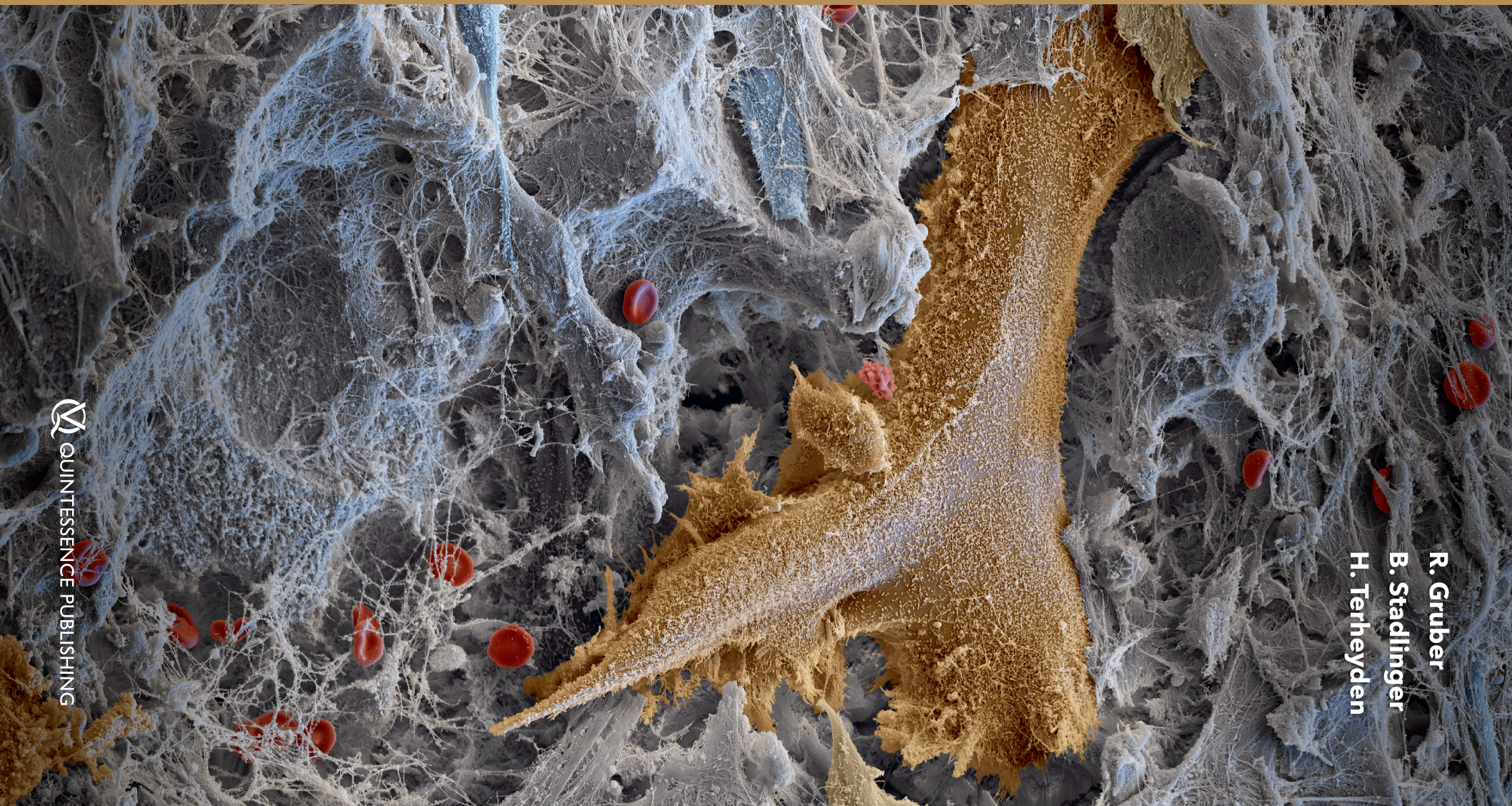
CELL-TO-CELL COMMUNICATION

CELL ATLAS – VISUAL BIOLOGY IN ORAL MEDICINE



R. Gruber
B. Stadlinger
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QUINTESSENCE PUBLISHING

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R. Gruber, B. Stadlinger, H. Terheyden (eds.)

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Preface by Quintessence Publishing with a special Reflection on the Idea of this Work

Speechless Intelligence

The UNESCO General Conference has proclaimed February 2021 of each year the International Mother Language Day, in memory of the importance of linguistic diversity as a cultural asset of humanity. Of the approximately 6,800 languages still spoken today, linguists estimate that more than 3,400 will suffer language death by the end of the 21st century.

To facilitate global cultural, social, and scientific communication in the face of language diversity and the uncertainties of language development, Ludwik Lejzer Zamenhof created Esperanto in 1887 as a constructed language designed to unite nations and to promote universal, neutral, fair, and equitable communication. Many Esperanto words were derived from Latin or Romance roots, with Germanic and Slavic admixtures. However, Esperanto has not gained international acceptance as a second language beyond a small community of enthusiasts.

The problem of human communication becomes manifest in the thesis by Ludwig Wittgenstein, one of the most important philosophers of the 20th century. In his epochal work *Tractatus Logico-Philosophicus*, he states, "The limits of my language mean the limits of my world." The iconic computer scientist Joseph Weizenbaum adds to this insight by pointing out that "the limits of my knowledge means the limits of my ability to interpret," because "if you don't understand anything about the outside world, you can't interpret it, probably not even perceive it."

This brings us to the problem of how to interpret intelligence. Intelligence can be grasped only by what we can interpret linguistically and what lies within the framework of our experienced implicit and explicit world and knowledge. If we are confronted with a different, speechless world, a world beyond our capabilities to articulate—in this case, the world of biochemical/biomedical cellular interactions—we will perceive nothing but chaos at first. The complex signal, information, and communication structures of that world will be closed to us. We therefore make an attempt to transfer this speechless world into a linguistic form, that is, to decode its signals and to interpret them as if they were cryptologic challenge.

In doing so, we have to attempt a cognitive differentiation between opinions, beliefs, and knowledge, the world that Immanuel Kant so cogently described in his *Critique of Pure Reason* (as translated by J.M.D. Meiklejohn):

- Opinion is a consciously insufficient judgment, subjectively as well as objectively.
- Belief is subjectively sufficient but is recognized as being objectively insufficient.
- Knowledge is both subjectively and objectively sufficient.

In this cascade, the linguistic ability to interpret increases from opinions as the lowest level to knowledge as the highest level of epistemology. Science always strives to gain knowledge that, starting with empiricism, is then evaluated by looking at evidence as the hardest currency of scientific knowledge, resulting in a reduced corpus of language with clearly defined theories, doctrines, and statements.

If the driving force behind our doubt is also the basis of any scientific efforts to arrive at new insights, then we have to admit that—if we fail to find an answer—"all knowledge begins with doubt and ends with faith" (Marie von Ebner-Eschenbach). This principle will never lose its validity, and the resulting faith will reveal the limits of our knowledge and, hence, the limits of our intelligence.

This is the conflict we as human beings find ourselves in. But is this conflict not a result of our biology, consisting essentially of the basic substances oxygen,

hydrogen, nitrogen, chlorine, fluorine, calcium, phosphorus, sulfur, potassium, sodium, magnesium, and iron? And is it not that speechless intelligence of biochemistry and biophysics that first enabled us to communicate using the full bandwidth of linguistic means? Should we not, therefore, be viewed as a product of our biology, our holobiont and thus as subsystems of ourselves, recognizing the speechless molecular biology as a higher intelligence? If the biologic essence of a human being is in their approximately 7.5×10^{27} atoms, we can barely begin to guess at the complexity of interactions and interdependencies in the speechless world of a biologic intelligence that lets us become what we are—according to Plato, the Being or Essence (οὐσία), and thus, according to Aristotle, "the whole [human being] is more than the sum of its parts."

The speechless intelligence of our cells has the advantage that these cells do not suffer from the language diversity prevailing amongst humans, so fraught with sociocultural problems. Communication in the molecular biologic network is universal (an Esperanto of biology) with an integrated "interpreter" that translates primary signals (ligands) from the extracellular space into secondary signals via receptors for intracellular reactions. The articulated human linguistic corpus is a limiting factor compared to the enormous power of the inarticulate world of communication in molecular biologic and biophysical interactions and biologic processes.

The mission statement for the present ambitious work should be evaluated against this background:

"Only if we understand the speechless world of the cells with their cellular interactions, messengers, and receptors, with intracellular signaling and communicating via extracellular matrices in healthy and diseased tissues, can we make the right diagnostic and therapeutic decisions—with humility and in harmony with biology."

This is also the primary objective of personalized and individualized medicine.

As publishers, we would like to thank the editors—Professors Reinhard Gruber, Bernd Stadlinger, and Hendrik Terheyden—for the immense enthusiasm they brought to this ambitious project. With their tireless dedication, unwavering passion, and clinical scientific expertise, they have taken up the challenge to guide us into the mysterious world of cell-to-cell communication, with its functions, interactions, and clinical relevance.

It is also thanks to the editors' extensive international network that they were able to enlist more than 47 contributors and experts from the U.S., Canada, Brazil, Europe, and Asia to contribute their knowledge for each cell type or cell formation. Our heartfelt thanks go to this "cell community" that has made this work so unique and that fills us with pride. We are honored to be able to realize this project and to take care of the publishing side.

Such an ambitious international project can hardly be realized without financial assistance; and thus, EMS and the SDA (Swiss Dental Academy) and their Chair, Bernd Bühner, deserve special thanks for their enormously important support. Bühner's entrepreneurial foresight when it comes to the topics that will define the future of dentistry/oral medicine, combined with his mission to promote prevention as the essential component in clinical therapy, supported by innovative technologies and concepts in oral medicine for the benefit of the patient, have all made him a highly distinguished personality in our professional world.

It is my personal wish to dedicate this preface, with heartfelt thanks, to our friend Dr Wolfgang Bengel, who passed away far too prematurely on October 10, 2014. With his passion for scientific photography, he had already conceived of the idea in his time to make the invisible visible and to visualize the fascination of science on this complex topic that Oliver Meckes with Nicole Ottawa have so fantastically staged with their scanning electron micropraph images.

We wish you an exciting and enlightening journey into the world of the speechless intelligence of cellular interactions in the oral cavity.

Alexander Ammann
Dr rer biol hum, Dipl Wirt Ing
Quintessence Publishing

Preface by the Editors

A Change of Perspective

A concept often mentioned by experienced physicians is the "top-down clinical approach." What this means is that each patient should primarily be perceived as a holistic entity. The examination of clinical details should wait until the subsequent diagnostic process. Figuratively speaking, the physician will begin each examination from a bird's-eye perspective. Even with all the detailed knowledge that we have, we must still first try to get a subjective general impression before we can add objective aspects to this impression, one at a time. Being able to alternate between the bird's-eye view and the frog's-eye view is the hallmark of the capable diagnostician.

Any first subjective impression is strongly influenced by individual clinical experience, enabling the clinician to "get a feel" for the health issue at hand based on medical experience and an understanding of the biologic processes involved in the development of pathologies. A sound mastery of the cosmos of findings and diagnoses (decision-making knowledge) must precede any therapeutic implementation (action knowledge or simply "know-how"). Any therapy in turn requires an understanding of the biologic healing processes. There is thus an ongoing transfer between explicit quantitative-positivist knowledge (how can I do something I want to do?), selective qualitative-orientational knowledge (what may I do and what must I not do?), and implicit action-oriented knowledge (what will I actually do?) that characterizes the clinical competence of the physician/dentist.

The progression of disease and healing is based on interactions between cells. In the world of cells and mediators, we are confronted with the interaction of an almost infinite number of individual factors. Usually this interaction works largely through symbiosis. Disease symptoms only arise when dysbiosis occurs, which can be triggered by exogenous factors such as bacteria or viruses. Their antagonist is our immune system, which protects the body in the form of innate and acquired immunity. Our oral system can be thought of as the immune system's first line of defense.

To achieve an understanding of complex systems, basic research always tries to subdivide and categorize in order to examine relationships under simplified model or laboratory conditions. The findings of basic research form the foundation for the promotion of medical knowledge. An important follow-up step is the transfer of these findings to the clinical setting.

The present volume intends to raise awareness of this essential connection. The chapters of this book are arranged alphabetically according to the different cell types that are of major relevance within the oral system. Each chapter is dedicated to a particular cell type, introducing its specific properties and its function within the cellular network.

The second part of each chapter highlights the role of the respective cell type in a clinical context. Each chapter has at least two authors, a basic researcher and a clinician. We made a conscious decision to let the basic researcher take the lead in each chapter, since high-quality clinical medicine invariably builds on a sound understanding of the underlying biologic principles.

The title of this book is Visual Biology. Each of its chapters begins with a colored scanning electron microscopic (SEM) image of a cell type. These SEM images (the magnification of the images refers to a picture width of 15 cm), created by Oliver Meckes and Nicole Ottawa, are intended to eloquently illustrate and explain the function of the depicted cell type. The osteocyte processes in Volkmann canals are an example in kind. Looking at these canals radiating out centrifugally from the osteocyte, embedded in the

bone, we can easily understand why a fracture that interrupts the canal will result in the release of mediators. Another example are fibroblastic processes that visualize being responsible for tissue elasticity. This visual understanding of form and function is intended to create a deeper awareness of biology. As this volume evolved, we had plenty of discussions between the science photographers, the editors, and the authors of the various chapters regarding the coordination of images and content.

Many of the cell types described are implicated in regenerative processes, such as neoangiogenesis by microvascular cells or bone regeneration by osteoblasts. Beyond the classic cell types addressed in the the first part of the book, organ systems or model systems of cell-to-cell communication of a more generic type are presented in four additional chapters in part two. For example, because 3D-printed hydroxyapatite can support bone healing, it is important for clinicians to know the possibilities and limits of related approaches in order to establish whether and to what extent their use may be indicated.

When observing healing processes and understanding the function of cell types, researchers are usually confronted with snapshots. Histologic images or cell cultures show us tissue at specific points in time. It is more difficult to develop a chronologic understanding of cell types and their interactions with other cells. This is where computer animations are helpful. Based on real scanning electron micropraph images, 3D reconstructions of cells can be designed and displayed as a film, i.e., a sequence of images.

The augmented reality (AR) tool is a distinctive feature of this publication, letting readers/viewers immerse themselves in an application that shows a 3D-animated cellular world of experience using bone regeneration as a salient example. In cooperation with my department at the University of Zurich, Professor Markus Gross and Dr Fabio Zünd and their team at the Game Technology Center (GTC) at ETH Zurich, and Dr Marko Reschke at iAS (interActive Systems), a subsidiary of Quintessence Publishing, this innovative AR tool has been developed to integrate knowledge gleaned from the world of gaming.

This book/AR project is part of the well-known and internationally acclaimed Cell-to-Cell Communication video and book series that aims to make invisible cellular interactions visible in photo-realistic 3D imaging.

The mission of this work is to re-experience the fascination of science, transferring knowledge from basic research to teaching and our everyday clinical work, in order to sharpen our clinical eye and provide a top-down clinical approach through a change of perspective.

Bernd Stadlinger
Professor, MD, DMD
University of Zurich

Using the Augmented Reality App

The book chapter on Osteoclasts / Odontoclasts by Riko Nishimura and Henrik Terheyden is accompanied by an augmented reality (AR) app for smartphones and tablets that allows you to experience the process of bone resorption virtually in your hands.

Download the app by scanning the QR code below or by searching for “AR Osteoclasts” in the app stores. Launch the app and point your device’s camera at Fig 1 in the chapter “Osteoclasts / Odontoclasts.” Immerse yourself in the process of bone resorption as you follow the story in the app and play augmented reality minigames on your book for each step in the process.

In this app, you are drawn into the microcosm of osteoclasts right above the bone surface. In the augmented display you are zoomed in to the level at which an osteoclast precursor cell appears to be the size of a hand (more than 600 times larger than in reality). You investigate the bone resorption process from all sides by moving around the augmented display on top of your book page and you interact with cells to learn more about their function.

The app is developed by the Game Technology Center (GTC) at ETH Zurich, Switzerland, in cooperation with iAS (interActive Systems), a subsidiary of Quintessence Publishing, and is available for iOS and Android smartphones and tablets.

GTC project website: https://gtc.inf.ethz.ch/publications/AROsteoclasts.html

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Prologue

Reinhard Gruber

The Language of Cells

We experience that human communication is complex, and so is cellular communication. Communication can be broken down into four elements: a Sender encoding a Message that is released and transported through a Channel to the Receiver (SMCR Model, 1960). The Receiver decodes the message and transfers it back to meaning. In analogy, the Sender is a cell releasing a Message, the signaling molecules. The Messenger in turn acts locally within the extracellular space or systemically when released into the blood stream. Through these Channels, the Messenger reaches the Receiver, the target cells, causing a response. Cell-to-cell communication encompasses the following four main communication networks: autocrine, paracrine, contact-dependent (juxtacrine), and endocrine communication.

Intercellular Communication

Autocrine signaling

Autocrine signaling is self-communication, when the Sender and the Receiver are of the same cell type. Examples of autocrine signaling are the activated T cells beginning to produce interleukin 2, which then supports the expansion of this particular clone of T cell. Further examples are monocytes and fibroblasts performing auto-amplification of original inflammatory signals in response to foreign clues.

Paracrine signaling

Paracrine signaling is communication in which the Sender and the Receiver are different cell types. For example, inflammation is a multicellular response, thus requiring the coordinated action of many cell types of the innate and the specific immunity. It is thus necessary to alarm nearby cells, spreading the Messenger to different Receivers (target cells). Another example of paracrine signaling is when osteocytes release key molecules controlling the formation, activation, and survival of bone-forming osteoblasts and bone-resorbing osteoclasts.

Contact-dependent (juxtacrine) signaling

Contact-dependent, or juxtacrine, signaling occurs when the Sender and Receiver are in close contact. In communication terminology, no Channel is required. An example is the presentation of foreign epitopes via the major histocompatibility complex II to the respective T cell receptor, a process supported by co-stimulatory molecules. Similar juxtacrine signals have been proposed to control osteoclastogenesis during bone remodeling, with mesenchymal cells being the respective partners.

Endocrine signaling

Endocrine signaling is communication via long distance between Sender and Receiver. The blood stream serves as the Channel. Endocrine signals are thereby distributed throughout the organism. Examples are steroid hormones, such as estradiol produced in the ovaries or parathyroid hormone produced in the parathyroid gland. Interestingly, osteocytes are endocrine organs as they release growth factors serving as hormones that control kidney function.

Signaling molecules

Signaling molecules, the Messages, are the universal language, “spoken and understood” by all cells in the

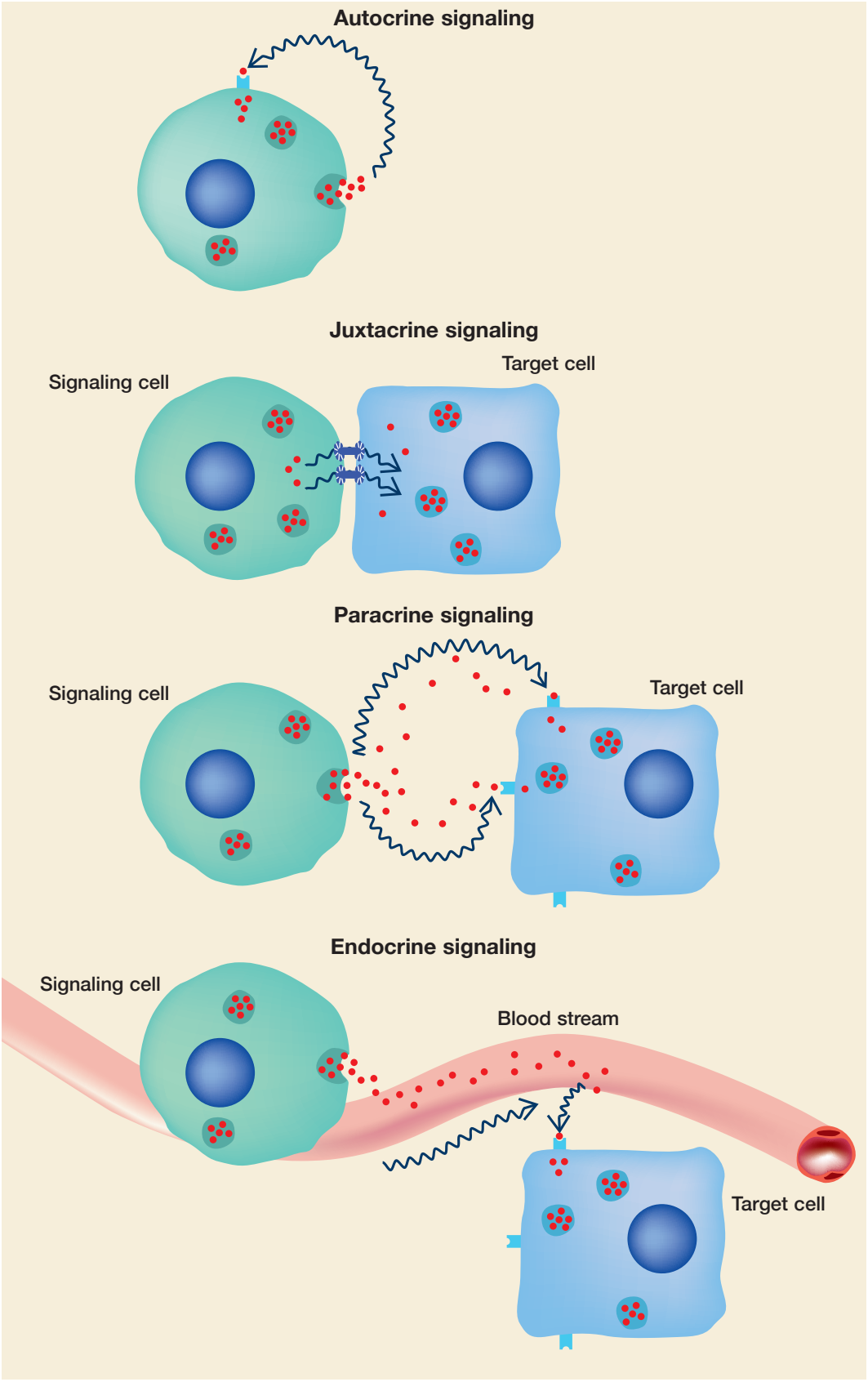

Fig 1 The language of cells: Networks of signal transmission.

body. The language is complex because of the large spectrum of signaling molecules, e.g., proteins, steroids, lipids, nucleotides, and even gases. The language of cells is a biochemical language. Sender cells produce and release signaling molecules. Sender cells decide when to communicate, the content, with whom, and when to stop to generate a desired effect. The Channels, which can be the local environment or the systemic circulation, carry the signaling molecules to the Receiver, the target cell. Signaling molecules bind specifically and with high affinity to receptors on the cell surface (e.g., growth factor receptors) or inside the cell (e.g., steroid hormone receptors) of target cells. Intracellular signaling cascades are initiated that can induce a feedback response, making the Receiver cell become a Sender.

Cell signaling

Cell signaling is the intracellular part of cell-to-cell communication, the conversion of the extracellular Messenger into an intracellular response. In analogy, it is the translation of a language spoken outside a cell to a language understood inside cell, the cytoplasm. Cell signaling is a sequential process that starts with the binding of an extracellular signal (also termed *ligand*) to a specific receptor, following the principle of the "key" shape fitting the receptor "lock." The receptor is activated, and signals are carried across the cytoplasm. This process is termed *transduction* and involves activation of receptor-coupled tyrosine kinases, G-proteins, or ion canals, which begin a cascade of phosphorylation events of protein kinases, similar to dominoes falling. The phosphorylation cascade running through the cytoplasm can be amplified or even suppressed before activating the final proteins, the transcription factors. Transcription factors enter the nucleus, bind to the DNA, and change gene expression. The transcribed mRNA is translated into proteins, which finally cause the cell response. When the cell produces, e.g., growth factors affecting autocrine and paracrine signaling, we ultimately return to the beginning of this chapter in a feedback loop. But what regulates the intensity of this communication?

Signal intensity

Signal intensity is regulated at the level of the extracellular communication but also by the intracellular part of cell signaling. Signal intensity controls the response of cells to changing environmental conditions. Cells have to adjust their behavior based on the intensity and duration of external signals, which represent multidimensional information integrating spatial and temporal gradients of agonists and antagonists. The coexistence of the various extra- and intracellular signaling molecules cannot be understood as a linear approach. The signaling molecules compete with their antagonists for the receptors outside the cell or meet with their agonists to intensify the original signal. The same is true for the multiple integrated intracellular signaling pathways. Extra- and intracellular signaling molecules can thus be envisioned as biologic clouds with the coexistence of increasing or decreasing signals competing for the overall cellular response.

Part 1:
Cell Atlas of the Oral System “A to Z”

Ameloblasts

[Ameloblast: Old English *amel* and ultimately Old French *esmail* "enamel" and Old Greek *βλάστη (blástē)* "germ"]

Richard J. Miron and Adrian Lussi

Ameloblasts and enamel (original magnification ×3500).

Molecular Aspects of Cell Communication

Amelogenesis

Odontogenesis starts in the human embryo 28 to 40 days after ovulation. Epithelial cells grow into the ectomesenchymal areas of the jaw. This gives rise to an epithelial bulge. Then, the dental papilla is formed by the continued penetration of epithelial cells into the ectomesenchyme.

At this stage, the cells destined for the formation of hard dental tissue are differentiated: the ameloblasts from the ectodermal cells and the odontoblasts from the adjacent ectomesenchymal cells of the dental papilla, in which a mutual induction chain controls their formation. In anterior teeth, the first enamel and dentin layers originate in the middle of the incisal margin; in the posterior teeth, they originate in the area of the cusp tips. With increasing growth, the various centers involved in the formation of dental hard tissue merge and thereby form the occlusal surface (see Lussi and Schaffner 2012).

The Hertwig epithelial root sheath, which is only two layers thick, results from the continued penetration of epithelial cells into the ectomesenchyme. It determines the size, form, and number of tooth roots that are formed. The ameloblasts with their Tomes processes are responsible for the formation of the prism rod and the interprismatic (inter-rod) enamel (Figs 1 and 2). Amelogenesis starts with the secretion

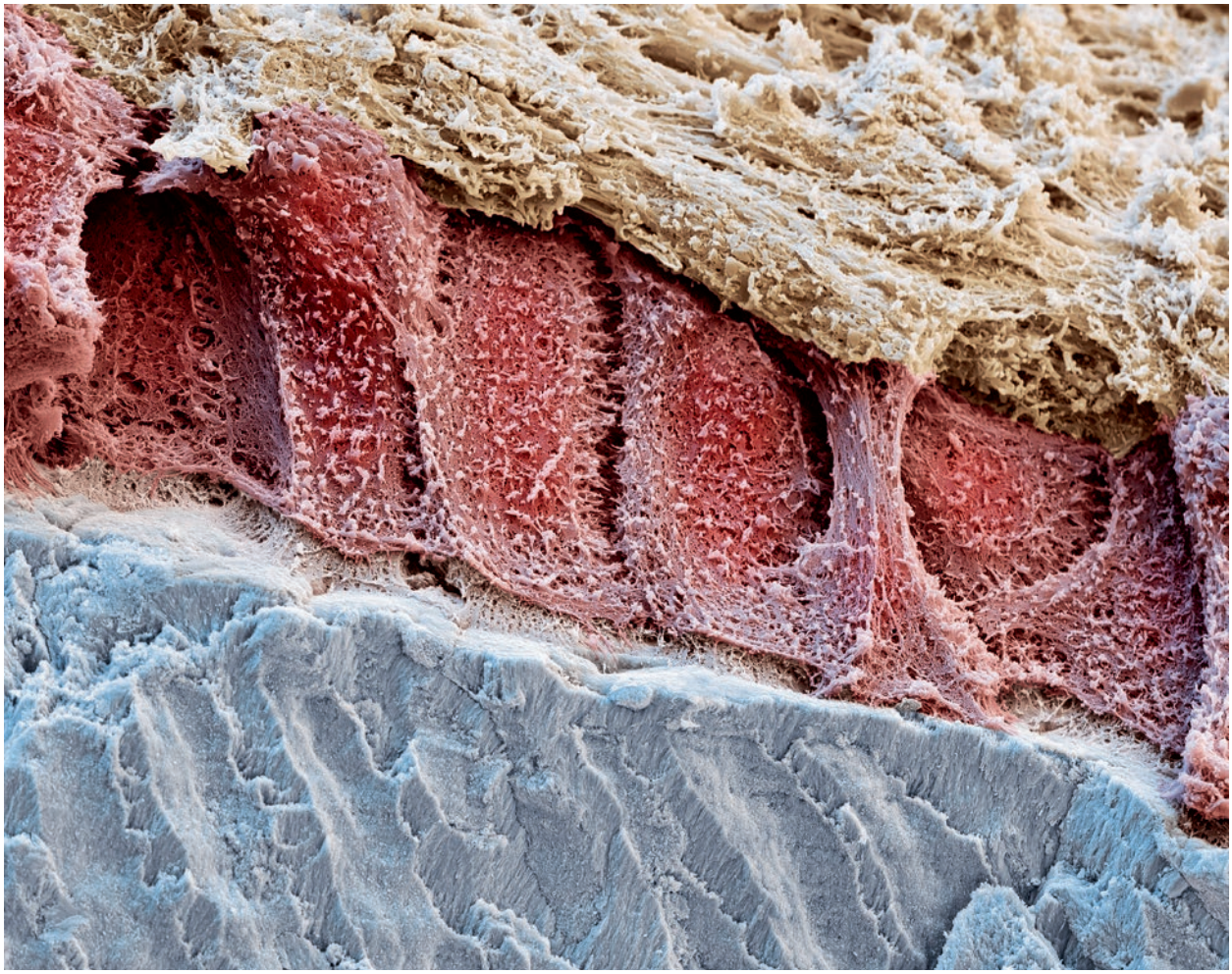

Fig 1 Freeze fracture through a molar in the final stage of enamel building (in a 17-year-old male). The ameloblasts *(red)* are shortened and are covered by the cells of the stratum intermedium *(beige)*. The orientation of the enamel *(blue-gray)* is clearly visible (original magnification ×2000). (Courtesy of eye of science.)

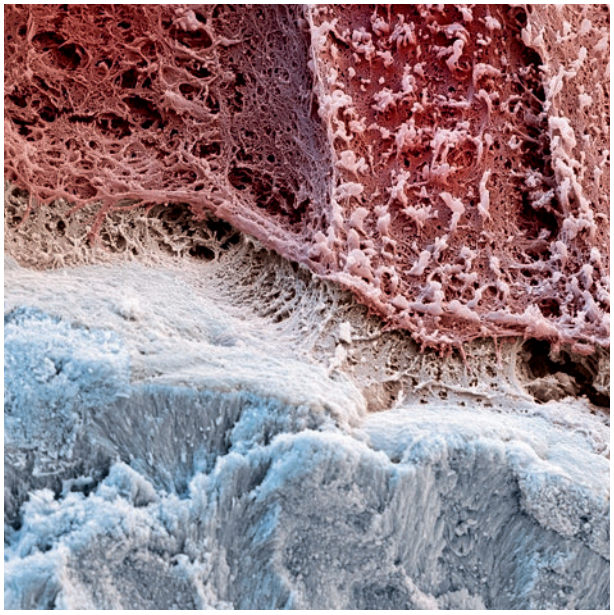

Fig 2 Contact area between ameloblasts *(red)* and enamel *(gray)* in high magnification. There is an extracellular area between the cells and enamel (original magnification ×8000). (Courtesy of eye of science.)

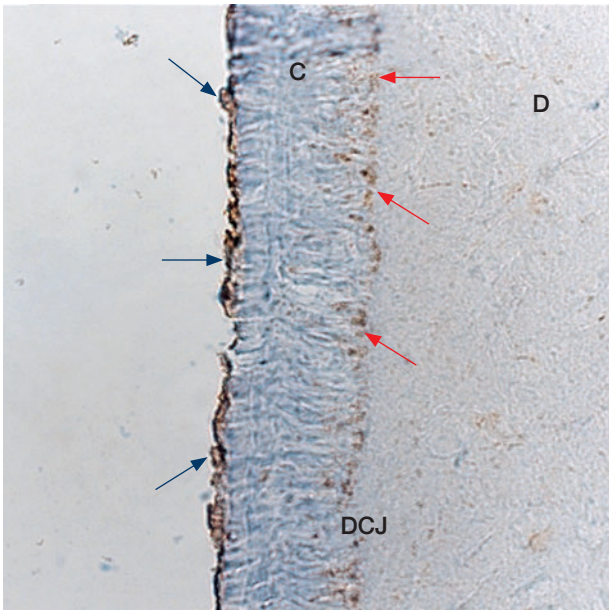

Fig 3 Histologic section depicting EMPs localized at the dentinocemental junction (DCJ). Results from the early 1990s demonstrated that enamel matrix proteins (EMPs) (which until then were considered enamel-specific proteins) were found also localized at the DCJ, forming the basis for the hypothesis that they may play a role during cementogenesis, which was investigated in numerous subsequent studies characterizing the role of EMPs in periodontal tissue differentiation. C, cementum; D, dentin; *blue arrow,* enamel matrix derivative (EMD) deposited on the root surface (*ex vivo* experiment); *red arrow,* EMPs found localized at the DCJ.

of enamel matrix by the ameloblasts. The average rate of amelogenesis is 4 µm per day. However, it varies widely, depending on the tooth and tooth surface that is being formed. The arrangement of the enamel prisms is not linear and can be interwoven in a kind of spiral or arranged in a wavelike fashion. The wave movement of the prism rods disappears in the outer third of the enamel. The periodic formation of enamel matrix by the ameloblasts, the variable production of enamel matrix in the area of the Tomes process, and the three-dimensional arrangement of the enamel prisms lead to the different structural characteristics of enamel seen under light and electron microscopy.

Cementogenesis

While ameloblasts are naturally associated with the enamel, it is important to note that they play a pivotal role during cementogenesis. Several decades ago, amelogenins were still considered enamel-specific proteins. Nevertheless, in the early 1990s a team of researchers in Sweden led by Lars Hammarström, Sven Lindskog, and Leif Blomlöf found that enamel matrix proteins (EMPs) were also deposited onto the surface of developing tooth roots prior to cementum formation (Hammarström et al 1991, 1992). It was hypothesized that based on their secretion, they may play a possible role during cementogenesis (Fig 3).

This relationship was further established by the fact that amelogenesis imperfecta or issues related to amelogenin production/synthesis may also lead to obvious deficiencies in cementum and/or tooth root development. Thus, the hypothesis that EMPs secreted by ameloblasts may play an integral role in the future differentiation of periodontal tissues was established (Hammarström 1997). A number of biologic and clinical studies thereafter have since demonstrated that EMPs are secreted by the Hertwig epithelial root sheath and are able to promote periodontal regeneration (Gestrelius et al 1997a, 1997b; Hammarström

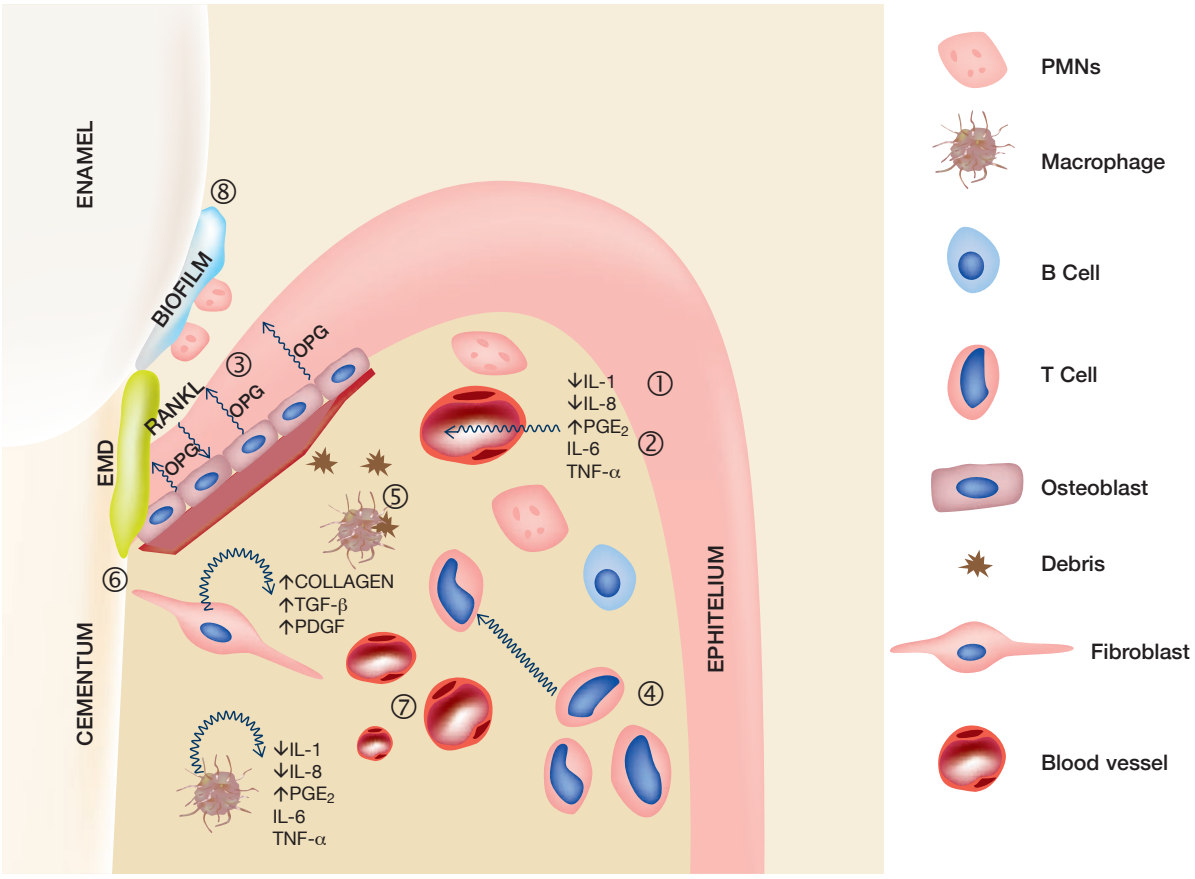

Fig 4 Diagram depicting inflammation-modifying changes induced by EMD (enamel matrix derivative). Following application of EMD, decreased production of interleukin (IL)-1b and IL-8 (1) and increased levels of prostaglandin E_2 (PGE_2) (2) are observed with little differences in tumor necrosis factor-α (TNF-α) expression. EMD also substantially changes the osteoprotegerin (OPG)/receptor activator of nuclear factor-kappa B ligand (RANKL) balance by increasing OPG and decreasing RANKL levels, resulting in diminished osteoclast formation/activity (3). EMD also increases the proliferation and migration of T lymphocytes (4), which enable tissue debridement by macrophages (5). Furthermore, EMD promotes mesenchymal cell differentiation into hard tissue–forming cells and also improves periodontal ligament cell regeneration (6). Microvascular cell differentiation and angiogenesis are improved following EMD application (7), and studies demonstrate that EMD also lowers bacterial numbers (8), resulting in a reduced inflammatory state. TGF-β, transforming growth factor β; PDGF, platelet-derived growth factor; PMNs, polymorphonuclear leukocytes. (Reprinted from Miron et al 2015 with permission.)

1997; Hammarström et al 1997; Heijl et al 1997; Zetterström et al 1997).

Key studies from basic science laboratories have determined that ameloblasts secrete an array of proteins, of which 90% are amelogenins, a family of hydrophobic proteins derived from different splice variants and postsecretory regulation from the expression of a single gene (Lyngstadaas et al 2009). These proteins self-assemble into supramolecular aggregates that form an insoluble extracellular matrix and function to control the ultrastructural organization of the developing enamel crystallites (Lyngstadaas et al 2009). Other proteins secreted by ameloblasts include enamelin, ameloblastin (also called *amelin* or *sheathlin*), amelotin, apin, and various proteinases (Bartlett et al 2006, Margolis et al 2006). Although these proteins are expressed in fewer quantities, further investigations have confirmed their valuable roles in various aspects of enamel, cementum, and periodontal regeneration.

Based on these combined results, a number of biologic and clinical experimental studies were created to investigate the role of EMPs during cementogenesis and periodontal regeneration. Years later, a purified fraction derived from the enamel layer of developing porcine teeth was given the working name

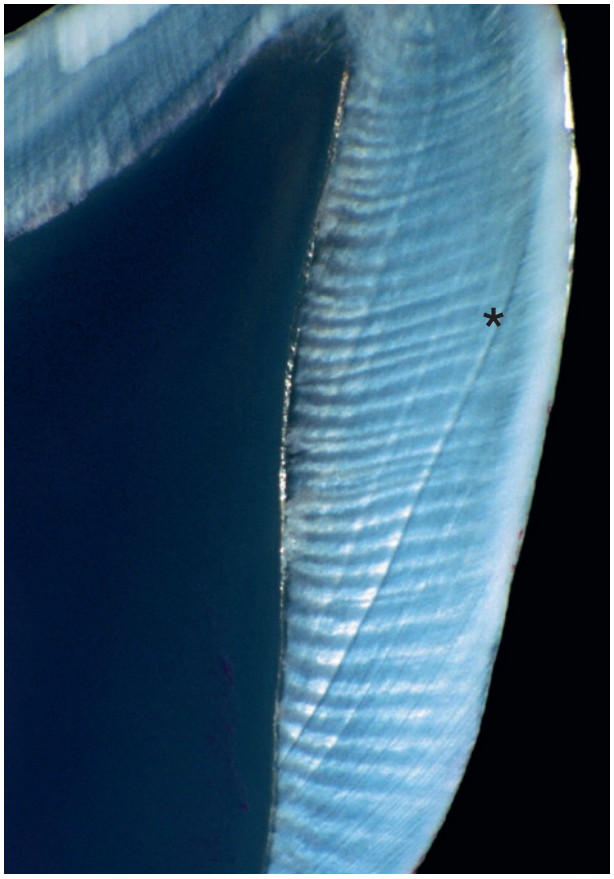

Fig 5 The lines of Retzius (*) extend from the dentinoenamel junction and may reach the surface, whereas the Hunter-Schreger bands are perpendicular to the latter and do not reach the surface (original magnification ×2.8).

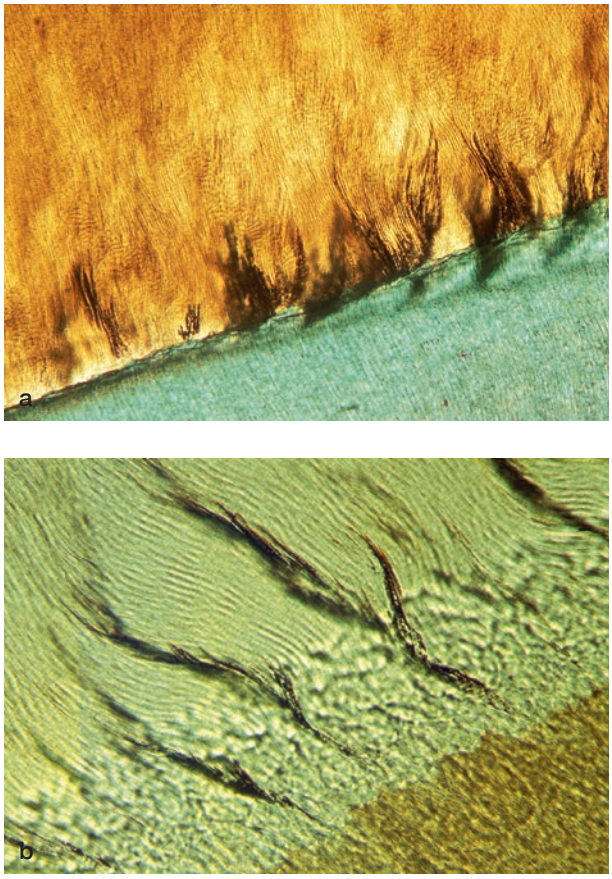

Fig 6 *(a and b)* Enamel tufts are hypomineralized areas of enamel, which look like tufts of grass under light microscopy (*fuchsine/light green*, original magnification × 65).

enamel matrix derivative (EMD) and has since been the basis of numerous publications investigating its use in periodontal regeneration as a biologic regenerative protein (Fig 4).

Clinical Relevance in Oral Medicine

Enamel: Structural characteristics

Under light microscopy in longitudinal sections, lines can clearly be seen running obliquely from the dentoenamel junction in an occlusal direction. The periodic laying down of enamel is expressed in the lines of Retzius resembling the growth rings of a tree. Where the lines of Retzius emerge at the enamel surface, the imbrication lines are formed. The perikymata lie between. These lines are clearly visible on newly erupted teeth. Viewing the longitudinal and transverse sections of enamel by light microscopy reveals light and dark striae in the inner two-thirds. These Hunter-Schreger bands are caused by the wavelike path of the enamel prisms (Fig 5). In most teeth, enamel structural defects can be identified by light microscopy. A large proportion of these defects arise during amelogenesis. These include enamel tufts and enamel lamellae

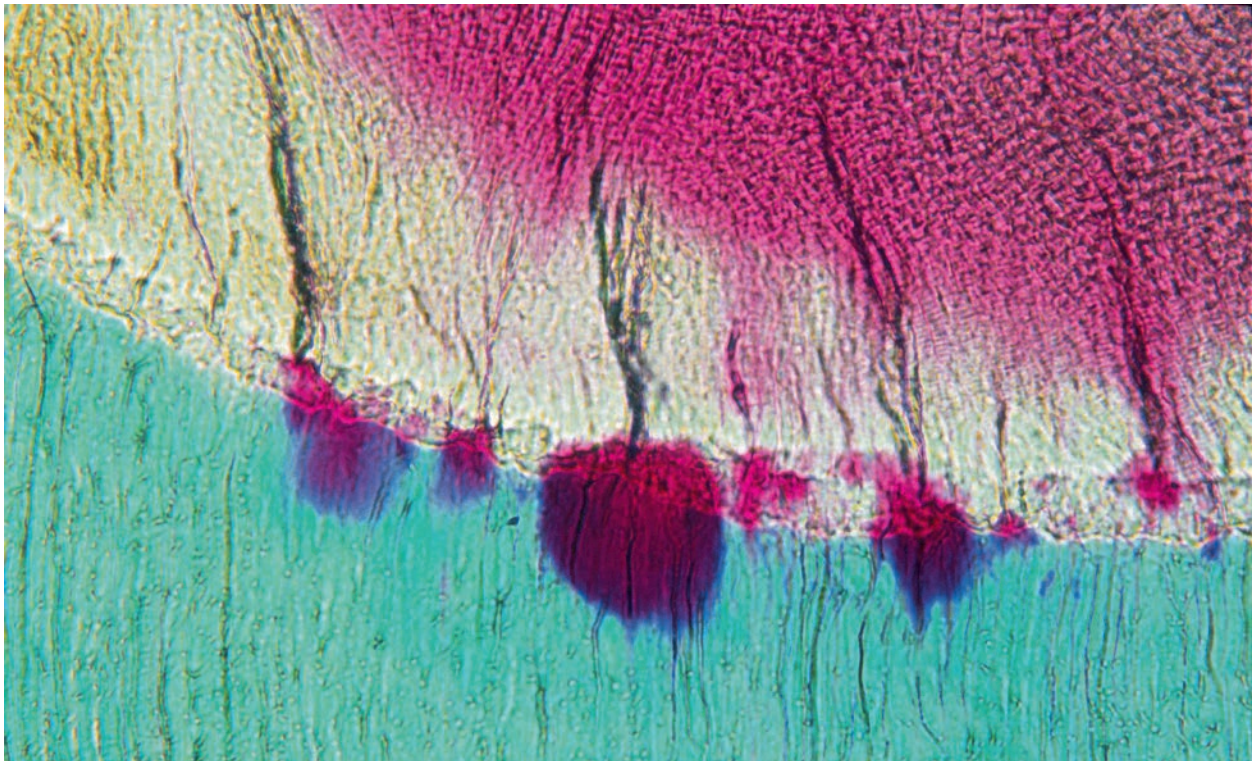

Fig 7 Enamel tufts can provide a location favorable to bacteria in the event of a carious attack. Caries is clearly visible in the histologic image (*fuchsine/light green*, original magnification ×65).

(Fig 6). Enamel tufts and lamellae can prove to be the line of least resistance in respect to the spread of caries (Fig 7). The enamel pearl is a paraplasia of the enamel, i.e., the formation of enamel in an atypical localization. Enamel pearls can cause isolated periodontitis in the area of the furcation (Fig 8).

Cementum

Since the working fraction of enamel protein was given the working name EMD, a great deal of publications (over 500) has since evaluated its role in dental medicine (Miron et al 2016). Approximately two decades ago, the first animal model investigating EMD as an adjunctive agent to periodontal surgery involved surgically created recession defects treated with a coronally advanced flap (CAF) either alone or in combination with EMD (Hammarström et al 1997). Following an 8-week healing period, the histologic evaluation revealed formation of acellular cementum, periodontal ligament, and alveolar bone in all defects treated with EMD. Following these original findings, subsequent animal experiments have evaluated the healing of different types of induced periodontal defects (i.e., fenestrations; recessions; and dehiscence-type, intrabony, and furcation defects) treated with EMD or guided tissue regeneration (GTR; Fig 9; Cochran et al 2003; Donos et al 2003; Ivanovic et al 2014; Nemcovsky et al 2006; Regazzini et al 2004; Sakallioğlu et al 2004; Sallum et al 2003, 2004; Sculean et al 2000a, 2000b). It was reported in these studies that application of EMD resulted in substantially larger amounts of new cementum, periodontal ligament, and bone formation when compared to controls (i.e., flap surgery alone).

EMD has demonstrated positive effects on general wound healing (Hagenaars et al 2004, Tonetti et al 2004, Wennström and Lindhe 2002), following nonsurgical periodontal therapy (Gutierrez et al 2003, Mombelli et al 2005), on intrabony and furcation defect regeneration with and without bone grafts (Fig 10;

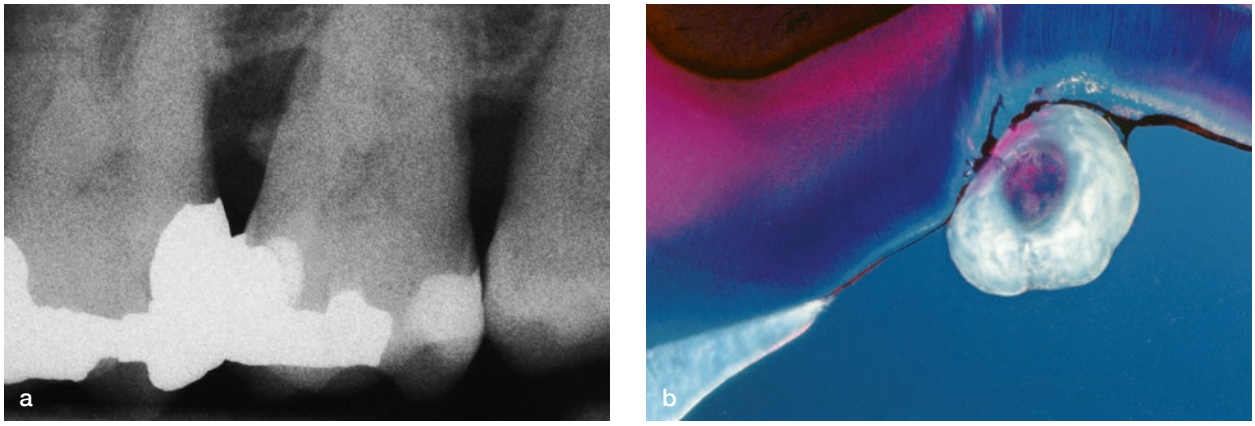

Fig 8 *(a)* Radiograph of an enamel pearl in the interproximal area distal to the maxillary left first molar. *(b)* Histologic section of an enamel pearl laying on cementum. At the top left of the image, enamel can be seen (*fuchsine/light green*, original magnification ×10).

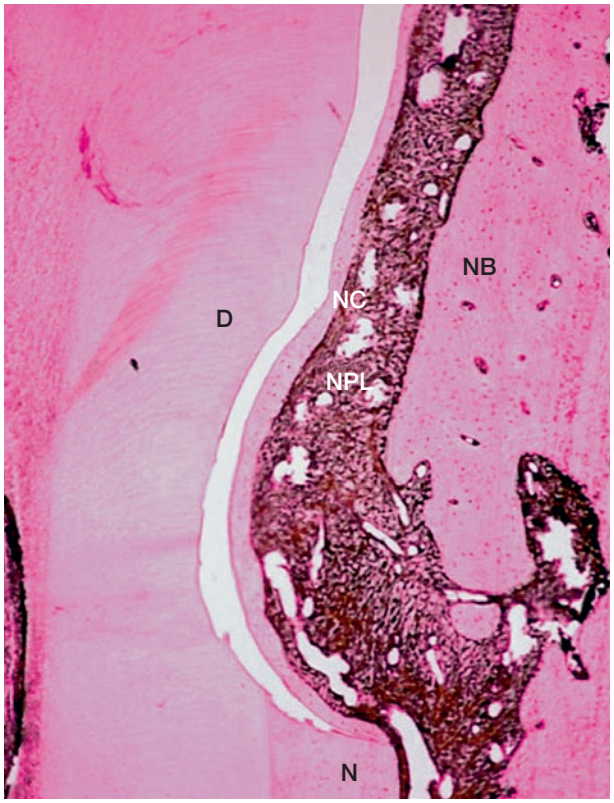

Fig 9 Micrograph showing regenerated monkey periodontium following treatment with EMP. A strong expression of vimentin can be observed in the newly formed periodontal ligament (NPL) coronal to the notch (N) indicating the apical part of the periodontal defect. Note that the NPL is in contact with the original periodontal ligament (PDL). No difference in terms of vimentin expression can be observed between the newly formed and the original PDL. NC, new cementum; NB, new bone; D, dentin (hematoxylin & eosin stain; original magnification ×50). (Reprinted with permission from Hammarström et al 2010.)

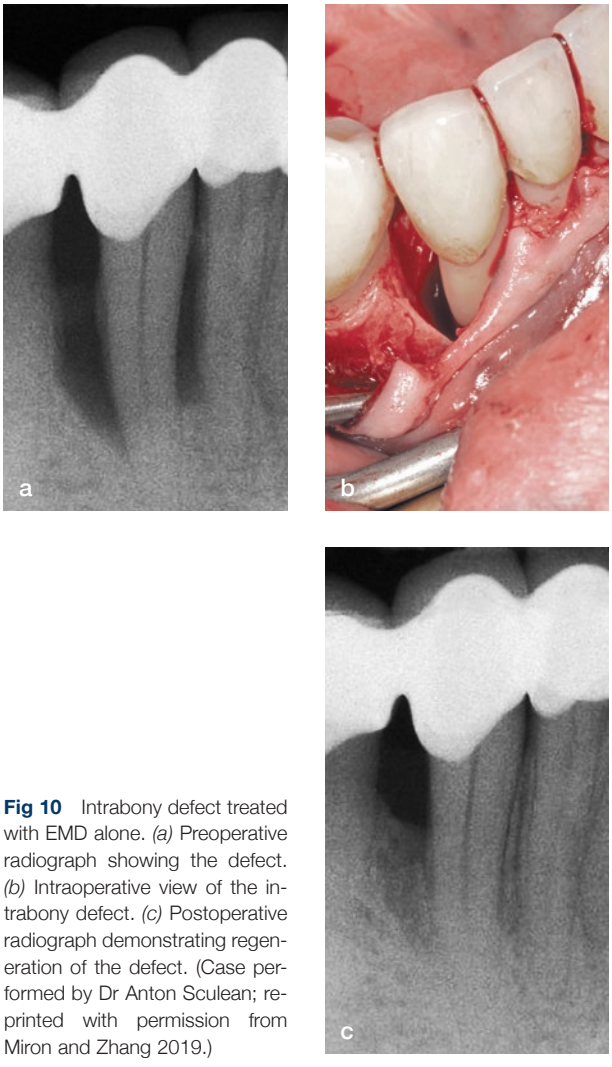

Fig 10 Intrabony defect treated with EMD alone. *(a)* Preoperative radiograph showing the defect. *(b)* Intraoperative view of the intrabony defect. *(c)* Postoperative radiograph demonstrating regeneration of the defect. (Case performed by Dr Anton Sculean; reprinted with permission from Miron and Zhang 2019.)

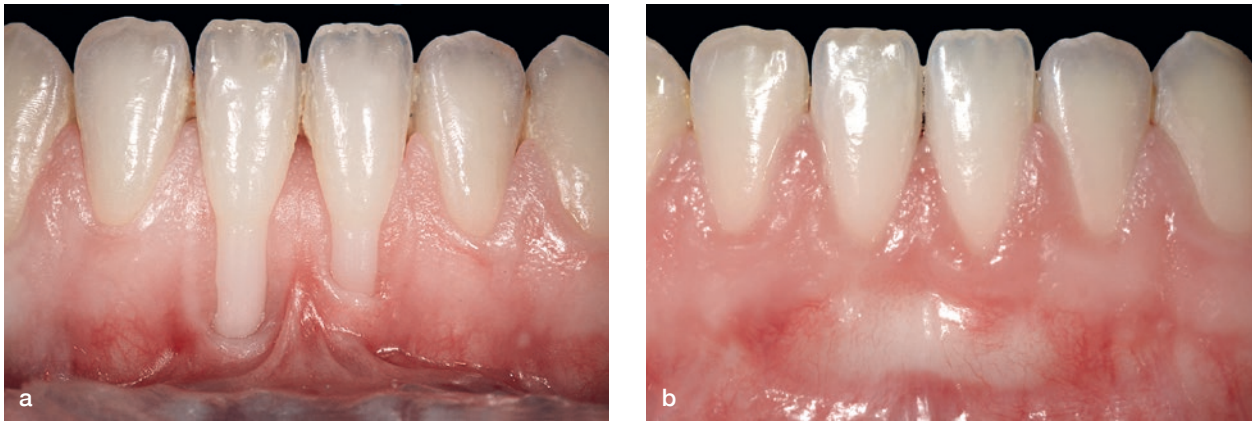

Fig 11 Use of EMD for the treatment of a Miller Class II recession. *(a)* Baseline photograph illustrating multiple Miller Class II recessions. *(b)* Two-year outcome following recession coverage with EMD and a connective tissue graft. (Case performed by Dr Anton Sculean; reprinted with permission from Miron and Zhang 2019.)

Miron et al 2016), and in recession defects either alone or as adjunct to soft tissue grafting (Fig 11; Cairo et al 2008, 2014; Castellanos et al 2006; Cueva et al 2004; Hägewald et al 2002; Pilloni et al 2006; Spahr et al 2005). Amazingly, the use of EMD was one of the first regenerative strategies derived from proteins used for clinical applications in dentistry. It is noteworthy that the breakthrough discovery of EMD was the result of a general understanding from basic research studies regarding the cell biology and communication of ameloblasts/amelogenins during cementogenesis. Millions of patients have since benefited from this concept.

Summary and Future Perspectives

Odontogenesis is a complex phenomenon that activates many cell types from many dermal layers. While its complexity has been revealed gradually over the years, it remains of interest to point out that much missing research remains needed that may eventually further improve tissue regeneration and potentially even the regrowth of teeth.

At the basis of enamel formation are the ameloblasts that not only play a pivotal role in enamel formation but also in cementogenesis. As highlighted within this chapter, EMPs secreted by the ameloblasts are responsible for periodontal regeneration, which has led to commercialization of EMD utilized in dentistry as a regenerative agent for more than 25 years.

Over the years, EMPs have also been shown to affect soft tissue wound healing (Miron et al 2015). Following much use of EMD in dentistry, these findings were extrapolated to general medicine, where EMD has also been utilized for the treatment of complex ulcers such as those found in diabetes on the lower limbs (Vowden et al 2006, 2007). Thus, it remains of interest to further evaluate how proteins secreted specifically by ameloblasts possess such wound healing properties.

Other investigators have focused on the various proteins and fractions of enamel matrix proteins to determine the effects of various proteins/fractions on biologic processes (Johnson et al 2009; Mumulidu et al 2007). For instance, Johnson and colleagues fractionated EMD into three major protein peaks following size exclusion chromatography with cross-linked dextran particle matrix. Peak I was associated with the column void volume, whereas peak III eluted near the salt volume. Peak II eluted between these two peaks. Proliferation and angiogenic activities were associated with peaks II and III with microvascular cells. The differentiation of osteoprogenitor cells was induced by EMD components present in peak I and the leading edge of peak II. The additional observation that this differentiation was inhibited by prior treatment of

the fractions with noggin suggested the activity was induced by bone morphogenetic protein rather than amelogenin or other unknown proteins. Gelatinolytic activities were detected in the early fractions of peaks I and II of gel-fractionated EMD.

In summary, ameloblasts prove to be an interesting cell type with various proteins secreted exclusively from their sources. These proteins, such as amelogenins, have been found to be associated with cementogenesis, periodontal regeneration, soft tissue healing, and various wound healing applications utilized in medicine. Future research will surely further uncover new keys to their function, role, and potential further applications.

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