CELL-TO-CELL COMMUNICATION

CELL ATLAS – VISUAL BIOLOGY IN ORAL MEDICINE

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 dis-play 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

Autocrine signaling

Juxtacrine signaling

Signaling cell   Target cell

Paracrine signaling

Signaling cell   Target cell

Endocrine signaling

Signaling cell
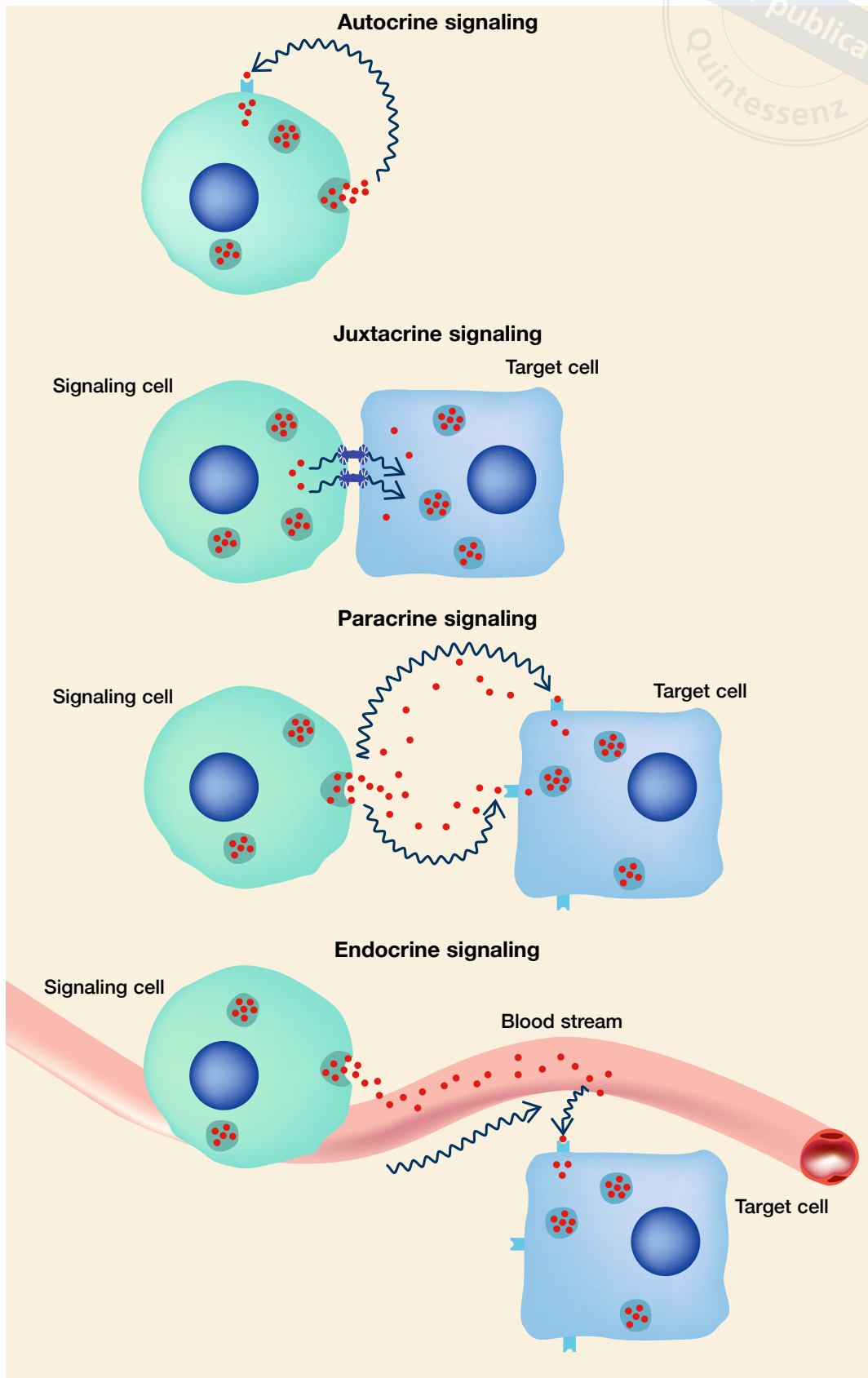
Blood stream

Target cell

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.

Osteocytes

[Osteocyte: Old Greek ὀστέον *(ostéon)* "bone" and κύτος *(kýtos)* "cell"]

Reinhard Gruber and Bernd Stadlinger

Osteocytes (original magnification ×3000).

Definition, Development, and Histologic Appearance

Osteocytes are long-living, former osteoblasts that became embedded in lacunae within the mineralized matrix and are connected via dendritic processes that run through canaliculi (Figs 1 and 2). Osteocytes build a syncytium, a dense interconnected network that spans the entire skeleton and thus also the skull bones with the periodontal structures. The syncytium also connects to blood vessels and the bone surface with its lining cells, osteoblasts, and osteoclasts (Fig 3). As recently summarized (Buenzli and Sims 2015), 1 mm^3 of bone hosts around 20,000 to 30,000 osteocytes, each with 100 dendritic processes and a radius of around 70 nm. The surface area of a lacuna

Fig 1   Osteocytes in cracked bone tissue (original magnification ×3600). (Courtesy of eye of science.)

measures around 300 µm². These numbers become particularly impressive when calculated for the entire skeleton, with around 40 billion (10^9) osteocytes making 20 trillion (10^{12}) connections and a total length of dendritic processes of 200,000 km. The surface area and the volume of the lacuno-canalicular network are around than 200 m² and 40 cm³, respectively. Osteocytes are not only interconnected via their dendritic processes; a liquid surrounds them and connects the cells to the overall circulation. Osteocytes are obviously predestined to control bone homeostasis at the local and systemic levels. In the newly formed woven bone, there is an increasing density of osteocytes that appear larger than those observed on the mature lamellar bone (Fig 4).

Fig 2 Osteocyte on the surface of an alveolar bone (original magnification ×4000). (Courtesy of eye of science.)
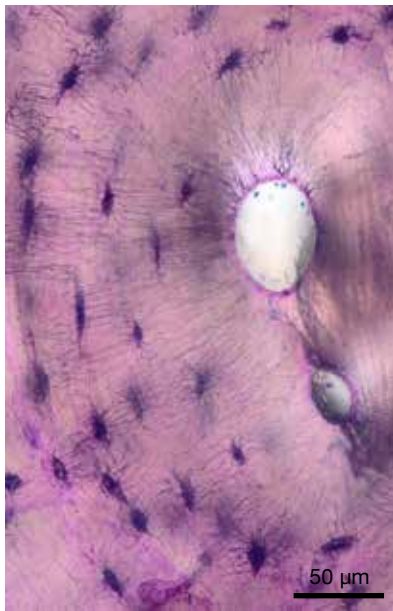
Fig 3 Osteocyte in lamellar bone (Lévai-Laczkó stain). The osteonal structure is characterized by the central canal leaving space for the blood vessels that provide the progenitor cells of osteoblasts and osteoclasts. Osteocytes, being former osteoblasts now embedded in the pink mineralized matrix, are stained purple. The dendritic processes forming an interconnected network that reaches toward the blood vessel space are clearly visible. (Courtesy of R. Gruber, T. Dobsak, and S. Tangl.)
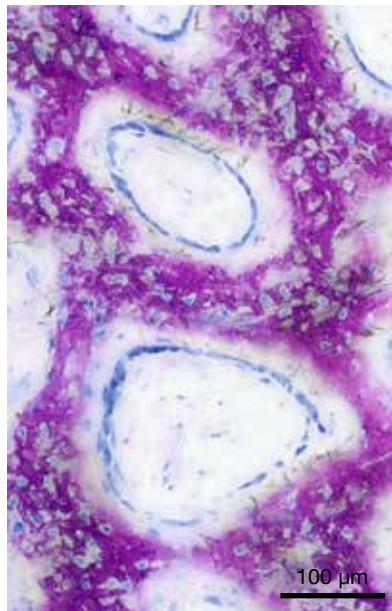
Fig 4 Osteocyte in woven bone (Lévai-Laczkó stain). Osteocytes are present in the newly formed woven bone that is characteristic for the early stages of bone regeneration, for instance around dental implants and at sites of bone augmentation. The purple stain shows the mineralized bone matrix hosting the numerous osteocytes with their large osteocyte lacunae. The blue ring indicates the bone-forming osteoblasts that produce the osteoid which later mineralizes. The supply of the osteoblasts is provided by a subtype of blood vessels, characterized by the H-type endothelial cells. (Courtesy of R. Gruber, T. Dobsak, and S. Tangl.)
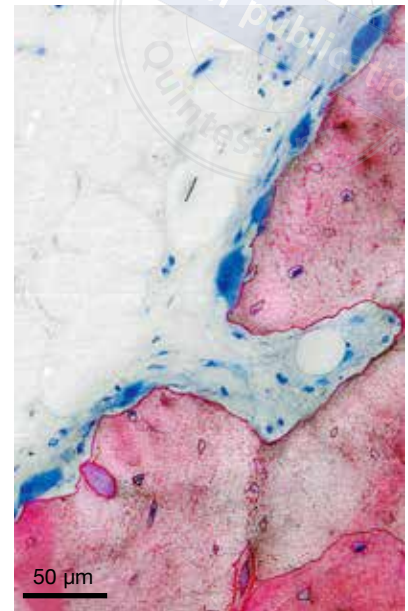
Fig 5 Osteocyte in communication with osteoclast. Osteoclasts are characterized by the multinucleated morphology in combination with their ability to resorb bone. There is increasing evidence that the formation of osteoclasts and their activation is controlled by the osteocytes, particularly dying osteocytes demarking necrotic areas and thus signaling the need to be replaced by new bone, which obviously requires the removal of the necrotic bone by the osteocytes (Lévai-Laczkó stain).
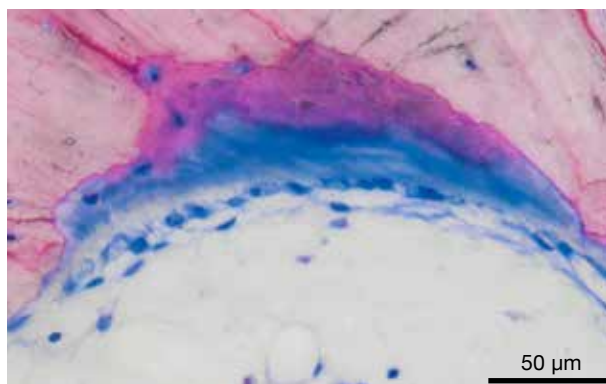
Fig 6 Osteocyte in communication with osteoblast. Osteoblasts are the cells actively forming bone, characterized by the non-mineralized osteoid matrix that within around 30 µm distance undergoes mineralization. Please note the cells that are now embedded in the osteoid later becoming an osteocyte, similar the other osteocytes that appear as blue dots with a lacuna surrounded by the pink staining of the mineralized bone matrix (Lévai-Laczkó stain).

Physiologic and Pathologic Aspects

At the local level, osteocytes signal the need for bone remodeling, including resorption of bone subjected to fatigue damage (Fig 5) and controlling bone formation to fill the gap (Fig 6; see reviews referenced at the end of the chapter). Under pathologic conditions, it is the osteocyte controlling the catabolic shift of bone remodeling, for example, in models of postmenopausal osteoporosis (Fujiwara et al 2016), secondary hyperparathyroidism (Xiong et al 2014), glucocorticoid osteoporosis (Piemontese et al 2016), and periodontitis (Graves et al 2018). Moreover, osteocytes are key sensors for mechanical
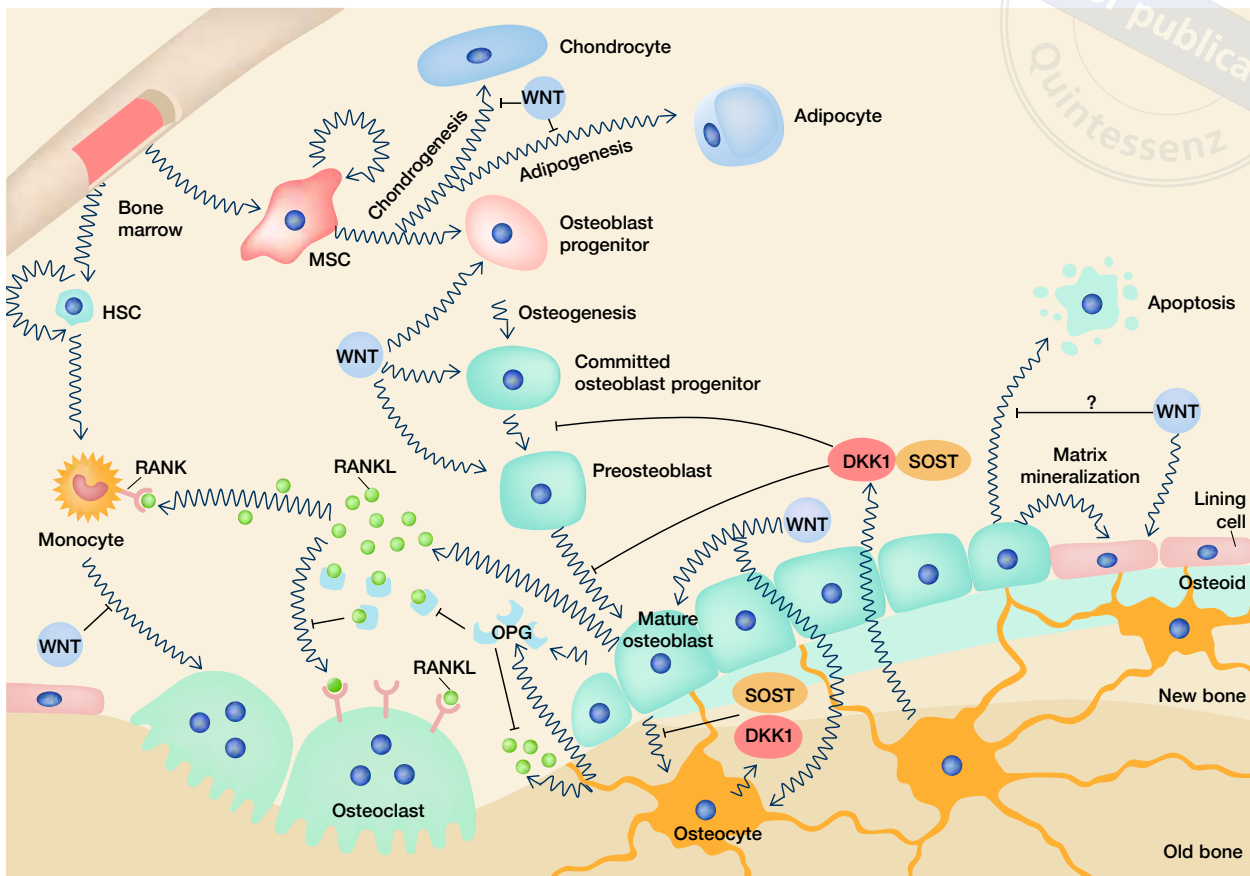
Fig 7 Osteocytes in control of the effector cells, the osteoclasts, and the osteoblasts. The schematic picture highlights the central role of the osteocytes to control osteoclastogenesis and osteoblastogenesis. It is particularly the osteocyte-derived RANKL in balance with OPG that drives the differentiation of blood-derived hematopoietic cells toward the osteoclastogenic lineage. On the other hand, the release of SOST and DKK1 by the osteocytes antagonizes WNT signaling and thereby the formation of osteoblasts originating from their mesenchymal progenitor cells that also come along with blood vessels but more like the pericytes on the outer surface. HSC, hemopoietic stem cell; RANK, receptor activator of nuclear factor-kappa B; WNT, Wingless/int-1; MSC, mesenchymal stem cell; RANKL, RANK ligand; OPG, osteoprotegerin; DKK1, Dickkopf-1; SOST, sclerostin.

loading, signaling the need for structural reinforcement of bone that is intensively loaded, as originally postulated by Frost in the mechanostat theory (Hughes and Petit 2010). At the systemic level, osteocytes produce fibroblast growth factor (FGF)-23, which targets the kidney and regulates phosphate homeostasis (Quarles 2012). Thus, osteocytes are not only susceptive of systemic hormones, local factors, and biomechanical stimuli; osteocytes integrate a large spectrum of signals, transforming them into key signals that control bone homeostasis. Understanding the function and the expression of these fundamental signals by osteocytes is the scientific foundation for therapeutic strategies, e.g., aiming to modulate bone remodeling.

Molecular Aspects of Cell Communication

As summarized in Fig 7, among the fundamental signals that are almost exclusively produced by the osteocytes is sclerostin, an antagonist of the Wingless/int-1 (WNT)/β-catenin signaling pathway (van Bezooijen et al 2004). Sclerosteosis and van Buchem disease are due to the loss of sclerostin expression or secretion, respectively; both diseases are characterized by bone overgrowth, including the jaw and facial bones (van Bezooijen et al 2005). Mouse models lacking the sclerostin gene (*Sost*) display high bone mass (Li et al 2008), and the alveolar bone and the cementum are increased (Kuchler et al 2014). Reduced pro-

duction of sclerostin by osteocytes is, for instance, a consequence of elevated parathyroid hormone (PTH) levels that increase bone mass (Keller and Kneissel 2005). Osteocytes are the main source of receptor activator of nuclear factor-kappa B ligand (RANKL) required for osteoclast formation during physiologic bone remodeling (Nakashima et al 2011; Xiong et al 2011, 2015) but also under pathologic conditions, including ovariectomy (Fujiwara et al 2016), secondary hyperparathyroidism (Xiong et al 2014), glucocorticoid excess (Piemontese et al 2016), and periodontitis (Graves et al 2018). Mice lacking osteocyte-derived RANKL even resist bone loss caused by tail suspension (Xiong et al 2015). In osteocytes, the receptor for PTH and PTH-related peptide regulates the respective anabolic but also the catabolic effects (Saini et al 2013). Also the mechanisms how dying osteocytes signal the need for bone resorption are beginning to be understood, including ATP, released from apoptotic osteocytes for triggering bystander osteocyte RANKL expression (McCutcheon et al 2020). Osteocyte necrosis triggers osteoclast-mediated bone loss through macrophage-inducible C-type lectin (Andreev et al 2020). Thus, osteocytes control bone formation and bone resorption in health and disease, including their expression of sclerostin and RANKL.

RANKL, sclerostin, and FGF-23, all molecules produced by osteocytes, are targets for pharmacologic therapies. RANKL is neutralized by denosumab (Prolia and XGEVA, Amgen), a monoclonal antibody approved for the treatment of osteoporosis and for the prevention of skeletal-related events in patients with bone metastasis from solid tumors (Lewiecki and Bilezikian 2012).

Romosozumab, a monoclonal antibody that neutralizes sclerostin, lowers the risk for new vertebral fractures and might become a future osteoporosis therapy (Cosman et al 2016). Teriparatide (Forteo, Eli Lilly), N-terminus 1-34 amino acids of PTH, is approved in the treatment of osteoporosis (Lindsay et al 2016). Its anabolic mechanisms may involve PTH lowering the expression of sclerostin by osteocytes (Keller and Kneissel 2005). KRN23 is a novel antibody to neutralize FGF-23 and thus to control renal maxi-

mum threshold for phosphate reabsorption as well as low serum phosphate and 1,25-dihydroxyvitamin D levels in X-linked hypophosphatemia (Carpenter et al 2014). Understanding the role of osteocytes both to control autocrine/paracrine mechanisms of bone remodeling and to regulate phosphate homeostasis allows the pharmacologic profile of the various therapies to be increasingly understood as well.

Preclinical Research Related to Dentistry

Osteocytes have a high implication in dentistry. For example, osteocytes play a role in catabolic processes on the pressure side in orthodontic tooth movement. Microcracks (Verna et al 2004, 2005) and dead osteocytes (Hamaya et al 2002, Sakai et al 2009) are found on the pressure side, together providing the signals that trigger massive resorption of the affected bone (i.e., osteocyte apoptosis and RANKL-related osteoclastogenesis).

Orthodontic animal models also show comparatively low sclerostin expression in osteocytes on the traction side (Nishiyama et al 2015) as there is osteocyte-periodontal ligament cross-talk in tooth movement (Odagaki et al 2018). Osteocytes are also of key relevance in implant dentistry based on the fundamental principle that dying osteocytes signal the need for bone to be resorbed. For instance, extensive zone of dying osteocytes occur upon high implant insertion torque, undersizing an implant osteotomy, and thermal damage upon drilling. This damaging process defines the area for osteoclast to be resorbed, a process that is clinically considered as the transition of the mechanical primary into the biologic secondary stability (Cha et al 2015, Chen et al 2018, Wang et al 2017, Coyac et al 2019). It is thus not surprising that new implants (Yin et al 2019) and novel osteotomy preparation techniques (Chen et al 2019) are designed to lower compressive strain and thermal injury with the overall goal to prevent osteocytes from undergoing apoptosis and thereby lower the peri-implant bone resorption. The dying of osteocytes is presumably also the main trigger that

Fig 8 *(a and b)* Orthodontic tooth movement, showing bone resorption on the pressure side and new bone formation of the tension side. (Courtesy of R. Patcas, Zurich.)
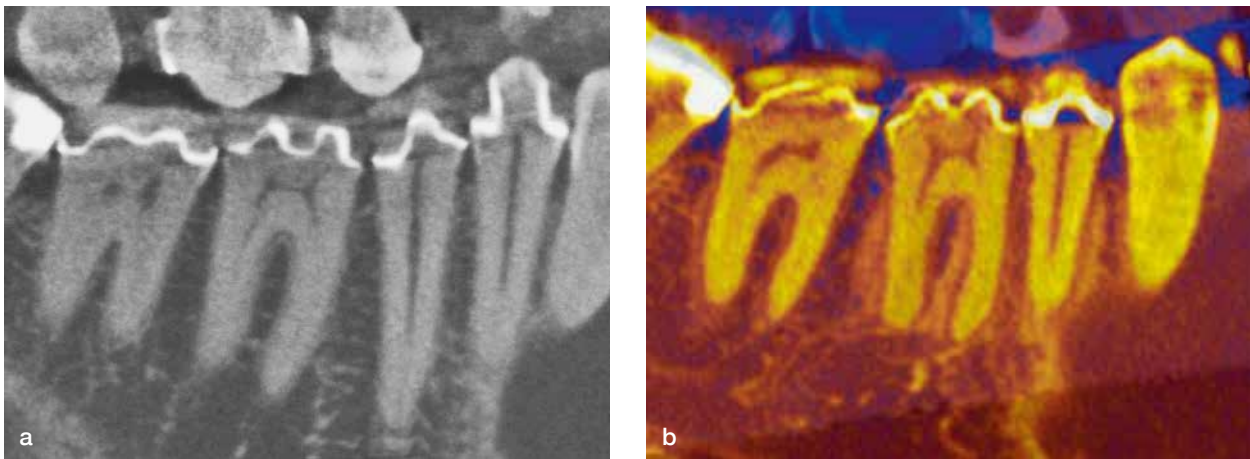
Fig 9 Orthodontic tooth movement, prior to movement *(a)* and after tooth movement *(b)*, showing alveolar thickening of bone around the tooth roots (color-coded fusion image).

causes the resorption of autologous bone grafts, particularly of bone chips with their large surface to be attacked by the osteoclasts (Saulacic et al 2015). Moreover, age-related degeneration of osteocyte networks may impair bone anabolic responses to loading, and sex differences in osteocyte cell body and lacunar fluid volumes may lead to sex-related differences in mechanosensitivity (Tiede-Lewis et al 2017).

Clinical Relevance in Dental Medicine

In clinics, aspects of mechanotransduction are of high importance in various oral and maxillofacial treatment procedures. Clinical examples of high relevance are orthodontic tooth movement, treatment of cystic lesions, bone augmentation, and implant placement with consecutive alveolar bone remodeling.

The *first* clinical example of osteocyte function is orthodontic tooth movement. As mentioned earlier, it is feasible through the exertion of forces on the tooth. Consecutive bone resorption is induced on the pressure side via osteoclasts, and bone formation is induced on the tension side via osteoblasts (Bumann and Frazier-Bowers 2017). The interplay of these two antagonists is orchestrated by the osteocyte, being a mechanosensing cell (Figs 8 and 9).

The *second* clinically interesting treatment procedure with regard to osteocyte function is the treat-
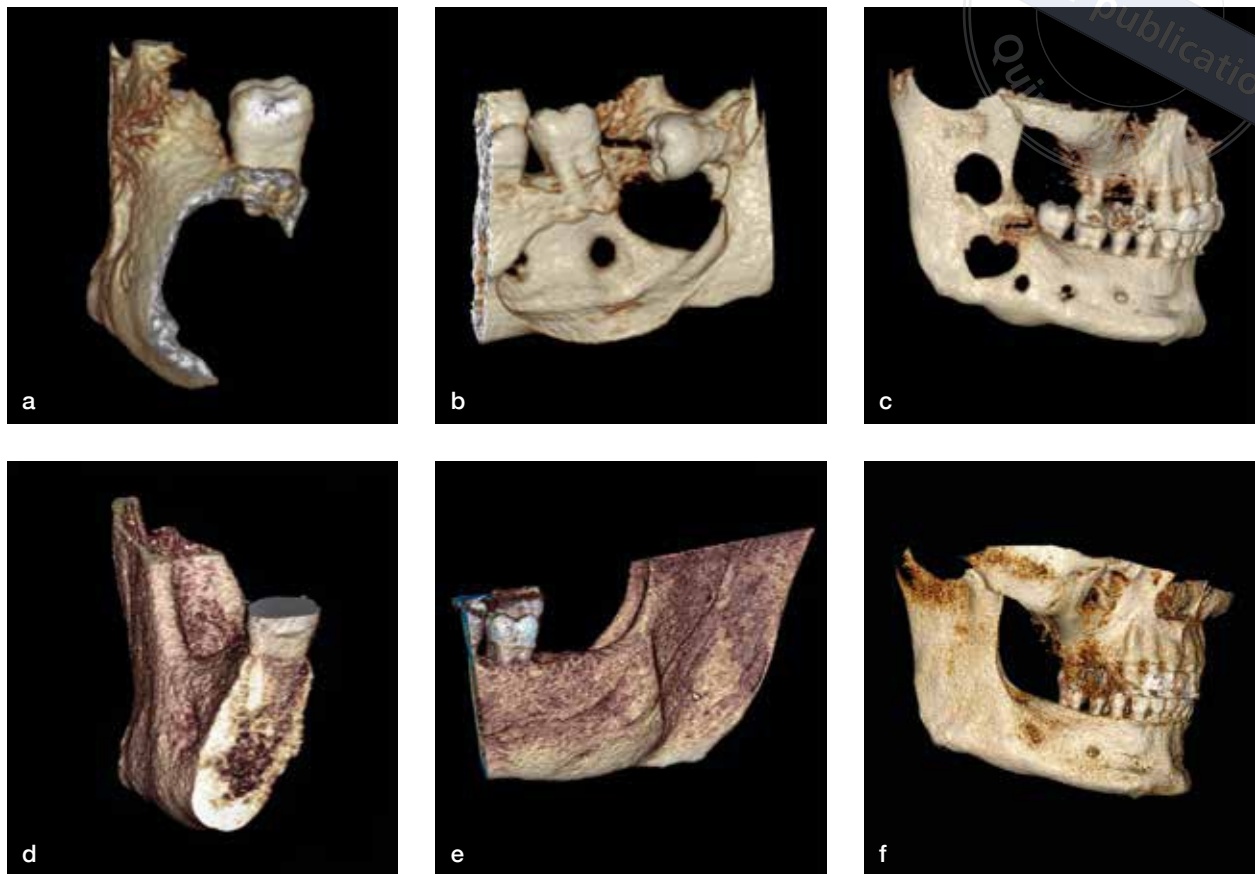
Fig 10 *(a to c)* Follicular cyst of the angle of the jaw due to an impacted third molar. *(d to f)* Same views 2 years after third molar removal and cystostomy, showing reossification. (Raw data of Figs 10a to 10c courtesy A. Stricker, J. Fleiner, Constance.)

ment of cystic lesions. Sclerostin staining was shown in aneurysmal and solitary bone cysts (Inagaki et al 2016). Most commonly encountered odontogenic cysts in the jaw, however, are radicular and follicular cysts. In these cysts, due to a continuous fluid uptake through the cyst membrane, pressure is exerted on the neighboring bone, leading to bone resorption. Following cystostomy or cystectomy, this pressure is relieved, mostly leading to new formation of bone within the former cavity. The switch from bone resorption to new bone formation is theoretically also induced by the osteocyte function in this case (Fig 10).

The *third* clinical example of the influence of mechanical forces on bone is dental implantology. In cases of bone atrophy, bone augmentation is required prior to implant placement. The transplantation of an autogenous bone block requires bone remodeling for the integration of this transplanted bone at the recip-

ient site. Following several months of remodeling, implant placement can take place. Implant placement causes controlled mechanical trauma, inducing further cortical remodeling due to microcracks in the peri-implant area (Wang et al 2014). Implant placement and consecutive osseointegration is strongly dependent on the mechanical forces being exerted on the peri-implant bone. A high implant torque value, leading to increased pressure, may lead to microfractures and bone necrosis due to the increased stimulation of osteoclasts by dying osteocytes (Insua et al 2017). Dead and dying osteocytes have been shown in increased amounts around implants with increased torque values (Cha et al 2015). In case of appropriate placement protocols, osseointegration will follow the phases of wound healing, leading to a balance of bone formation and bone resorption (Fig 11; Terheyden et al 2012).

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Fig 11 Anterior maxilla (a) before bone augmentation, (b) 5 months after bone block augmentation, and (c) with 3D digital implant planning (SMOP, Swissmeda).

Dental implants provide a protective effect for the surrounding bone, as bone atrophy within this area will be impacted (Boven et al 2014).

Summary and Future Perspectives

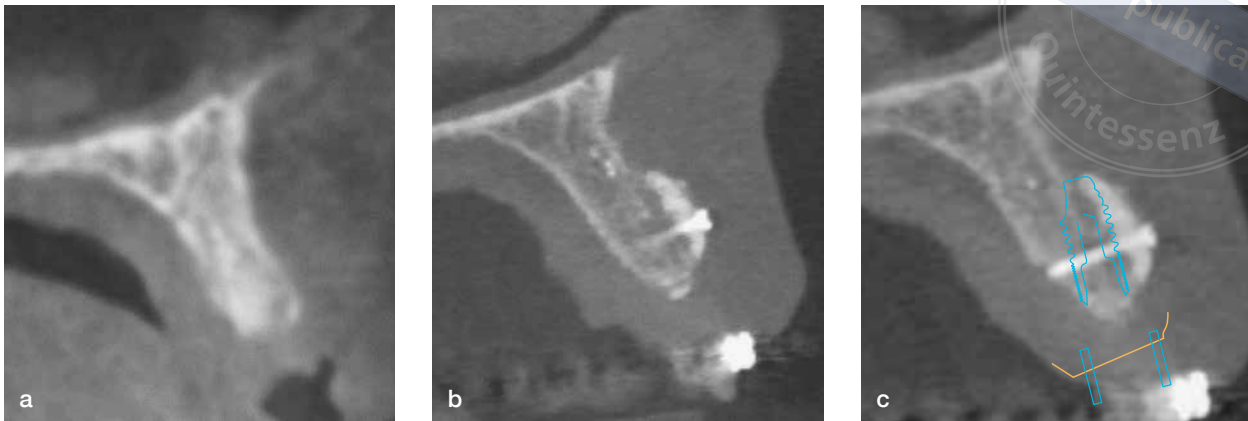Osteocytes have recently been considered "amazing" cells as they are in control of the effector cells of bone remodeling, the bone-forming osteoblasts, and the bone-resorbing osteoclasts. In physiology, osteocytes sense the need to replace damaged bone (see reviews referenced below), while in pathology, dying osteocytes can also cause a catabolic shift of bone remodeling that culminates in bone loss and atrophy. Osteocytes are sensitive to drugs controlling bone remodeling. Osteocytes also have an endocrine function, but that was not the focus of this chapter. The critical role of osteocytes to release molecular signals such as SOST and RANKL, controlling cell-to-cell commutation, have been decoded. Today, the challenge is to take advantage of this molecular information to understand the role of osteocytes in local and systemic phenomena that are relevant in dentistry, for example, bone atrophy, osseointegration, graft consolidation, and orthodontic tooth movement.

Readers are referred to recent reviews on osteocytes for further details (Bellido 2014, Bonewald 2011, Dallas et al 2013, Plotkin and Bellido 2016, Shah et al 2018, Zhao et al 2016).

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Epilogue

Hendrik Terheyden

By viewing the cellular level with the artistic scanning electron micrograph (SEM) images of Oliver Meckes and Nicole Ottawa, the highly qualified creative experts of the "eye of science" team with over 25 years of experience, the clinician and reader may develop their personal approach to the cells, at first on an esthetic level. This understanding was further deepened in this book by introducing the cells as individuals, like actors in a film, based on morphologic criteria and their histologic tissue products. The deepest understanding of the cells will be found in decoding the communication between cells and seeing how it is regulated. The human body is in this view not a single individual, but a holobiont of a billion eukaryotic and even more prokaryotic cells (bacteria). It is clear that such a holobiont can only exist if these single individual cells are controlled and regulated, and this requires cell communication between them. Cells talk in a language, the language of cytokines and other molecules. Prof Gruber has addressed this in the prologue of this book.

Once we have understood the language of cells, clinicians might be able to talk to cells and control their action. After browsing through this book, the reader may get some impression of how far we are from understanding this language. A modest contribution this book can offer is to bring us from the level of unconscious to conscious incompetence in terms of speaking the language of the cells. But this book also can show us the direction in which research and therapy will go in the future, described at the end of each chapter. The cells are the key for therapeutic influence in the future. This book helps to open the vision of how we can regenerate tissues and heal diseases by controlling the language of the cells. We already have been clinically successful in applying certain cytokines, for example, bone morphogenetic protein 2, to stimulate osteoblasts to successfully fill a bone defect. Instead of invasive bone tissue transplantations, we can stimulate the body's own regenerative potential, elegantly filling a bone defect without foreign materials or open surgery. In the opposite direction, also molecular osteotomies are imaginable, with topically applied cytokines, which promote the differentiation and activation of osteoclasts. We can imagine the revolution this might cause in orthodontics. The reason why such therapeutic approaches are not available today or only at a very rudimentary level is the complexity of the cellular regulation in our body. This book has demonstrated the level of complexity, of which there are mainly three sources.

The first source of complexity is the multitude of cell types, which have been introduced in this book based in morphologic criteria. Behind these cell types stands a cellular differentiation during the development of the particular cell, which is based on specific activation of a few of the 40,000 coding and noncoding human genes. We are far from understanding all of these genes and their interactions, which are the product of millions of years of evolution of life. Their pure number explains the complexity.

A second source of complexity is the multitude of interactions between cells and their control circuits. To every action there is always an equal counteraction, Sir Isaac Newton said. This is especially true for the cellular metabolism and control. Thinking at the cellular level means to think in balances. For example, periodontal bone loss is not only action of osteoclasts, it is more an overemphasis on bone resorption, a slight shift in the physiologic balance of bone remodeling over time. A multitude of interdigitating circuits control bone remodeling, with some promoting bone formation and some promoting more bone resorption. Anti-inflammatory conditions promote osteoblasts and

pro-inflammatory conditions promote osteoclasts. In addition, the activation of a cell surface receptor activates a second messenger system inside the cells, which can amplify a signal or dampen it and which stands under the influence of multiple other intracellular control circuits. Even the genes inside the cell nucleus interact at the next level. Obviously, in a complex organism, uncontrolled action of a cell type is a severe threat if one thinks of cancer. Therefore, inhibitory control circuits outweigh stimulatory circuits. That is the reason why it is not easy to address the one stimulatory influence to heal a defect; one also has to dampen the inhibitory competitive circuit.

A third source of complexity is the small size of the signaling molecules and the unimaginable high numbers in the atomic scale. In some chapters of this book, some cytokines are displayed in sketches and schematic drawings; this can easily cause a wrong impression in the reader. A cell is already small at 10 microns, but a cytokine molecule is smaller by a factor of 1000. In terms of volume three dimensionally, this means a size difference of 10 to the ninth between cell volume and the volume of a cytokine molecule. A good way of visualizing the interaction of a cell with a cytokine is if we imagine moving through a fog. We can hardly distinguish single fog particles, but we can identify where the fog is denser or lighter, similar to chemotaxis for the cells. This picture also explains why the cell is never exposed to only one signal, but in a fog of molecules, some signals can become overemphasized. It also explains, based on the size of the signaling molecules, that most of this stimulation has to occur within the cellular distance of

the molecular reach of a cell. That often means a topical action in the tissue context. For instance, osteoclast precursors need to sense collagen for their development, which is bound to the bone surface and not soluble. That limits their differentiation to the exposed local bone surface and prevents uncontrolled body-wide bone resorption.

It is fascinating to follow how quickly modern research makes discoveries. This book by world-renowned experts, with at least one clinician and one basic scientist for each chapter, highlights a reliable and actual state of research that quickly moves forward. Medicine and dentistry are applied natural sciences with a strong foundation in biology. Dentistry in particular has moved from a material-based profession into a biologically oriented specialty. Where in the past dental treatment in the form of prostheses and tooth restorations were applied outside the ectodermal barrier, today dentists enter into the human body, for instance, by placing intraosseous implants or for surgical treatment of periodontitis. Furthermore, the success of dental therapies more and more depends on biologic factors, like antiresorptive medications, and other health risk factors. Consequently, biologic knowledge has a strong place in dental education.

Finally, the authors and editors of this book are deeply convinced that the success and quality of the clinical therapies improve with the awareness of the cells as our clinical partners. This book has tried to contribute to our biologic imagination, and we would like to thank the kind reader for sharing that fascination with us.

List of Abbreviations

A

AC acinus cell
AChR muscarinic acetylcholine receptor
ACPA anti-citrullinated peptide antibodies
ADAMTS a disintegrin and metalloproteinase with thrombospondin motifs
Adipo-CAR adipogenic CAR cell
AE2 anion exchange protein 2
AP-1 activating protein-1
APRIL a proliferation-inducing ligand
AQ5 aquaporin 5
ARC adventitial reticular cell
ASIC acid-sensing ion channel
ATP adenosine triphosphate

B

BAFF B cell activating factor of the TNF family
BCL-6 B cell lymphoma 6
BCR B-cell receptor
BCSP bone cartilage stromal progenitor
BLIMP1 B lymphocyte maturation-induced protein-1
BLNK B cell linker protein
BLyS B-lymphocyte stimulator
BM bone marrow
BM-MSC bone marrow–derived mesenchymal stromal cell
BMP bone morphogenetic protein
BMU bone multicellular unit
Breg regulatory B cell
BSP bone sialoprotein
BTK Bruton's tyrosine kinase

C

Ca^{2+} calcium ion
CAF coronally advanced flap

CaMKIV calcium/calmodulin-dependent protein kinase IV
cAMP cyclic adenosine monophosphate
CAP cementum attachment protein
CAR Cxcl12-abundant reticular
CCD charge-coupled device
CCL C-C chemokine ligand (e.g., CCL2)
CDGF cementum-derived growth factor
CEMP1 cementum protein 1
CFU-F colony-forming unit fibroblast
CGRP calcitonin gene-related peptide
Cl^- chloride ion
Col1A1 collagen type $I\alpha1$
COX-2 cyclooxygenase-2
CP-23 cementum protein 23
CPC calcium phosphate cement
CREB cyclic adenosine monophosphate response element binding protein
Cx43 connexin 43
Cxcl12 C-X-C motif chemokine ligand 12
cysC cystatin C

D

DAG diacylglycerol
DAP12 DNAX activation protein of 12 kDa
DC-STAMP dendritic cell-specific transmembrane protein
DDR-2 discoidin domain receptor 2
DEL-1 developmental endothelial locus-1
DESC dental epithelial stem cell
DKK1 Dickkopf-1
DMSC dental mesenchymal stem cell
DNase deoxyribonuclease
DNMT3A DNA methyltransferase 3A
DP-MSC MSC-related cell harvested from dental pulp
DPSC dental pulp stem cell

E

ECM	extracellular matrix
EMD	enamel matrix derivative
EMP	enamel matrix protein
ENPP1	ectonucleotide pyrophosphatase/ phosphodiesterase 1
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
ERM	epithelial rests of Malassez

F

FcγRIII	Fc gamma receptor III
FcRγ	gamma subunit of immunoglobulin Fc receptor
FFOD	familial florid osseous dysplasia
FGF	fibroblast growth factor
FISH	fluorescence *in situ* hybridization
fMLP	formyl-methionyl-leucyl-phenylalanine
Foxp3	forkhead box P3
FSP-1	fibroblast-specific protein-1

G

GACI	generalized arterial calcification in infancy
GAG	glycosaminoglycan
G-CSF	granulocyte colony-stimulating factor
GFR	growth factor receptor
Gli1	glioma oncogene homolog 1
GM-CSF	granulocyte-macrophage colony-stimulating factor
Grem-1	gremlin-1
GTP	guanosine triphosphate
GTR	guided tissue regeneration

H

H&E	hematoxylin & eosin
HA	hyaluronic acid
HA/TCP	hydroxyapatite tricalcium phosphate
hBD	human beta-defensin
hCAP18	human cationic antimicrobial peptide-18
HCO_3	bicarbonate
HERS	Hertwig epithelial root sheath
HIES	hyper IgE syndrome
HPP	hypophosphatasia

hrCEMP1	recombinant human cementum protein 1
HSC	hemopoietic stem cells

I

ICAM-1	intercellular adhesion molecule 1
IEE	inner enamel epithelium
IFN-β	interferon-β
IFN-γ	interferon-γ
Ig	immunoglobulin
IGF-1	insulin-like growth factor-1
IL	interleukin
IMRT	intensity-modulated radiotherapy
IP_1	inositol monophosphate
IP3R	inositol trisphosphate receptor
IPEX	immune dysregulation polyendocrinopathy enteropathy X-linked
IRF	interferon regulatory factor
ISCT	International Society for Cellular Therapy
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibition motif

K

K^+	potassium ion

L

LAD	leukocyte adhesion deficiency
LAP	localized aggressive periodontitis
Lepr	leptin receptor
LFA-1	lymphocyte function-associated antigen-1
LL-37	leucine-leucine-37
LPS	lipopolysaccharide
LYST	lysosomal trafficking regulator

M

M1Φ	M1 macrophages
Mac-1	macrophage antigen 1
MAFB	musculoaponeurotic fibrosarcoma oncogene homolog B
MALT	mucosa-associated lymphoid tissue
MAPK	mitogen-activated protein kinase
MaR1	maresin 1

MCAM	melanoma cell adhesion molecule
MCC	mandibular condylar cartilage
M-CSF	macrophage colony-stimulating factor
MHC	major histocompatibility complex
micro-CT	microcomputed tomography
MITF	microphthalmia-associated transcription factor
MMP	matrix metalloproteinase
MΦ	macrophages
MPO	myeloperoxidase
MRI	magnetic resonance imaging
mRNA	messenger RNA
MRONJ	medication-related osteonecrosis of the jaw
MSC	mesenchymal stem cell
MSC	mesenchymal stromal cell
Mx1	myxovirus resistance 1
MyD88	myeloid differentiation factor 88

N

Na^+	sodium ion
NADPH	nicotinamide adenine dinucleotide phosphate
Nes	nestin
NET	neutrophil extracellular trap
NFATc1	nuclear factor of activated T cells 1
NF-κB	nuclear factor-kappa B
NHE1	sodium hydrogen exchanger isoform 1
NK	natural killer
NKCC1	$Na^+/K^+/2Cl^-$ co-transporter 1
NLR	NOD-like receptor
NO	nitric oxide
NOD	nucleotide-binding oligomerization domain
NOS2	nitric oxide synthase 2
Nrp1	neuropilin1

O

OCN	osteocalcin
OEE	outer enamel epithelium
OPG	osteoprotegerin
OPN	osteopontin
OSCAR	osteoclast-associated receptor
Osteo-CAR	osteogenic CAR cell

P

PAMP	pathogen-associated molecular pattern
PCM	pericellular matrix
PDB	Paget disease of the bone
PDGF	platelet-derived growth factor
PDL	periodontal ligament
PDLSC	PDL-derived mesenchymal cell
PDPN	Podoplanin
PGE_2	prostaglandin E_2
PHEX	phosphate-regulating endopeptidase homolog X-linked
pIgR	polymeric immunoglobulin receptor
PIP_3	phosphatidylinositol trisphosphat
PIR-A	paired immunoglobulin-like receptor type A
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PLCγ	phospholipase C-gamma
PLL1	poly-L-lysine 1
PLS	Papillon-Lefèvre syndrome
PMN	polymorphonuclear leukocyte
poly I:C	polyinosinic:polycytidylic acid
PP_i	inorganic pyrophosphate
PRP	platelet rich plasma
PRR	pathogen recognition receptor
PTH	parathyroid hormone
PTHrP	parathyroid hormone–related peptide

R

RANK	receptor activator of nuclear factor-kappa B
RANKL	receptor activator of nuclear factor-kappa B ligand
RER	rough endoplasmic reticulum
RGD	arginine-glycine-aspartic acid
RNA-seq	RNA sequencing
ROS	reactive oxygen species
RWV	rotating wall vessel

S

SEM	scanning electron micrograph/microscopy

Sema3A	semaphorin 3A
SH3BP2	SH3 domain-binding protein 2
SHH	sonic hedgehog
SIRPβ1	signal regulatory protein beta1
SLP76	SH2 domain-containing leukocyte phosphoprotein of 76 kDa
SNARE	soluble N-ethylmaleimide-sensitive factor attachment receptor
SOST	sclerostin
SPIM	selective plane illumination microscopy
SPM	specialized pro-resolving lipid mediator
SR	stellate reticulum
SSC	skeletal stem cell
sTREM-1	soluble triggering receptor expressed on myeloid cells 1
SYK	spleen tyrosine kinase

T

TAK1	transforming growth factor β-activated kinase 1
TAMP	tissue-associated molecular pattern
Tc	cytotoxic T cell
TCR	T-cell receptor
TEC	tyrosine kinase expressed in hepato-cellular carcinoma
TEM	transmission electron microscopy
Tfh	T-follicular helper cell
TGF-β	transforming growth factor β
TG-PEG	transglutaminase cross-linked poly(ethylene glycol)
Th	T helper (e.g., Th1)
TIMP	tissue inhibitors of matrix metalloproteinase
TLR	Toll-like receptor (e.g., TLR2)

TMEM16A	transmembrane member 16A
TMJ	temporomandibular joint
TNAP	tissue nonspecific alkaline phosphatase
TNF-α	tumor necrosis factor α
TRAF6	tumor necrosis factor receptor-associated factor 6
TRAM	TRIF-related adaptor molecule
TREM2	triggering receptor expressed on myeloid cells 2
Treg	regulatory T cell
TRIF	TIR-domain-containing adapter-inducing interferon-β
TRP	transient receptor potential [channels]
TRPA1	transient receptor potential ankyrin 1
TRPM8	transient receptor potential melastatin 8
TRPV1	transient receptor potential cation channel subfamily V member 1

U

UBC-GFP	ubiquitin C –green fluorescent protein
USP6	ubiquitin-specific protease 6

V

VAMP	vesicle-associated membrane protein
VE	vascular endothelial
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal polypeptide
VIPAC	vasoactive intestinal peptide receptor

W

Wnt / WNT	Wingless/int-1

X

XLH	X-linked hypophosphatemic rickets