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The development of the periodontium – a largely ectomesenchymally derived unit

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The periodontium is simply defined as the tissues supporting and investing the tooth. The tissues supporting the tooth are developmentally derived from the dental follicle proper, whereas those investing the tooth, that is the gingiva, are an adaptation of the oral mucosa. Apart from the gingival epithelium, which includes the dentogingival junction, and the epithelial cell rests of Malassez formed from the fragmented root sheath, the functioning periodontium is almost entirely derived from ectomesenchyme, embryonic connective tissue derived from neuroectoderm, if neural and vascular elements are ignored. Epithelial tissue is, however, involved in directing ectomesenchyme to form some of the connective tissue components of the tooth. To explain how this takes place, some basic tenets of development must be described.

Induction, competence and differentiation

Induction, competence and differentiation are important concepts in embryology. All the cells of an individual stem from the zygote and somehow differentiate into populations that assume particular functions, shapes and rates of turnover. Such populations are compartmentalized; successive generations of cells in a compartment may remain constant or differentiate, that is, change their characteristics and establish a new population of cells. The process that initiates differentiation is termed induction and an inducer is the agent that persuades cells to differentiate. Furthermore, each compartment of cells must be competent to respond to the induction process.

With the advent of recombinant DNA and immunocytochemical techniques, which enable precise identification of gene expression and localization of various signaling molecules, a much clearer understanding has been gained of the mechanisms involved in induction, competence and differentiation (44). Thus regulatory homeobox genes are now recognized (31). These genes are associated with patterning and generate transcription proteins that bind to downstream genes to regulate their expression. One set of homeobox genes (the Hox genes) are associated with anterior-posterior patterning in embryos and are highly conserved, that is, they occur in the most primitive organisms through to humans. This set of genes is, however absent from the most anterior part of the developing mammalian embryo, where they are replaced by a different set of homeobox genes (which include Msx and Dlx genes) developed later in evolution that are associated with the development of the head (32).

Gene expression is also regulated by two groups of regulatory molecules: growth factors and the steroid/thyroid/retinoic acid superfamily. Growth factors are polypeptides and belong to a number of families (Table 1). Three such families, transforming growth factor, nerve growth factor and platelet-derived growth factor are similar in structure, indicating their derivation from the same ancestral gene. Furthermore, many growth factors are homologous to growth factors involved in signaling between tissue layers in the developing *Drosophila* embryo, indicating that signaling mechanisms during embryogenesis are also highly conserved.

For growth factors to exert an effect, cells must express membrane receptors to capture them (making the cell competent) and, once captured, the receptor must also be able to interact with both membrane and cytoplasmic bound compounds to bring about, after a complex set of intracellular events,

Table 1. Growth factor families[a]

Transforming growth factors-beta
transforming growth factors-beta 1–5
bone morphogenetic proteins 2–8
growth and differentiation factors 1–7
Epidermal growth factors
epidermal growth factor
transforming growth factor-alpha
amphiregulin
epidermal growth factor
Fibroblast growth factors
fibroblast growth factors 1–8
Insulin-like growth factors
insulin-like growth factors 1–2
Platelet-derived growth factors
platelet-derived growth factors A and B
Neurotrophins
nerve growth factor
brain-derived neurotrophic factor
neurotrophins 3 and 4

[a] Some growth factors in all these families have been implicated in craniofacial and tooth morphogenesis.

alteration in gene function. The members of the retinoic acid family, on the other hand, freely enter the cell, where they complex with intracellular receptors that go on to alter gene function.

Neural crest and ectomesenchyme

Now that the concept of regulatory genes and signaling molecules as important factors in embryogenesis has been introduced, the role of neural crest in cephalogenesis can be explained. In simple vertebrates, the connective tissue elements (including cartilage and bone) and muscle are derived from segmented blocks of mesoderm lying beside the notachord known as somites. As the head region evolved, it became necessary to find an alternative source for the derivation of these tissues (with the exception of a partial contribution to the muscular component continuing to come from partially segmented blocks of mesoderm, the somatomeres, which flank the anterior portion of the neural tube). This new source is the neuroectoderm of the neural tube from which cells detach (Fig. 1) and migrate and differentiate to form a variety of different tissues including mesenchyme or embryonic connective tissue. This mesenchyme is designated as ectomesenchyme to reflect its origin from the neuroectoderm.

A good deal is now known about the pattern of migration of the neural crest cells to the developing head region (19, 20, 36). As the neural tube forms, it becomes partly segmented in the region of the developing hindbrain to form a series of eight bulges or rhombomeres, and it is from these rhombomeres that the neural crest cells emerge in a very specific pattern (described later) to migrate into the developing facial region. It is also known that the rhombomeres express homeobox genes in various combinations or codes and that these codes are maintained and carried by the neural crest cells as they migrate to initiate further downstream patterning (32).

The branchial arch system

As the head fold occurs early in the development of the embryo, the primitive stomatodeum is established, with its lateral boundary initially consisting of a sandwich of ectoderm (endoderm caudal to the bucopharyngeal membrane), a thin layer of mesoderm and finally the ectoderm covering the external aspect of the embryo. The thin layer of mesoderm in this sandwich becomes reinforced by a stream of neural crest cells, with the result that a series of swellings form on the lateral aspect of the stomatodeum and extend into its floor. These swellings are the branchial arches.

It is now established that the neural crest tissue invading and establishing each branchial arch arises from specific rhombomeres. Thus, the neural crest cells populating the first branchial arch emanate specifically from the mid-brain and between rhombomeres 1 and 2, that of the second arch from rhombomeres 4 and that of the third and fourth arches from rhombomeres 6 and 7. It has already been mentioned that the neural crest cells, as they migrate, carry with them the homeobox codes ex-

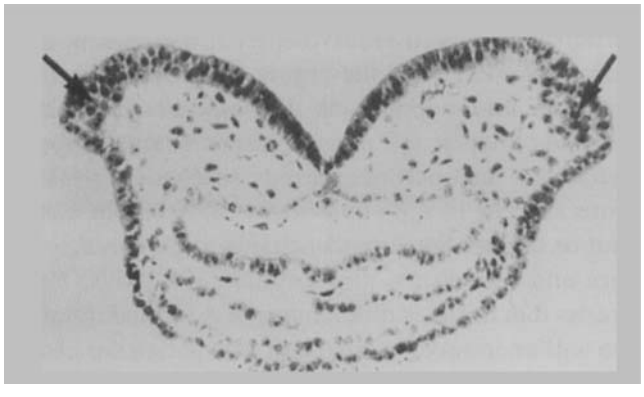

Fig. 1. Mouse embryo. Differentiation of normal crest cells (arrows) from the lateral aspect of the neural plate. Source: Ten Cate AR. Oral histology, development, structure and function. St. Louis: Mosby, 1994.

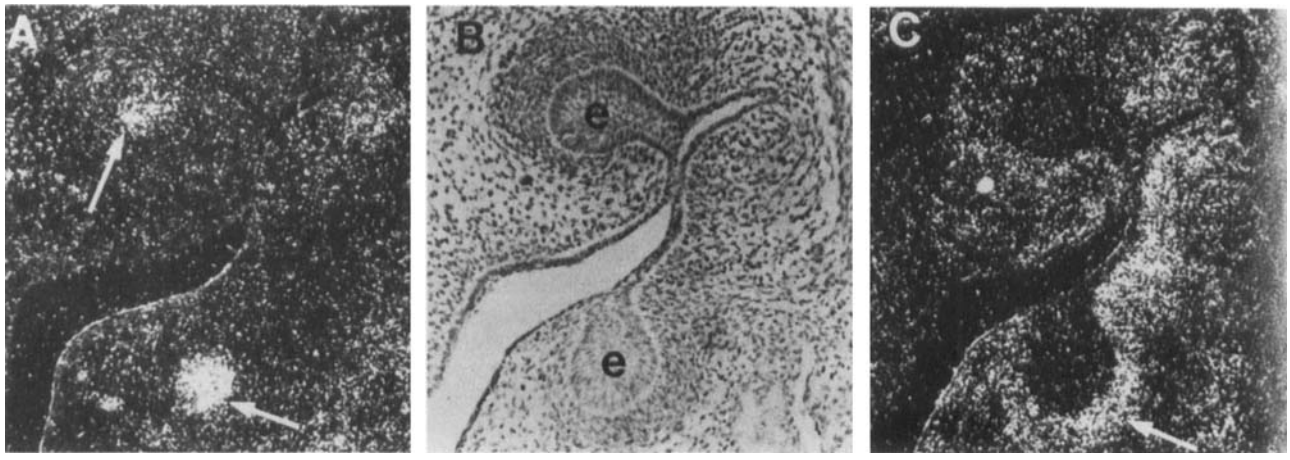

Fig. 2. Expression of the growth factors *Bmp-2* and *Bmp-4* (bone morphogenetic proteins) in mouse embryonic molar tooth germs at the bud stage. A. *Bmp-2* is expressed in the dental epithelium (arrows). B. A comparable section to those in A and C, as seen in bright-field illumination. C. *Bmp-4* is expressed in mesenchymal cells (arrow). Source: Thesleff I. Acta Odontol Scand 1995: 53: 129–134.

pressed in the neuroectoderm. In the case of the second and later arches, this code represents a combination of expressions of the Hox genes. Such genes are not expressed by rhombomeres 1 and 2. Instead a new set (in evolutionary terms) of homeobox genes has been developed that includes three families, the Msx genes, the Dlx genes and the Goosecoid gene. These patterning genes, originally expressed and coded in the neuroectoderm, are carried to the first arch and are later expressed in the ectomesenchyme. Analysis of the expression of these various genes in first arch ectomesenchyme has revealed the existence of a simple homeobox code associated with the eventual development of the dentition (14, 32).

Odontogenesis

Odontogenesis is the term used to describe the development of teeth. It is a complex process involving the gamut of induction, differentiation and morphogenesis. The initiation of tooth development has long been a matter of debate, with experimental evidence presented to support either a lead role for the epithelium of the first arch or, conversely, the ectomesenchyme of first arch. This debate has been reviewed recently (40) and is only summarized here. Initially, the bulk of experimental evidence indicated a lead role for ectomesenchyme in the initiation of odontogenesis. For example, the recombination of first arch mammalian ectomesenchyme with avian epithelium (9) produced "hen's teeth"; recombination of first arch mesenchyme with embryonic plantar (foot) epithelium changed the developmental direction of the epithelium to form an enamel organ (8), and recombination of a molar papilla with an incisor enamel organ resulted in molar development (7). On the other hand, when neural crest tissue from differing sites was combined with epithelium from different sites, dentinogenesis was initiated only with first arch epithelium (13).

These apparent differences were then explained by the recognition that the temporal factor is of importance (17). Thus, when first and second branchial arches of mouse embryos of 9–13 days gestational age (E9–E13) were dissected out, their epithelial and ectomesenchymal components separated and recombined heterotypically, the outcome was that when mandibular arch epithelium was combined with second arch mesenchyme, teeth formed only in E9 through E12 day material, with the highest incidence occurring at E11 days. No teeth formed in E13 day material. On the other hand, when mandibular arch ectomesenchyme was recombined with second arch epithelium, tooth formation resulted only in E12 and E13 day grafts. These results indicate that first arch epithelium has odontogenic potential up to E12 days of gestation, and is able to elicit a reaction from ectomesenchyme of the second arch. Thereafter, this odontogenic potential is lost from the epithelium but interestingly is now assumed by the ectomesenchyme. This finding not only indicates an epithelial role in odontogenesis, but emphasizes the importance of chronology in experimental design.

But it is now understood that the situation is a little more complex than either/or. Earlier it was explained that, as the neural crest cells migrated from

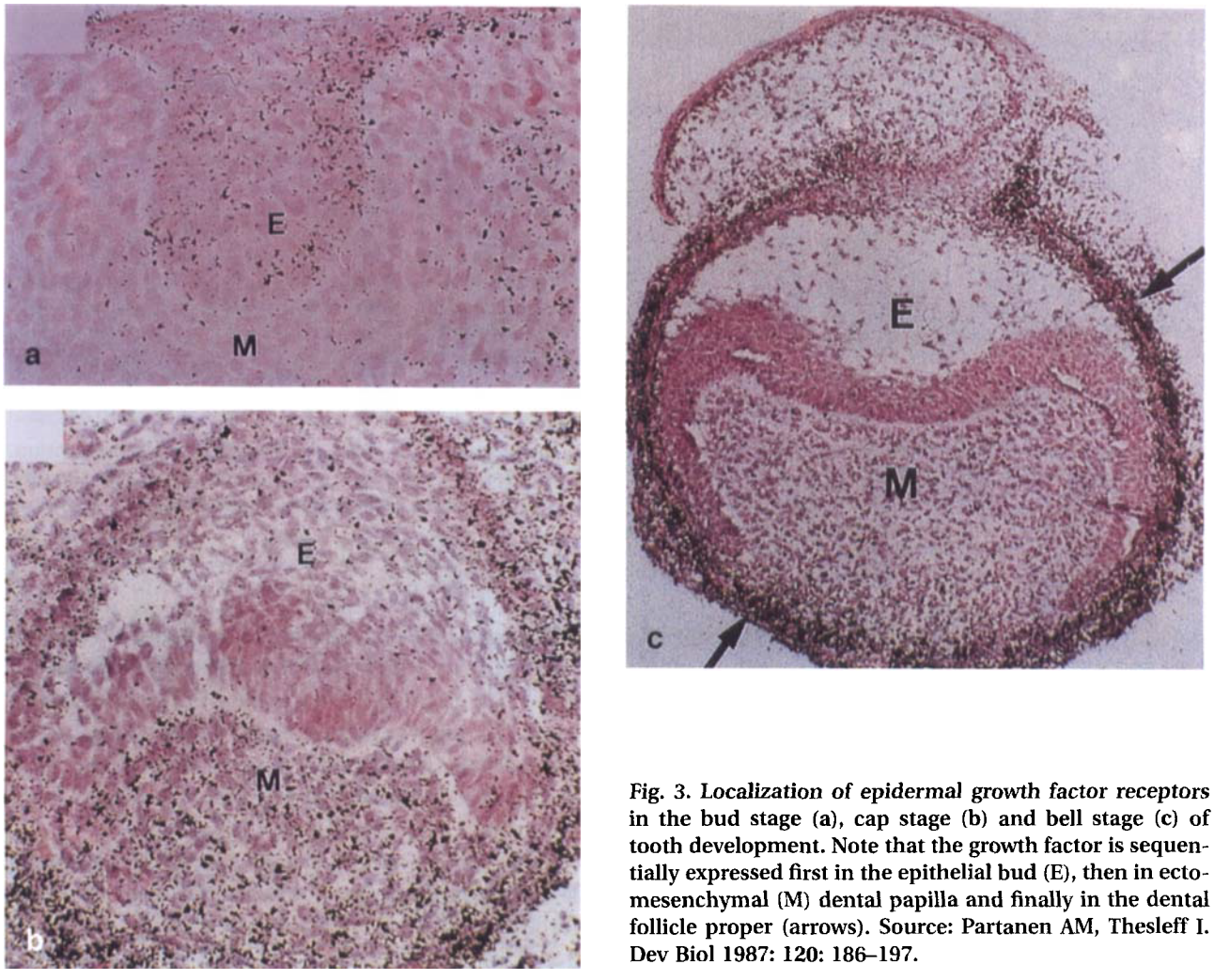

Fig. 3. Localization of epidermal growth factor receptors in the bud stage (a), cap stage (b) and bell stage (c) of tooth development. Note that the growth factor is sequentially expressed first in the epithelial bud (E), then in ectomesenchymal (M) dental papilla and finally in the dental follicle proper (arrows). Source: Partanen AM, Thesleff I. Dev Biol 1987: 120: 186–197.

the rhombomeres, they carried with them homeobox genes coded to initiate dental development by signaling the overlying first arch epithelium to thicken and form the primary epithelial band. This expression is then down-regulated (14). Within the primary epithelial band, tooth development is initiated at specific sites by the expression of various growth factors in the epithelium, in particular BMP2 (45) which, in turn, locally affects the ectomesenchyme to once again up-regulate and express Msx-1 and Msx-2 and subsequently BMP (Fig. 2). In other words, there is a transfer of patterning and signaling mechanisms from the epithelium to the ectomesenchyme. The important point is that this local up-regulation involves not only the ectomesenchyme, which forms the dental papilla, but also the ectomesenchyme, which forms the dental follicle proper. Indeed, all the expressions of the dental mesenchyme, be they further expression of homeobox genes, growth factors or the expression of various extracellular matrix molecules, such as syndecan or tenascin, always involve the dental follicle proper. A good example of the cascade of signaling is provided by the shifts in the expression of epidermal growth factor (Fig. 3) during early tooth development.

The dental follicle proper is the formative organ for the tissues of tooth support

Twenty-five years ago, knowledge of the development of the periodontium was limited, imprecise and based almost entirely on descriptive studies (37). The definition of the term dental follicle from which the tooth-supporting tissues supposedly derived was generally considered to describe the tissue between the tooth germ and the forming bone of the jaw and was subdivided into either two compartments (bone and tooth) or three (bone, tooth and intermediate). Nor was the contribution of these varying zones to the development of tooth support

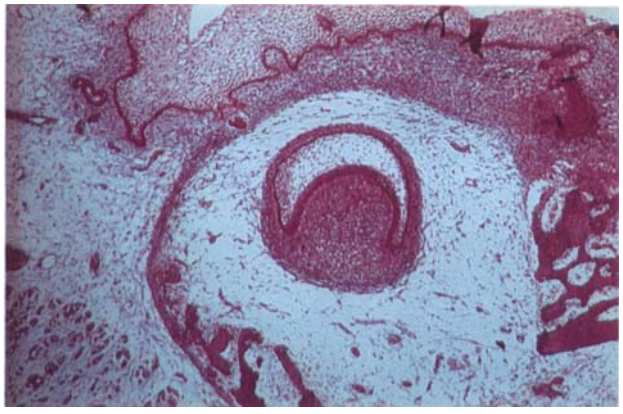

Fig. 4. Human tooth germ at the bell stage of development. This slightly oblique section clearly shows the continuity of the dental follicle proper with the dental papilla.

understood. As to the origin of the follicle, Scott (30) indicated that it was derived from the dental papilla, although the evidence for this was not presented. On the basis of a careful descriptive analysis of histological sections and some histochemical observations, I argued (37) that the term dental follicle should be reserved for the inner or investing layer (the tooth-related compartment), as there seemed to be sufficient evidence to indicate that this layer gave origin to both the cementum and periodontal ligament. It was pointed out that this layer was continuous with the dental papilla (Fig. 4), but no comment was made as to its possible origin from the papilla, as claimed by Scott (30). Rather, it was assumed that just as the enamel organ initiates the formation of the dental papilla, it also initiated the formation of the investing layer.

The only experimental evidence available at that time contradicted the conclusions drawn from a descriptive analysis. In these experiments (5) developing molar teeth, removed from their follicles, were transplanted from newborn hamsters into a subcutaneous site in adult animals. Development continued in this ectopic location with the formation of cementum, periodontal ligament and "alveolar" bone, and it was therefore argued that the enamel organ and dental papilla had the ability to differentiate the tissues of tooth support from ectopic connective tissue and that the formation of tooth supporting tissue was the "effort of a morphogenetic field to complete itself" (5). However, it was properly noted that:

> ... additional evidence should be obtained to establish conclusively that no transplanted cells were the precursor to the periodontal tissues formed. Without doubt, a certain few cells adhered to the outer enamel epithelium and were transplanted. It seems improbable that these cells could have been responsible for the extensive formation of the periodontium routinely seen around transplanted teeth after 28 days in the host subcutaneous tissues (37).

This caution was more than appropriate, for my previous experience in the dissection of tooth germs suggested that the presence of a follicle was key to maintaining the integrity of the tooth germ. At that time I commented that:

> ... it is possible that in removing the tooth germs from their dental sacs Hoffman (5) obtained the same results as removal of human tooth germs, and that the 'certain few cells' represented the ectomesenchymal cells of the investing layer" (37), that is, the dental follicle proper.

To determine the correctness of the matter, Hoffman's (5) experimental approach was modified (41). Murine tooth germs were first dissected out and flash labeled with tritiated thymidine in culture medium. Control sections assured that the follicular cells had captured label. The tooth germs were then transplanted to a subcutaneous location where development continued with the formation of cementum, periodontal ligament and alveolar bone (Fig. 5a, b). The demonstration of labeled cementoblasts and ligament fibroblasts (Fig. 6) established their derivation from donor material, namely the tooth germ. The origin of bone was not established with certainty as labeling was minimal and could be attributed to background, although the demonstration of lymphocytes on the external surface of the bone (42) suggested the initiation of a rejection response and therefore its origin from donor tissue.

The issue of whether this bone derived from follicle was seemingly nicely settled by altering the experimental design so as to grow teeth in an intraocular location rather than subcutaneously (44). A problem with the subcutaneous location is that it is known that a number of foreign agents can induce bone in this location by marshaling osteoblasts from presumably undifferentiated mesenchymal cells. In the anterior chamber of the eye, such potential osteogenic precursor cells are absent. When various recombinations of dental follicle proper, dental papilla and enamel organ were transplanted intraocularly, teeth formed with associated supporting tissues, including bone. Significantly, with this ex-

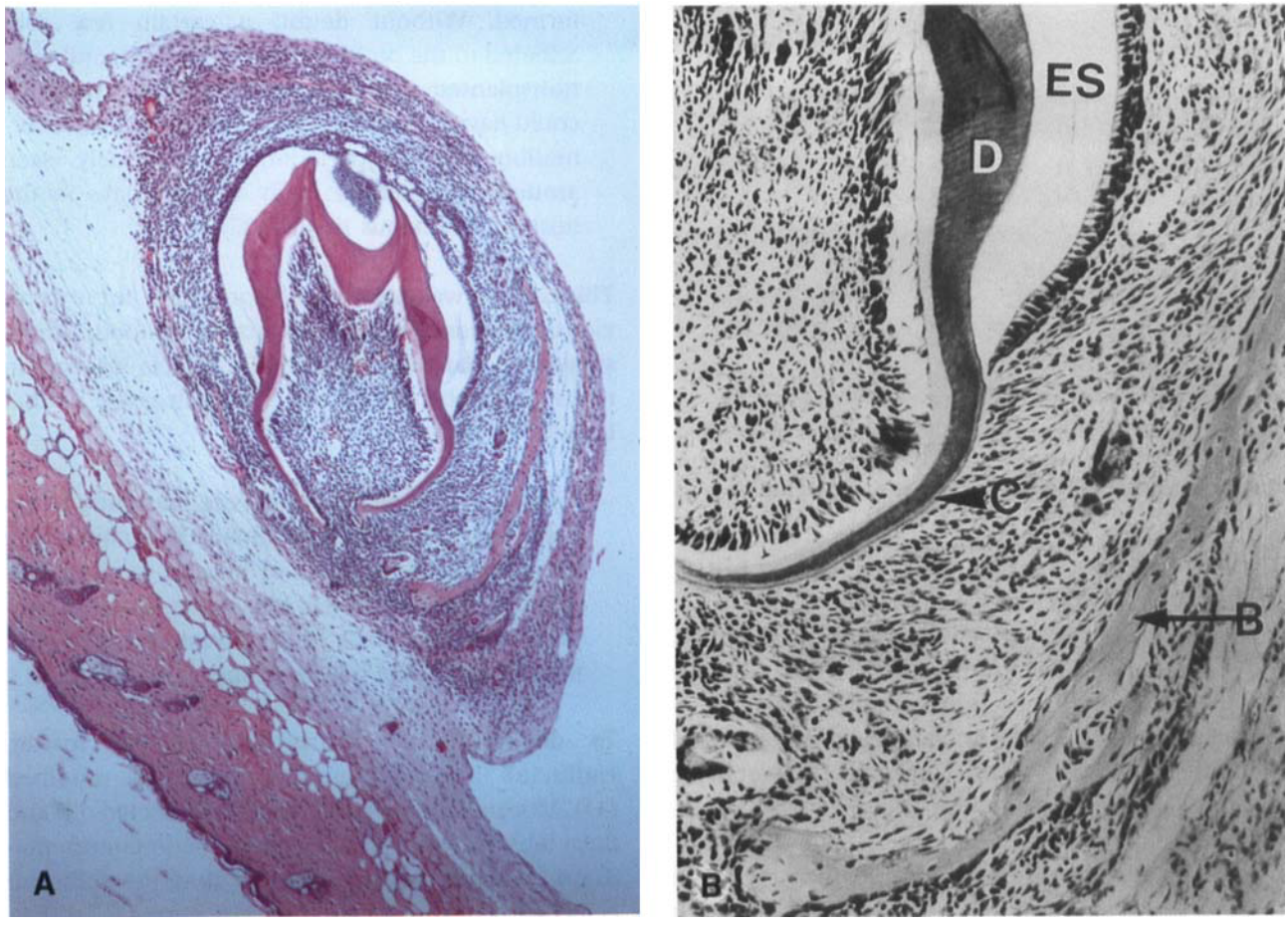

Fig. 5. Three-week subcutaneous implant of a 1-day-old mouse molar tooth germ. A. Development has continued with the formation of the tissue of tooth support. B. D: dentin; ES: enamel space; C: cementum; B: alveolar bone. Source: Ten Cate et al. (41).

perimental design, Yoshika & Kollar (47) were able to show that a recombination of enamel organ and dental papilla alone produced all the diverse, fully differentiated structures of tooth support, including bone, indicating that follicle derives from papilla.

This observation fits very nicely with both a theoretical (27) and experimental analysis (28) of the evolution of various forms of tooth attachment. In the theoretical analysis, a cladistic interpretation of development was presented to explain the various forms of tooth attachment that exist in animals. Clades (clones) describe populations of cells that, during evolution and development, become committed sequentially to form the various components of the tooth and its attachment. To substantiate this theoretical analysis, it was essential to show some form of sequential differentiation of cell populations in odontogenesis, and this was achieved when it was shown that papillal cells (the attachment clade) migrated into the follicle at the bell stage of tooth development (28), thereby confirming Yoshika & Kollar's (47) *in vitro* (ocular) findings. This demonstration of papillal cells migrating into the dental follicle proper with clearly the ability to differentiate into the tissues of tooth support raises the question as to the role of the initial condensation of ectomesenchyme around the epithelial tooth bud forming at the same time as the dental papilla. It is only possible to speculate on this question, as it has not been addressed experimentally, and it may be that this initial condensation does no more than define the boundary of odontogenic tissue.

Does alveolar bone proper derive from dental follicle?

There is one remaining problem before it can be stated equivocally that all the tissues of tooth support stem from a particular clade of cells: whether alveolar bone proper is a developmentally precise

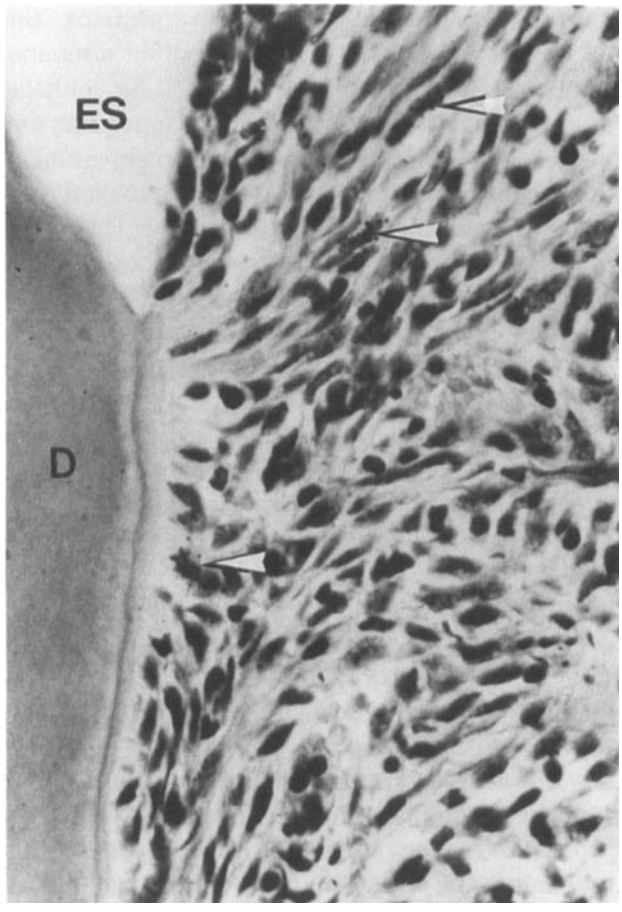

Fig. 6. Autoradiograph of a 3-week subcutaneous implant of a 1-day-old mouse first molar tooth germ. A cementoblast and two fibroblasts (arrows) are labeled indicating origin from donor tissue. Source: Ten Cate et al. (41).

tissue of tooth support. This dubiety arises from a single piece of work by Palmer & Lumsden (29). They extended the experimental design of Yoshika & Kollar (47) to investigate in more detail temporal effects and found that, in recombinations of early embryos, bone formation only occurred when the follicle proper is included in the recombinations, raising the possibility of contamination of the odontogenic tissue during dissection. On the other hand, it has been clearly shown (38) that, as the periodontal ligament forms, bone is deposited on the crypt wall to establish the width of the periodontal ligament and a root-analogous socket. It has also been shown (15) that, when disassociated enamel organ and dental papillal cells are recombined in an ectopic location, bone forms. Osborn (27) also argued that alveolar bone evolved from the late periodontal clade and that its lineage is different from that of jaw bone and that this clade fills a gutter between buccal and lingual plates of jaw bone and spreads over the coronal crest of the jaw bone. In support of his argument, he quotes Mummery's (18) observation that the lower posterior molars of the manatee develop within a shell separated by soft tissue from the ramus of the jaw and his own observation that the successional teeth in the elephant develop within the ramus of the mandible encased in bone, which is quite separate from the bone of the jaw. Assuming that Palmer & Lumsden's (29) finding is not an experimental aberration, it is possible that alveolar bone stems from the initial condensation of ectomesenchyme around the early tooth germ and that the ligament and cementum stem from papillal cells that have migrated into the follicle.

In sum, all the evidence to this point indicates that the tissues of tooth support, that is, cementum, periodontal ligament and alveolar bone, represent a functional unit, the dental follicle proper, defined developmentally as odontogenic tissue, and a product of the cascade of signaling initiated initially in the first arch epithelium and responded to by the ectomesenchyme of the same arch surrounding the early tooth germ. Based on the evidence presented, it is both logical and consistent to reserve the term dental follicle proper for this layer immediately encapsulating the tooth germ as it is clearly odontogenic. Thus, the enamel organ is the formative organ of enamel, the dental papilla the formative organ of the dentine pulp complex and the dental follicle proper, the formative organ for the tissues of tooth support.

The hyaline layer of Hopewell Smith as a tissue of tooth support

Classically, the tissues of tooth support consist of cementum, periodontal ligament and alveolar bone. It needs to be considered whether a fourth tissue needs to be added to this list: the hyaline (homogeneous) layer of Hopewell Smith, sometimes also erroneously called intermediate cementum. This hyaline layer is found on the surface of root dentine extending (at least in single-rooted teeth) from the cementoenamel junction to the apical third of the root where its identity is generally lost. Since its first description by Hopewell Smith (6), the nature and function of this layer was largely ignored except by a few professional dental histologists, who largely debated whether it was a variation of either cementum or dentine. Owens (22–25) first established that the hyaline layer was most likely a form of dentine after studying ground sections of teeth exposed to suc-

cessive doses of tetracycline. He was able to show essentially two things. First, that the mineralization front of dentine (as revealed by the tetracycline staining) continued into the hyaline layer by sharply changing direction and running coronally (see Fig. 10 of Bosshardt & Selvig in this volume). Second, that there was an apparent continuity between predentine and the hyaline layer. Furthermore, it was argued that, as some thickness of dentine is formed before Hertwig's epithelial root sheath fragments, and because of the demonstrated continuity of the layer with predentine, the hyaline simply could not be cementum. These observations, based on light microscopic studies, were essentially confirmed by Bosshardt & Schroeder (1, 2) in their ultrastructural studies of root formation in human premolar teeth. They found that an unmineralized layer exists between the forming mantle dentine and the root sheath, which was continuous with predentine. However, morphological continuity between these two tissues does not necessarily imply structural and compositional similarity, and differences have been determined following developmental and analytical studies. Thus, independent studies (11, 26, 39) describing root formation in rat, mouse and dog all found that, following the initiation of root-forming odontoblasts and the deposition of radicular mantle dentine, root sheath cells develop the armamentarium for secretion. Further, all recognized that the first formed radicular mantle dentin was not deposited directly against the basal lamina of the root sheath (as is the case in coronal mantle dentinogenesis) but a few microns away from it. The "gap" so formed initially consists of papillal extra-cellular material consisting of a few fine collagen fibers and noncollagenous extracellular matrix molecules. It is into this milieu that the epithelial cells secrete. However the studies of Bosshardt & Schroeder (1, 2), although essentially confirming the presence of an unmineralized layer on the root surface of human teeth, suggest that in humans there may be some developmental differences. First, the root sheath is not in contact with the root surface and second, the unmineralized layer resembles predentine in having a high collagen fibril content rather than the more structureless milieu described in other species. Even so the fact remains that the root sheath cells were once in contact with papillal tissue (to initiate the differentiation of odontoblasts), and the potential for the secretion of epithelial products exists.

Attempts at determining the nature of this epithelial secretory product first began in 1976 when Slavkin (33) indicated that the lingual surface of the rabbit incisor contained enamel-like proteins. This is perhaps not the best animal model for a number of reasons, but it was assumed that these epithelial products were confined to acellular cementum, and the prediction was made that acellular cementum on the forming root surfaces of other mammalian teeth would also contain enamel-like proteins. That this is the case was established by a further series of papers coming from Slavkin's laboratory (12, 33, 35). Thus, in 1988 the secretion of enamel-like proteins by cells of the root sheath onto the root surface in mouse molar tooth germs grown in a chemically defined medium using immunocytochemical techniques was demonstrated (34). Biochemical and immunochemical characterization of this secretory product revealed it to be a distinct class of protein within the family of enamel proteins, and it was suggested that it resulted from alternate splicing of the structural gene for enamel protein. The only dissension to these findings comes from Lou et al. (12), who were unable to detect the transcription of amelogenin by root sheath cells, but nevertheless postulated that root sheath cells synthesize proteins which contain amelogenin domains. Recently Nanci (21) has confirmed the findings emanating from Slavkin's laboratory and, using the technique of immunogold labeling, has determined in the rat the presence of enamel-like proteins at the surface of root dentine. Finally Slavkin et al. (35) have detected proteins immunologically related to enamel proteins in human cementum.

If it is accepted that the hyaline layer is a reality, that it is neither a form of cementum or dentine (with the possible exception that it is a form of predentine in the human) and that it is a tissue in its own right consisting of an admixture of papillal products and enamel-like proteins, a function for this layer needs to be determined. What evidence there is would seem to indicate that the hyaline layer is involved in "cementing" cementum to radicular dentine. Thus, Owens (25) described the formation of a fibrous fringe against the hyaline layer in dogs, and Yamamoto & Wakita (46) described the initial attachment of collagen fibers to root dentine in the rat as mediated through a "ruthenium red" stained layer. Bosshardt & Schroeder (1), in the human developing premolar, also described tiny bundles of collagen fibers becoming stitched to the non-yet mineralized dentinal surface (read hyaline layer), and these bundles subsequently become part of the matrix of acellular cementum. Further circumstantial support for a cementing role for the hyaline layer can be drawn from experimental data derived from

studies of periodontal repair. Such studies involve root planing and subsequent deposition of reparative cementum on the treated root surface. An artifactual split is found between the newly formed reparative cementum and the prepared root surface in paraffin-embedded sections, whereas no such split seems to occur in plastic-embedded sections. As shrinkage is a well established feature in the preparation of paraffin sections, it is entirely possible that, because of root planing and the removal of the hyaline layer, reparative cementum is simply opposed to the root surface rather than attached to it. This consequence again has significance in any consideration of periodontal repair.

In conclusion, the hyaline layer seems to be involved in tooth support and, while largely ectomesenchymally derived, it does contain epithelial derived enamel-like proteins, whose role has yet to be determined.

Is cellular cementum derived from the dental follicle?

Based on morphology, many observers consider cellular cementum to be bone-like, and this consideration is strengthened when the phenotypic expression of cementocytes is studied, where the results show that cementoblasts share many phenotypic characteristics with osteocytes (3, 43). In particular, a monoclonal antibody (E11), which specifically stains osteocytes and osteoblasts, also stains cementoblasts and cementocytes of cellular cementum (43). This monoclonal antibody does not stain the cells associated with acellular cementum formation.

Not only may the two populations of cementoblasts be phenotypically distinct, but they also may have different developmental origins. The case for the cells associated with the development of acellular cementum being derived from follicular cells is strong. Equally, an almost as strong a case can be made to indicate that cells forming cellular cementum might be derived from extraligamentary sources. For example, progenitor cells in endosteal spaces can migrate via communicating channels into the periodontal ligament (16). A recent article (10) demonstrated that, when cells from alveolar bone were cocultured with extracted dental roots, a tissue resembling cellular cementum is deposited. In contrast, when ligament cells were cocultured in a similar manner, no calcified tissue formed and instead the cells synthesized a connective tissue with orientated fiber bundles attached to both host bone and root, resembling periodontal ligament. Of particular interest here is the unexplained mechanism by which the fiber bundles attach to the cementum.

In sum, the real possibility exists that acellular and cellular cementum represent distinct tissues formed by cells with different phenotypes and possessing different developmental origins.

Why should alveolar bone proper form as odontogenic tissue?

The origin of alveolar bone proper has already been discussed and shown to derive from the follicular layer. Why is it necessary to form this bone rather than harness the activity of the bone cells lining the cryptal wall? A case can be made that the formation of alveolar bone proper is essential to establish attachment. It has been shown (2) that ligament fiber bundles, as they form, gain attachment by remodeling to the dense collagen fiber bundles that are packed and orientated nearly perpendicular to the root surface and that extend from the surface of the acellular cementum. A similar situation may well exist on the bone surface. Grant & Bernick (4) have described the development of ligament fiber bundles, and an essential feature is the initial development of fibers protruding from the bone surface a short distance into the ligament to only later become part of the principal fiber bundles. The similarity of this developmental sequence is strikingly similar to the events occurring at the cemental surface and calls for an ultrastructural investigation of the events occurring as alveolar bone proper forms and whether the Sharpey fibers also are a product of the dental follicle proper.

Conclusion

The combination of newer investigative techniques of recombinant DNA technology and immunocytochemistry with the more established techniques of tissue recombination have clearly established that the dental papilla and dental follicle proper are derived from embryonic connective tissue derived from neuroectoderm (the neural crest). It has also been shown that a cascade of signals (not all as yet worked out) exists involving homeobox genes and growth factors, to initiate odontogenesis and the dif-

ferentiation of dental tissues. These tissues include those of tooth support, namely acellular cementum, periodontal ligament and alveolar bone proper (bone of attachment?).

While there is still some discussion as to the exact nature of the root surface, it is clear that it is specialized in such a way so as to permit the firm union of acellular cementum to it.

A fundamental question that needs to be addressed is whether the cascade of signals required to bring about normal odontogenesis, which includes the development of tooth support, is further required to initiate regeneration or repair of the supporting apparatus of the tooth. While it may be a matter of semantics, it is unlikely that regeneration, defined as the restoration of the normal architecture of a tissue, can be achieved. The biological signaling mechanisms that have been described for odontogenesis also occur in the development of many other organs ranging from limb bud development to gland formation. In these latter situations no evidence exists that regeneration is possible and it is therefore difficult to argue that the tissues of tooth support represent a unique situation. Put another way, is there any evidence that acellular cementum, a true odontogenic tissue, re-forms? The extensive literature on restoration of tooth support would indicate that, when restoration does occur, it is achieved by a reparative process that involves the deposition of cellular cementum which, it has been argued, is likely not to be a true odontogenic tissue.

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