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A Survey of Cell Biology

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Biological Problems of Regenerative Cementogenesis: Synthesis and Attachment of Collagenous Matrices on Growing and Established Root Surfaces

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I. Introduction

During the past decade, regeneration of periodontal tissues has received increasing and worldwide attention, from both oral biologists and dental clinicians. "Periodontal regeneration" is defined as the restoration of the various components of the periodontium, i.e., alveolar bone, periodontal ligament, root cementum, and gingiva lost due to disease, "in their appropriate locations, amounts, and relationships to each other" (Aukhil, 1991). In contrast to more simple goals such as the reattachment of the periodontal ligament and supraalveolar connective tissue to the torn cell/fiber tissue at the dental root surface, following their short-term separation, or as the spontaneous repair resulting from unguided wound healing, "periodontal regeneration" requires an enormously well-conducted action of various cell populations to appear and function in space and time in order to reconstitude both structural normality and functional integrity.

As the development, structure, and function of the human periodontium, being dissimilar in details to that of laboratory animals such as rodents, dogs, and non-human primates, are immensely complex biologically and not entirely understood (Schroeder, 1986), clinical and laboratory experiments in humans and other animals designed to study the regenerative potential of the various tissue components under conditions of different defects and treatment modalities are exceedingly difficult to analyze. In fact such experiments necessarily address the periodontium as a whole rather than being able to examine separately the response of component tissues. In addition, the situation is rendered even more complex by the

fact that the creation of artificial defects or the pretreatment of spontaneous lesions caused by bacterial infection and inflammation introduce artificial tissue alterations that interfere with repair and regeneration. For these reasons, clinical and laboratory experiments on periodontal regeneration, reviewed by Egelberg (1987), Nyman *et al.* (1989), and Minabe (1991), have yielded widely different results that remain insufficient from a clinical, and invalid from a biological, point of view, results that to a varying extent appear as biologically undecodable messages from a black-box world. Not surprisingly, periodontal regeneration is loaded with problems, clinical, such as reinfection and mechanical disturbance of the wound healing processes, as well as biological. The latter include the physical and chemical denaturation of treated root surfaces; epithelial migration over the exposed root surface sites that should be the substrate for regenerative tissue formation; the different turnover and growth potentials of the four periodontal tissue components; and innumerable problems regarding cells, mediators, growth factors, etc. (Abdallah *et al.*, 1988; Terranova *et al.*, 1989a; Messadi and Bertolami, 1991; Aukhil, 1991).

One reason for the unsatisfying situation was and still is the fact that human cementogenesis remained undiscovered for too long. Indeed, at the beginning of the research focusing on periodontal regeneration, our knowledge of dental root cementum was incredibly meager, resting on antique rather than medieval information. Root cementum represents, however, the most cardinal periodontal tissue component that is primary and indispensible for regenerating the tooth–bone connection, i.e., for reconstituting tooth anchorage. This review provides some of the missing data needed to discuss spontaneous and regenerative cementogenesis and unveils some of the biological problems involved.

II. Origin, Types, and Function of Root Cementum on Human Teeth

A new and increasingly accepted classification of root cementum on human teeth was proposed by Jones (1981) and with modifications adopted by Schroeder (1986). It differentiates among four varieties according to the absence or presence of cells and to the source of collagen fibers, i.e., the major matrix component contained within. Consequently, this classification distinguishes between acellular afibrillar cementum (AAC); acellular extrinsic fiber cementum (AEFC); cellular intrinsic fiber cementum (CIFC) that may also occur as an acellular variety (AIFC; Bosshardt and Schroeder, 1990); and cellular, mixed stratified cementum (CMSC). These varieties are summarized in Table I.

TABLE I
Types of Human Root Cementum

Terms	Abbreviation	Organic components	Location	Function
Acellular, afibrillar cementum	AAC	Homogeneous matrix, no cells, no collagen fibrils	At dentino–enamel junction, on enamel	Unknown
Acellular, extrinsic fiber cementum	AEFC	Collagen fibrils as Sharpey's fibers, no cells	Cervical to middle root	Tooth anchorage
Cellular, intrinsic fiber cementum	CIFC	Intrinsic collagen fibrils and fibers, cementocytes	Apical and interradicular root surfaces, resorption lacunae, fractures	Adaptation, repair
Acellular, intrinsic fiber cementum	AIFC	Intrinsic collagen fibrils and fibers, no cells	Apical and interradicular root surfaces	Adaptation
Cellular, mixed, stratified cementum (AEFC + CIFC/AIFC)	CMSC	Intrinsic collagen fibrils and fibers, collagen fibrils as Sharpey's fibers, cementocytes	Apical and interradicular root surfaces	Adaptation, root anchorage

Apart from a homogeneous and mineralized ground substance of unknown composition, AAC contains neither cells nor collagen fibrils. In humans, it is found as coronal cementum covering patchwise the cervical enamel, and as an occasional part of cervical AEFC (Schroeder 1986, 1988). Its origin and function are unknown, but it may represent serum-derived organic material coprecipitated with mineral (Beertsen and Van Den Bos, 1991). The AEFC lacks cells and is composed entirely of densely packed, well-oriented bundles of collagen fibrils, i.e., the so-called fibers of Sharpey. These fibers continue into the periodontal ligament and connect the root to the alveolar bone. Thus, all AEFC fibers are extrinsic. About 30,000 fibers insert into 1 mm^2 of AEFC surface, each fiber being about 4 μm in diameter (Schroeder, 1986). In humans, the rather thin (20 to 250 μm), densely mineralized AEFC shows parallel incremental lines and is found primarily on the cervical and middle root regions, but it may extend further apically. It is formed by fibroblasts of the dental follicle

proper, i.e., ectomesenchymal derivative, and later of the periodontal ligament, and serves exclusively for tooth anchorage (Schroeder 1986, 1988). The CIFC contains cells, the cementocytes, but its collagen fibers, being again the major matrix component, are all intrinsic and run a circular or spiral course around the root, i.e., more or less parallel to the root surface. Thus, CIFC lacks Sharpey's fibers. In humans, the less well mineralized CIFC is found mainly in situations of repair, filling resorption lacunae or connecting root fracture fragments. It is also part of CMSC, forming initially on apical root portions and patchwise between layers of AEFC. The CIFC is formed by cementoblasts of the dental follicle proper and later of the periodontal ligament; its function is associated with repair and adaptation. On particular portions of the root, AIFC may form without leaving cementocytes behind. CMSC is a mixture of pure AEFC and CIFC/AIFC; the latter part may contain cementocytes with uneven distribution and density. The CMSC is usually a stratified tissue, with consecutive or alternating layers of AEFC and CIFC/AIFC being unpredictably superimposed on one another. In humans, the inhomogeneously mineralized and, in part, porous CMSC is variably thick, ranging from 100 to 600 μm or more, and occurs primarily in the apical third of the roots and in the furcations. It serves the functions of adaptation, i.e., a dynamic reshapening of the root surface as the tooth shifts and drifts in its socket, and, if superficially covered by AEFC, of root anchorage (Table I; Schroeder, 1986, 1988).

Measurements of sequential fluorochrome labeling lines in all deciduous teeth and the permanent molars of one ~13-month-old *M. fascicularis* monkey (Bosshardt *et al.*, 1989) and in the alveolar bone surrounding the first molars (Schroeder *et al.*, 1992) provided data for the formation rate of two cementum varieties, in comparison to that of dentine, and bone

TABLE II

Rates of Formation of Cementum, Dentine, and Bone

Tissue	Abbreviation	Formation rate (μm/day)[a] $\bar{x} \pm s$
Acellular, extrinsic fiber cementum	AEFC	$< 0.10 \pm 0.02$
Cellular, intrinsic fiber cementum	CIFC	
Initial layer		0.4–3.1
Appositional layers		0.1–0.5
Crown dentine (first molars)	CD	3.1 ±0.2
Root dentine (deciduous teeth)	RD	2.7–4.6
Root dentine elongation (deciduous teeth)	RDE	12.0–36.0
Alveolar bone crest (first molars)	ABC	5.0–14.0
Alveolar bone septum (first molars)	ABS	13.0–22.0

[a] Measured in one sequentially fluorochrome-labeled *M. fascicularis* monkey; from Bosshardt *et al.* (1989) and Schroeder *et al.* (1992).

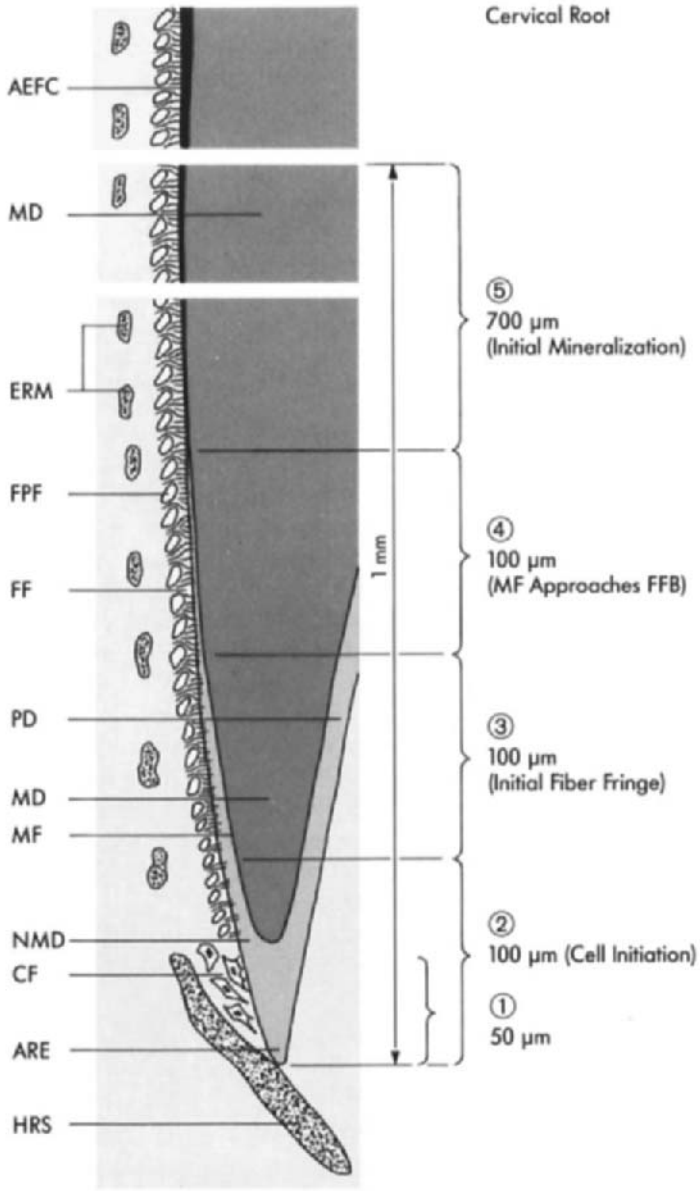
and to root elongation (Table II). These data demonstrated that AEFC is an extremely slowly forming tissue, initially as well as later in life (Sequeira *et al.*, 1992). In humans, its daily rate of formation, i.e., increase in thickness, is smaller that 0.1 μm, possibly as low as 0.005 to 0.01 μm (Dastmalchi *et al.*, 1990; Sequeira *et al.*, 1992). In contrast, initial CIFC is formed at a fast rate, ranging from 3.1 to 0.4 μm/day. Subsequently, appositional cementum layers, possibly of the AIFC variety, may still form at a faster rate than AEFC, i.e., 0.1 to 0.5 μm/day. Comparatively, CIFC may form as rapidly as crown and root dentine and not much slower than alveolar bone (Table II).

III. Spontaneous Cementogenesis and Matrix Formation on Growing Root Surfaces

In contrast to amelogensis and dentinogenesis, i.e., to rather well-defined developmental systems characterized by particular classes of cells and their morphologically and biochemically defined matrix products, cementogenesis on human teeth was essentially unknown until 1985, although some fragmentary information was available for other mammalian species such as rodents. This information had been derived from studies on mouse incisors and molars (Selvig 1963, 1964, 1967) and on rat molars (Paynter and Pudy, 1958; Diab and Stallard, 1965; Lester, 1969; Formicola *et al.*, 1971; Owens, 1980). Although such molars are also covered by both acellular and cellular cementum, on their roots, and albeit most recent investigations of Cho and Garant (1988, 1989) and Yamamoto and Wakita (1990, 1991, 1992) demonstrated some similarity in matrix production and attachment to dentine, there are a number of reasons for the argument that root development and cementogenesis in rodent molars might be unlike that in humans (see below). Therefore, this review focuses primarily on cementogenesis in human teeth and recent observations in rodents will be used only comparatively.

A. Acellular Extrinsic Fiber Cementum

In human teeth, AEFC covers the cervical root surfaces and extends from the cemento–enamel junction apically. In single-rooted teeth (i.e., incisors, canines, and most premolars), AEFC coats 60 to 90% of the total root length that varies between 13 (central incisors) and 15.5 mm (canines; Schumacher and Schmidt, 1983; Schroeder, 1988). AEFC is first formed while the roots develop. AEFC formation begins at and along the growing root edge and the AEFC slowly increases in thickness in the coronal direction. As shown in human premolars with incomplete roots developed

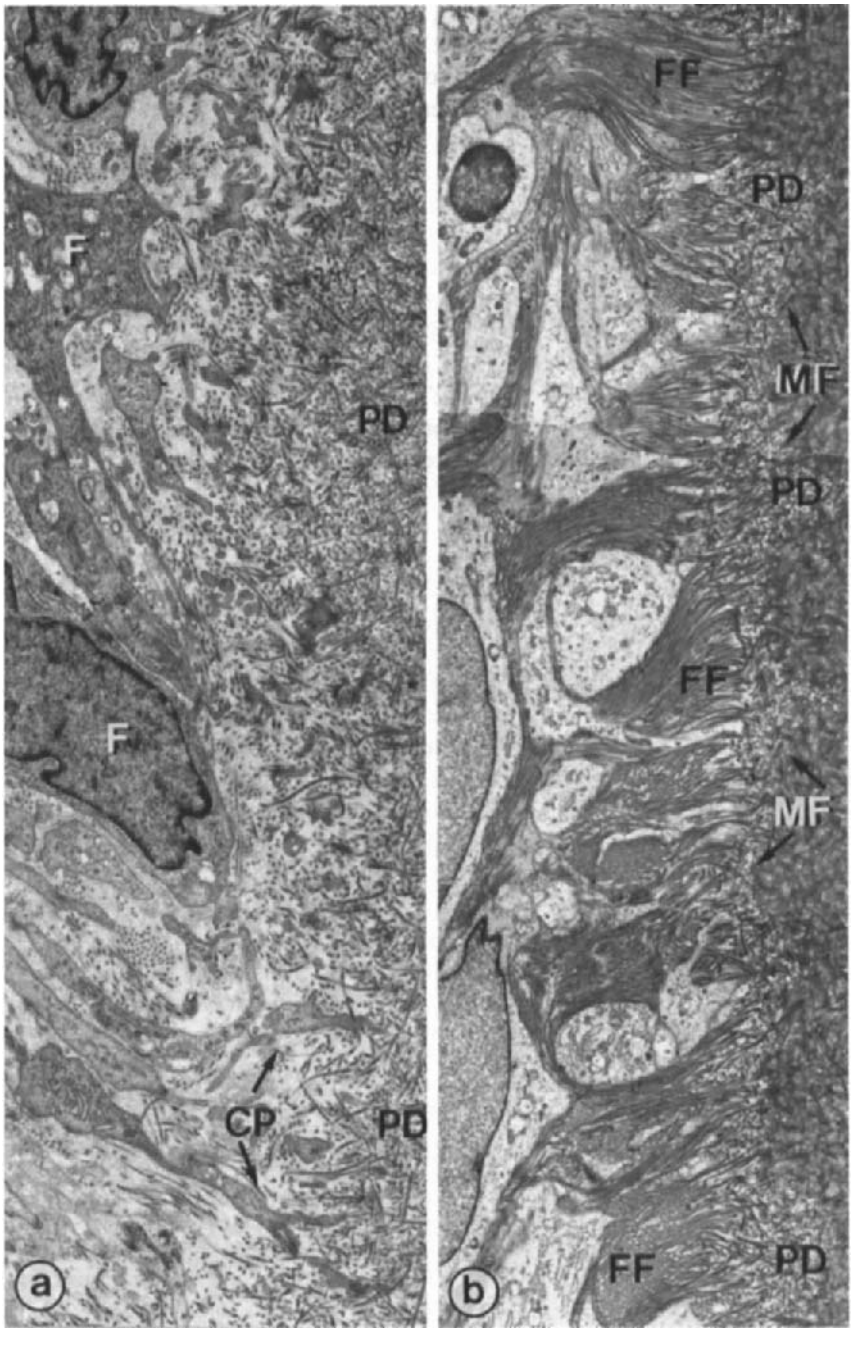

Cervical Root
AEFC
MD
ERM
FPF
FF
PD
MD
MF
NMD
CF
ARE
HRS
1 mm
⑤
700 µm
(Initial Mineralization)
④
100 µm
(MF Approaches FFB)
③
100 µm
(Initial Fiber Fringe)
②
100 µm (Cell Initiation)
①
50 µm

50 to 60% of their final length (Bosshardt and Schroeder, 1991a), initiation of AEFC formation, early matrix production, and its attachment to root dentine all take place within a zone of about 300 μm, extending coronally from the advancing root edge (Fig. 1). This zone probably first develops at the outer surface of the initially formed root portion and later shifts in the apical direction as the root grows in length. As a consequence, thin layers of established AEFC are encountered in coronal root regions, while initial AEFC genesis continues apically, i.e., near the advancing root edge.

From data defining the time period necessary for root development, it is known that human premolars attain their complete root length within about 6 years following the completion of their crown (Schroeder, 1987). Based on a rough calculation, the time period during which AEFC is formed initially on the growing root spans 43 to 65 months, i.e., 60 to 90% of 6 years. Because AEFC coats 8 to 10 mm (i.e., 60 to 75%) of the final root length in human premolars, AEFC development at and along the growing root may proceed at a rate of 4.6 to 6.9 μm/day (Schroeder, 1987, 1988). These rates, calculated from clinical and morphometric measurements rather than based on direct evidence which is unavailable, are very much lower than the rates of root elongation measured in all deciduous and permanent molar teeth of the *M. fascicularis* monkey, ranging between 12 and 36 μm/day (Table II). As a preliminary estimate, it can be inferred from these data that initiation of AEFC formation within the 300-μm-wide zone proceeds apically with an average speed of 5 to 6 μm/day.

In all probability, AEFC is a product of a particular class of fibroblasts (Beertsen and Everts, 1990; Bosshardt and Schroeder, 1991a,b). The advancing root edge includes the leading edge of newly produced predentine and the inorganic edge of mineralized dentine. The latter follows the former within a short distance of up to 50 μm. From this edge, predentine continues both over the pulpal surface along the dentine–odontoblast interface and over the external surface of the newly formed root dentine. At the latter site, it can be followed coronally over a distance of about 250

FIG. 1 Schematic drawing illustrating topographically the initial stages of AEFC genesis on human premolars developed to 50–60% of their final root length: 1, fibroblasts contact root/predentine and become committed; 2, fibroblasts start to form and attach collagen fibrils; 3, inital fiber fringe with maximum fiber density is established; 4, cell fiber fringe meshwork is established and the mineralization front approaches the base of the fringe; 5, mineralization front progresses into initial fiber fringe. AEFC, acellular extrinsic fiber cementum; MD, mineralized dentine; ERM, epithelial rests of Malassez; FPF, fringe-producing fibroblasts; FF, collagenous fiber fringe; PD, predentine; MF, mineralization front; NMD, nonmineralized dentine or predentine; CF, committed fibroblasts; ARE, advancing root edge; HRS, Hertwig's epithelial root sheath; FFB, fiber fringe base. Modified from Bosshardt and Schroeder (1991a).


F
PD
F
CP
PD
a
FF
PD
MF
PD
FF
MF
FF
PD
b

to 300 μm from the advancing edge (Fig. 1). At this edge, the diaphragm, i.e., the most apical part of Hertwig's epithelial root sheath, touches the predentine but, lateral or external to this edge, the root sheath deviates from the surface of newly formed dentine, continues coronally as a short strand, and eventually breaks up into the discontinuous epithelial rests of Malassez (Fig. 1). In humans, Hertwig's root sheath, including its diaphragm, consists of the former inner and outer layers of the enamel epithelium, extends by continuous proliferation of the diaphragm (Diab and Stallard, 1965; Kenney and Ramfjord, 1969; Formicola *et al.*, 1971), disintegrates coronally in accordance with its rate of proliferation, and is surrounded by a basal lamina. The latter actually contacts the leading root edge of predentine (Schroeder, 1986). In contrast to previous statements in most current textbooks, Hertwig's root sheath does not cover much of the external surface of newly formed predentine, at least in human premolars. Rather, that surface at the advancing root edge is almost from its beginning accessible to connective tissue cells of the dental follicle proper.

In the triangular region between the laterally deviating root sheath and the surface of newly formed predentine, connective tissue cells with the morphological appearance of fibroblasts can always be encountered. These cells are slender or bulky, are basophilic, and reveal an activated euchromatin-rich nucleus, displaying an ~50-nm-thick nuclear fibrous lamina and a cytoplasm with numerous strands of rough endoplasmic reticulum cisternae and a prominent Golgi field. These cells are connected to one another by desmosome-like junctions and project numerous, slender cytoplasmic processes that contact and insert between collagen fibrils of the not yet mineralized outer predentine matrix (Fig.2a). These features are typical for the most apical 30 to 50 μm along the surface of the newly formed root. In about that distance to the advancing root edge, cells of similar appearance begin to produce the first AEFC matrix portions in the form of tiny but discrete bundles of collagen fibrils, slowly increasing in length and density in the apico–coronal direction. Thus, a short fiber fringe is produced, with most of the collagen fibrils arranged in parallel and oriented more or less perpendicular to the root surface. These fibrils

FIG. 2 (a) Electron micrograph depicting the most apical zone of not yet mineralized predentine matrix (PD) contacted and penetrated by cytoplasmic processes (CP) of fibroblast-like cells (F) that begin to produce cemental collagen fibrils (see Fig. 1, parts 1 and 2). (b) Electron micrograph of the initial fringe of collagen fibrils and fibril bundles (FF) that insert into the not yet mineralized predentine (PD); the mineralization front (MF) has not reached the future dentino–cemental junction (see Fig. 1, parts 3 and 4). Magnification: a,b, ×6700. (a) From Bosshardt and Schroeder (1991a). (b) Courtesy Dr. D. D. Bosshardt.