showed that they differentiated into odontoblasts, forming dentine matrix. In similar earlier experiments, it was shown that bone marrow contains stromal stem cells that differentiate into osteoblasts and give rise to bone after transplantation [2]. Stem cells for ameloblasts have been identified in the cervical loop epithelium of rodent incisors [3].

The Stem Cell Niche

For most stem cells there are no known markers which could be used for the localization of stem cells in tissues. However, evidence from a variety of studies indicates that the cells reside in specific locations called stem cell niches [4, 5]. The microenvironment in these niches supports the maintenance of stem cell characteristics as well as their self-renewal. The differentiation of the cells first to transit amplifying cells and then to terminally differentiated cell types is stimulated by specific signal molecules, called growth and differentiation factors.

Usually the stem cells in different tissues give rise to one or a few cell types. For example, stem cells in the hair follicles give rise to hair matrix cells, sebaceous gland cells and epidermal cells of the skin [6]. Recently, however, evidence has accumulated that stem cells in adult tissues may have the potential to give rise to a variety of different cell types, as was shown when labelled stem cells were transplanted to different locations. It appears that when stem cells are removed from their original niche and encounter a new environment they can be reprogrammed and cross lineage boundaries. For instance, brain stem cells may give rise to haematopoietic cells, and bone marrow cells to epithelium [7, 8]. Hence, there seems to be much more plasticity in the reprogrammable capacity of the stem cell than previously thought.

Embryonic Stem Cells

At present there are no methods available for the isolation of stem cells from their niches in adult tissues, and hence it is not possible to collect them for tissue engineering purposes. Stem cells from human embryos have recently become an alternative source for the regeneration of human tissues. It has been known for more than a century that the cells in the early embryo are totipotent, i.e. they have the capacity to form all tissues. For example, one isolated cell of an 8-cell embryo can give rise to a whole organism. In the blastocyst stage embryo, the cells of the inner cell mass contribute to all tissues of the adult [9]. Within the last 20 years, it has been possible to culture inner cell mass cells as continuous growing stem cell lines. In fact the mouse embryonic stem cell lines form the basis for the production of transgenic mice, in which specific genes are targeted and modified. For instance, the technique of gene targeting in embryonic stem cells is used to produce so-called knockout mice, in which the function of a specific gene is deleted. When the modified cells are mixed with cells from the inner cell mass of a wild-type mouse embryo, they contribute to the newly formed embryo and can differentiate to all embryonic tissues. Some of these cells end up in the germ line and then the genetic modification can be passed on to the offspring.

Successful culture of stem cells from human embryos was reported for the first time in 1988 [10], and since then

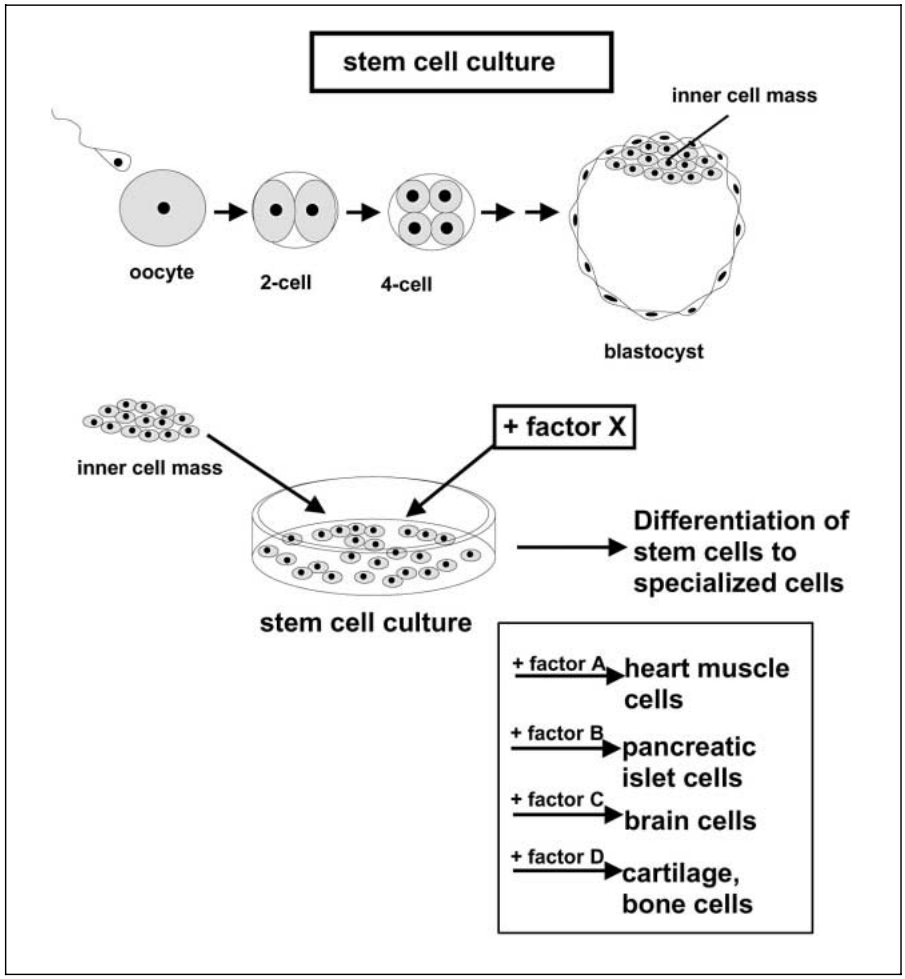

Fig. 1. Schematic depiction of stem cell culture. Fertilized oocytes are cultured until the blastocyst stage. The inner cell mass, which will give rise to the embryo, is collected and cultured. By adding specific growth and differentiation factors to the culture medium the stem cells are forced to differentiate into certain specialized cell types.

the technical and ethical aspects of their use in tissue engineering have been actively discussed in professional journals as well as in the media. For the first time it is possible to analyse the differentiation of human embryonic cells experimentally. Although the mechanisms of embryonic development are astonishingly similar in all mammals, including mice and human beings, there are nevertheless differences; it is very important to learn these differences for the development of stem cell technologies, because small regulatory differences during development can ultimately lead to hugely different results. Therefore, the first application for human embryonic stem cell lines and the first order of business is the fundamental study of human development and cellular differentiation [11], which is essential for gaining knowledge on how to differentiate tissues and maybe even create organs from human cells in the future. It is apparent that this will be a long endeavour and much effort will be required before there is sufficient knowledge of the molecular mechanisms of human cell differentiation.

As a second and more practical application in the future, human embryonic stem cells could provide a source of cells for tissue regeneration. Stem cells could be exposed to specific combinations of growth and differentiation factors in vitro, which would induce their differentiation in desired directions (fig. 1). Different types of tissues could then be grown in culture and afterwards transplanted to the patients. Another possibility is that the totipotent embryonic stem cells could be directly implanted into the patient's tissues, where they would then differentiate into specific cell types after encountering the appropriate niche. There is, in fact, some evidence that damaged tissues may exert chemotactic influences on stem cells and, thereby, stem cells might be specifically guided to sites where they are needed for tissue regeneration.

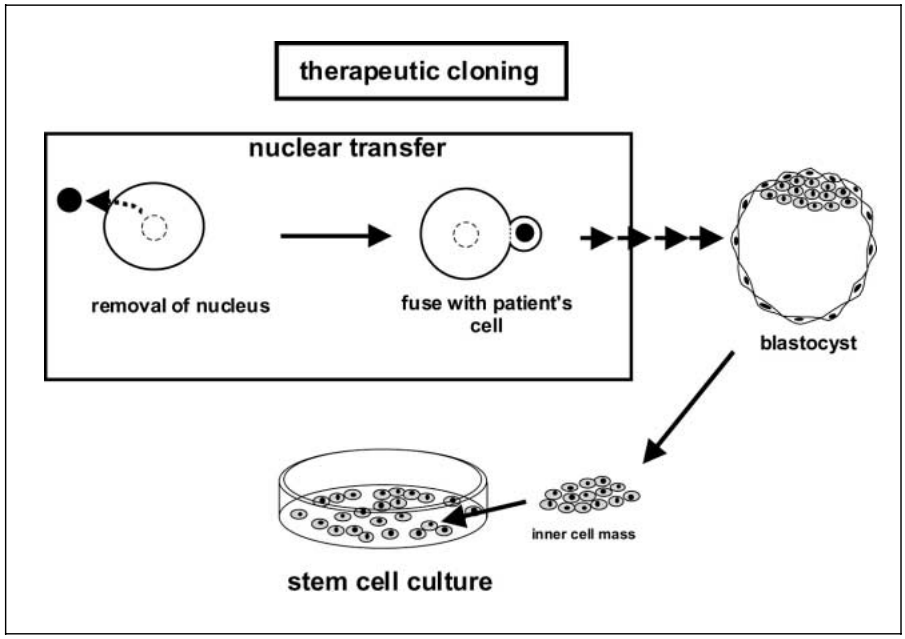

Fig. 2. The technique of therapeutic cloning. The nucleus is removed from a donor oocyte, leaving it without DNA. This empty cell is subsequently fused with a cell from the patient and therefore also with the DNA of the patient. In the environment of the oocyte this DNA is reprogrammed and can regulate embryonic development. The embryo can now be grown until the blastocyst stage, after which the inner cell mass can be collected and used for creating stem cell lines by culturing them. The tissues created with these cell lines will be immunologically identical to that of the patient.

Therapeutic Cloning

One problem of using embryonic stem cell lines for tissue engineering is the potential rejection of these cells by the patient. It could be possible to overcome this problem by producing embryonic stem cells from the patient's own cells by so-called therapeutic cloning (fig. 2), based on a nuclear transfer technique similar to the one developed for cloning the sheep Dolly. The nucleus of a somatic cell of the patient is transferred to an enucleated oocyte and cultured in vitro until the blastocyst stage, from which a stem cell line is derived. Since the nucleus contains the genetic information of the patient, any tissue produced by this method will be immunologically identical to and not rejected by the patient. The technology behind the method is obviously demanding and at present only partially exists; hence therapeutic cloning is not yet feasible in practice. In addition, it requires donated oocytes and raises a number of ethical issues that are currently being actively discussed.

Future Vision of Stem Cell Therapy

The optimal way to produce stem cells would be to generate undifferentiated cells from a patient's own differentiated tissue, thereby avoiding the use of donated human oocytes and human embryonic stem cells. The cloning of Dolly showed that the cytoplasm of the oocyte provides an environment which can turn back the developmental clock of a transplanted adult nucleus so that it 'forgets' its previous state and history and becomes totipotent. If we could learn enough about the factors that turn back the cellular clock, it may be possible in the future to experimentally dedifferentiate adult cells, for example by treating them with a set of specific factors. In fact, the recent findings that suggest a previously unanticipated level of plasticity in some adult cells and their capacity to differentiate in multiple directions (see above) give some indication that this could be possible in the future. This would bring us closer to a scenario as simple as, for instance, taking a skin biopsy of the patient, dedifferentiating the cells, and then inducing their differentiation into desired cell types, such as heart muscle cells, bone cells or brain cells.

Growth and Differentiation Factors

The ability to induce the differentiation of stem cells in desired directions is still a major challenge in stem cell research. Whether the stem cells are derived from embryos or from adult tissues, the molecular mechanisms that guide the differentiation of various types of cells must be understood before specific tissues can be regenerated. The mechanisms that regulate morphogenesis and cell dif-

Table 1. Most commonly studied growth and differentiation factors

Growth and differentiation factor families
Transforming growth factor-β (TGF-β)
Bone morphogenetic protein (BMP)
Activin
Wnt (Wingless)
Retinoic acid (RA)
Fibroblast growth factor (FGF)
Platelet-derived growth factor (PDGF)
Hedgehog

ferentiation in the developing embryo have been a subject of intensive research for many decades. One of the pioneers in this field was Hans Spemann, who won the Nobel Prize in 1935 for studies that he performed in the early 1900s on amphibian embryos. He showed that inductive signals generated in one embryonic tissue could regulate the differentiation of neighbouring tissues [9]. These findings initiated a long search for the identity of the actual inductive signals, but it was only after the technological advances in molecular biology that the molecular nature of the signals was discovered. The last 15 years have been an era of intense activity in the field of developmental biology, and a number of scientific breakthroughs have contributed to the current understanding of the molecular signals that regulate cell differentiation. It is now evident that most signals are small secreted molecules called growth factors or differentiation factors. They are hormone-like molecules which are produced in one cell, released outside this cell and then act on other cells; through binding to specific receptors they regulate gene expression, which subsequently affects cellular differentiation (fig. 3). The most commonly studied growth and differentiation factor families are listed in table 1. Growth and differentiation factors have a variety of effects on cells and, typically, the same molecules affect the differentiation of many cell types and organs.

The understanding of how growth and differentiation factors regulate cell differentiation has increased rapidly during recent years. There are even some examples of successful use of growth and differentiation factors in the stimulation of mouse embryonic stem cell differentiation. However, we are still far from a situation where we could apply specific cocktails of factors to undifferentiated cells and stimulate their differentiation into specific pathways, thereby producing the desired replacement tissues for patients, or even for experimental animals.

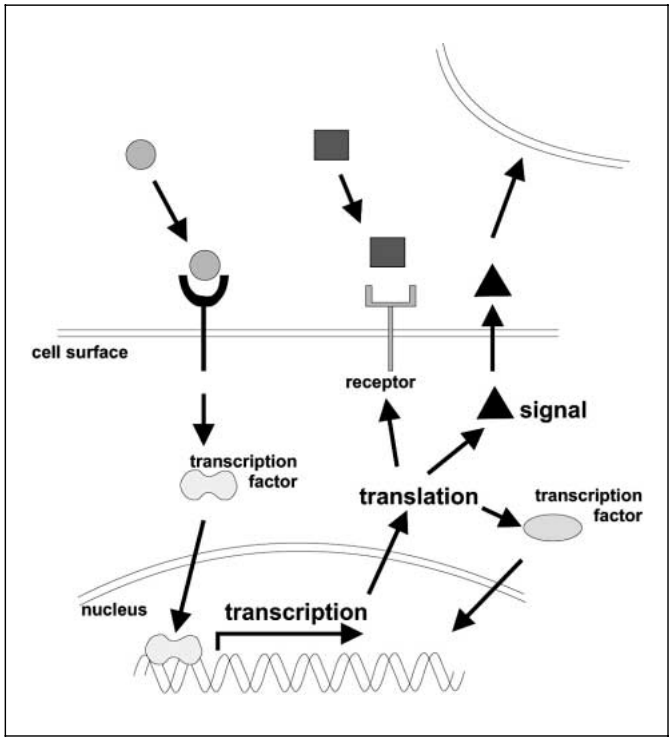

Fig. 3. Signalling molecules outside the cell transduce their signal through specific transmembrane receptors into the cell. The signal is then forwarded to the nucleus where it activates the transcription of specific genes. The mRNAs of these genes are translated into functional proteins such as receptors, signalling molecules, or transcription factors. The transcription factors will modulate the expression of certain genes in the same cell. The receptors will move to the cell membrane and are then capable of receiving a signal from outside the cell, and the signalling molecules will be transported out of the cell and hence can communicate with other cells.

Regeneration of Bone, Dentin and Periodontal Ligament by Bone Morphogenetic Proteins

Bone is a very special type of tissue. It undergoes constant turnover, resulting from apposition by osteoblasts and resorption by osteoclasts, and it also has a fantastic ability to regenerate after injury. It has been known for a long time that bone matrix is rich in growth and differentiation factors, and today the molecular nature of the most important of these factors is known. It has been shown that they can stimulate the differentiation of stem cells into bone-forming osteoblasts. The ability of bone matrix to stimulate the formation of new bone was experimentally proven several decades ago by transplanting demineralized bone matrix into muscle [12]. After many decades of intense biochemical research the active sub-

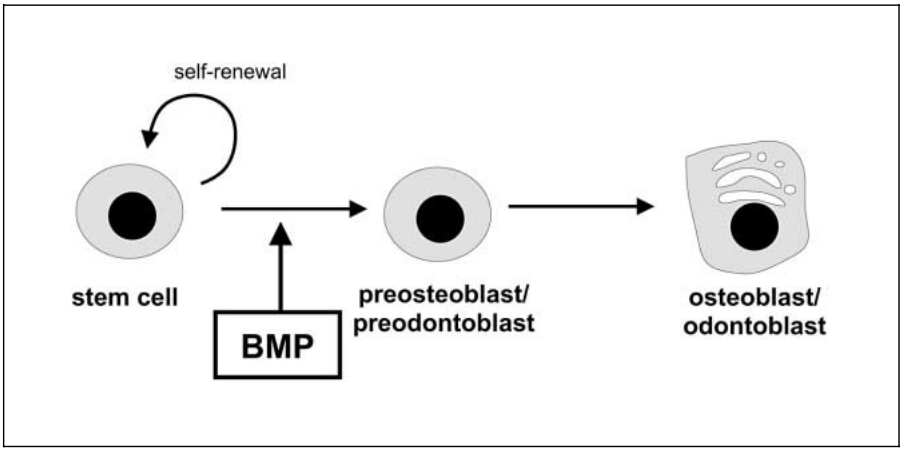

Fig. 4. The differentiation of stem cell progeny. During stem cell divisions new stem cells are created and cells that will continue to differentiate. In this particular case BMPs are capable of inducing the differentiation of stem cells into preostoblasts and preodontoblasts, which will then continue differentiating into osteoblasts and odontoblasts, respectively.

stance, called bone morphogenetic protein (BMP), was finally isolated and cloned [13]. There are more than 30 BMPs known at present, and many of them induce bone and cartilage formation in vivo when transplanted with carrier substances to soft tissues such as muscle. They also induce the differentiation of osteoblasts and chondrocytes in a variety of cultured cells in vitro. Animal experiments over many years have shown that BMPs have the capacity to stimulate bone formation in different bones, including jawbones. Thus, BMPs stimulate alveolar bone formation around teeth and induce the regeneration of periodontal attachment. They apparently have stimulatory effects on cementoblast differentiation [14, 15]. Clinical trials in humans are presently ongoing in which the effects of BMPs are tested on bone formation in various orthopaedic applications as well as for periodontal regeneration.

Although the morphology of dentin matrix differs from that of bone, their biochemical compositions are very similar. Hence, dentin matrix also contains BMPs, and demineralized dentin matrix can stimulate bone formation in muscle. Interestingly, both bone and dentin matrices stimulate dentin formation when implanted into the dental pulp, and this effect can be mimicked by recombinant BMP [16]. Dentin does not normally undergo turnover like bone, but it has the well-known ability to regenerate after pulpal injury. As described above, osteoblastic stem cells have been localized in bone marrow, whereas odontoblast stem cells were recently discovered in the dental pulp. It appears, therefore, that BMPs are growth and differentiation factors which can stimulate the differentiation of pulpal stem cells into odontoblasts and bone marrow stem cells into osteoblasts (fig. 4).

Could Enamel Be Regenerated?

The regeneration of enamel is clearly more problematic than that of dentin and bone. The enamel organ epithelium, including the ameloblasts, remains as a protective layer on the tooth crown only until eruption, at which point it is lost. Therefore, in contrast to dentin, enamel does not regenerate after traumatic injury. There are conceivably no stem cells for the enamel-producing ameloblasts in adult tissue. It is not impossible, however, that some oral epithelial cells could have the ability to transdifferentiate into ameloblasts under favourable circumstances. Work in our laboratory has led to the identification of epithelial stem cells in the cervical loop of mouse incisors [3]. These teeth erupt continuously, and enamel production continues throughout the life of the animals. Thus, by definition there have to be stem cells for ameloblasts present. Stem cells for ameloblasts apparently reside in the germinative end of the incisors among the stellate reticulum cells located in the centre of the cervical loop epithelium. We have suggested that the maintenance and/or differentiation of these stem cells is stimulated by fibroblast growth factors (FGFs), which are a family of well-known growth and differentiation factors required for the development of a variety of organs, including teeth [17]. We also suggested that FGFs regulate the Notch pathway, which is a molecular signalling pathway involved in stem cell development in other animals and organs [18]. The understanding of the molecular pathways that regulate the differentiation of dental epithelial stem cells into ameloblasts in vivo may lead to the generation of tools whereby oral epithelial cells or other epithelial stem cells could be induced to form enamel.

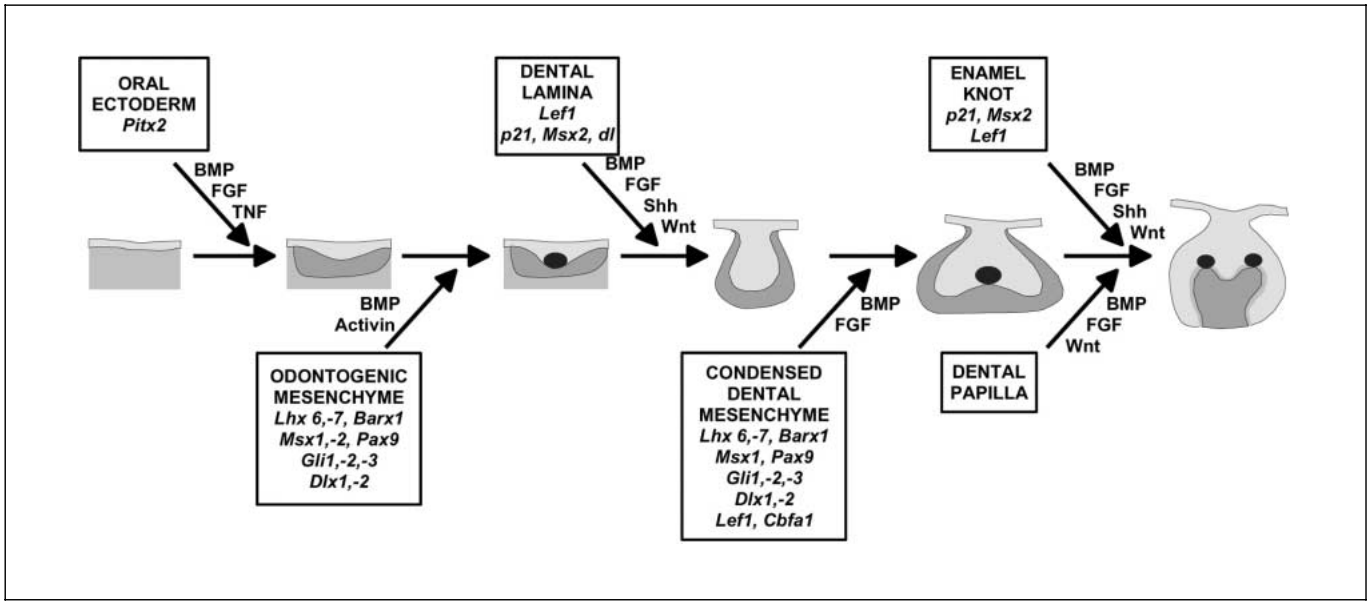

Fig. 5. Signalling between mesenchyme and epithelium plays an important role in the development of epithelial-mesenchymal organs like the tooth. The same growth and differentiation factors regulate different stages of development. The top panels are epithelial signals and the bottom panels mesenchymal signals. In the boxes are transcription factors regulated by the signals.

Prospects to Grow New Teeth?

The regeneration of whole organs like teeth is certainly much more demanding than the regeneration of individual tissues like bone or dentin. However, this goal may not be as distant as it appeared to be a few years ago. This is due to the rapid progress in the understanding of the molecular mechanisms that regulate the development of embryonic organs in general and of teeth in particular. Teeth develop from oral ectoderm and the underlying mesenchymal cells, and at the time of tooth initiation the epithelial and mesenchymal cells are undifferentiated. Dental epithelium consists of cuboidal cells that form the thickened dental lamina, and the dental mesenchymal cells underneath are morphologically similar to the rest of the jaw mesenchyme. A chain of signaling events, taking place mainly between the epithelium and mesenchyme of the tooth germ, guides morphogenesis through several stages of increasing complexity, accompanied by the progressive differentiation of cells [19].

Numerous molecules that are involved in the complex process of tooth morphogenesis have been identified during the last 20 years. Many growth and differentiation factors have been shown to act as signals mediating the morphogenetic cell-cell interactions during tooth development. For instance, our laboratory has discovered signaling centres in the tooth germ epithelium, called enamel knots. Composed of small aggregates of epithelial cells which simultaneously produce more than 10 growth and differentiation factors [20], the enamel knots regulate the formation of tooth cusps and are therefore important organizing centres of tooth development. The targets of the signals regulating tooth development have been identified in some cases, and hence we are beginning to understand the molecular and genetic networks that regulat odontogenesis. Figure 5 shows a schematic illustration of the molecules regulating early tooth morphogenesis.

Our laboratory has created a database on the expression patterns of genes associated with tooth morphogenesis (http://bite-it.helsinki.fi). As of July, 2001, patterns of 230 genes are shown in this database. It is conceivable that the completion of the genome project will result in a rapid increase in the number of these genes. Molecular genetic studies of human syndromes have revealed novel genes regulating tooth development. In addition, the genetic analysis of mouse development, in particular the production of transgenic mice, will certainly result in the discovery of more genes that are required for normal tooth development. This accumulation of molecular information will contribute to a more complete understand-

ing of the mechanisms that regulate tooth morphogenesis and the roles that growth and differentiation factors play in these processes.

Despite the accumulation of molecular information and our understanding of the regulation of tooth development, it is not clear how teeth could be grown in practice. Perhaps one day we will be able to isolate cells that have the capacity to form teeth, and then tooth development could be initiated in vitro. Such multipotential stem cells could be obtained by some of the methods described above. After initiation, the tooth germ could either be transplanted into the mouth or it could be cultured in vitro. This approach would be the most difficult since it would require a thorough knowledge of all processes that govern the formation of the proper three-dimensional structure of the tooth. Alternatively, it is possible that tooth development could be initiated in vivo by applying specific growth and differentiation factors. Some years ago we developed a technique whereby growth factors were introduced locally to embryonic tissue in vitro by small agarose beads that released the factors to surrounding cells [21]. Could it be possible to induce the formation of new teeth in vivo by such beads? It is intriguing that FGF-releasing beads induced extra limbs in chicken embryos when they were implanted in the flank between the wings and legs [22]. The potential feasibility of tooth regeneration gains additional credibility from the fact that teeth are continuously being replaced in some animals such as fish or amphibians. Furthermore, teeth are often found in human teratomas. These tumours consist of a variety of differentiated tissues, and it is noteworthy that the teeth found in these tumours have quite normal shapes and structures, thus indicating that the program for development is conceivably present very early in the tooth-forming tissue and is therefore not influenced by the surrounding tissues to a significant extent. Therefore, with this approach we would ideally require only to introduce the signal that starts tooth formation and then let nature run its course, without having to worry about the complex processes that occur during tooth development.

Conclusions

Despite the rapid advances in the fields of developmental biology and regenerative medicine, much research is still required before tooth regeneration in dental practice is a reality. In particular, we need a more thorough understanding of the mechanisms of tooth initiation and the characteristics of dental stem cells.

References

1 Gronthos S, Mankani M, Brahim J, Robey PG, Shi S: Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 2000;97:13625–13630.
2 Bianco P, Robey PG: Stem cells in tissue engineering. Nature 2001;414:118–121.
3 Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I: Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 1999;147:105–120.
4 Watt FM, Hogan BL: Out of Eden: Stem cells and their niches. Science 2000;287:1427–1430.
5 Spradling A, Drummond-Barbosa D, Kai T: Stem cells find their niche. Nature 2001;414:98–104.
6 Fuchs E, Segre JA: Stem cells: A new lease on life. Cell 2000;100:143–155.
7 Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL: Turning brain into blood: A hematopoietic fate adopted by adult neural stem cells in vivo. Science 1999;283:534–537.
8 Anderson DJ, Gage FH, Weissman IL: Can stem cells cross lineage boundaries? Nat Med 2001;7:393–395.
9 Gilbert SF: Developmental Biology, ed 6. Sunderland, Sinauer, 2000, pp 314–318.
10 Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–1147.
11 Donovan PJ, Gearhart J: The end of the beginning for pluripotent stem cells. Nature 2001;414:92–97.
12 Urist MR: Bone: Formation by autoinduction. Science 1965;150:893–899.
13 Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA: Novel regulators of bone formation: Molecular clones and activities. Science 1988;242:1528–1534.
14 Talwar R, Di Silvio L, Hughes FJ, King GN: Effects of carrier release kinetics on bone morphogenetic protein-2-induced periodontal regeneration in vivo. J Clin Periodontol 2001;28:340–347.
15 Ripamonti U, Heliotis M, Rueger DC, Sampath TK: Induction of cementogenesis by recombinant human osteogenic protein-1 (hOP-1/BMP-7) in the baboon *(Papio ursinus)*. Arch Oral Biol 1996;41:121–126.
16 Rutherford RB, Wahle J, Tucker M, Rueger D, Charette M: Induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. Arch Oral Biol 1993;38:571–576.
17 De Moerlooze L, Spencer-Dene B, Revest J, Hajihosseini M, Rosewell I, Dickson C: An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signaling during mouse organogenesis. Development 2000;127:483–492.
18 Artavanis-Tsakonas S, Rand MD, Lake RJ: Notch signaling: Cell fate control and signal integration in development. Science 1999;284:770–776.
19 Thesleff I, Nieminen P: Tooth morphogenesis and cell differentiation. Curr Opin Cell Biol 1996;8:844–850.
20 Jernvall J, Thesleff I: Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 2000;92:19–29.
21 Vainio S, Karavanova I, Jowett A, Thesleff I: Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. Cell 1993;75:45–58.
22 Cohn MJ, Izpisua-Belmonte JC, Abud H, Heath JK, Tickel C: Fibroblast growth factors induce additional limb development from the flank of chick embryos. Cell 1995;80:739–746.