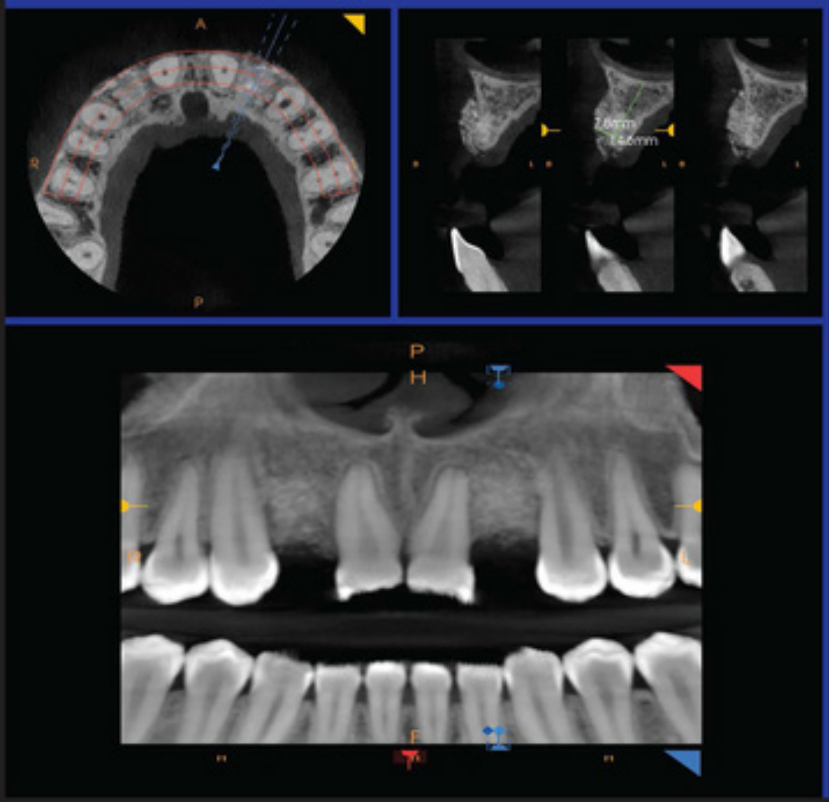
Second Edition

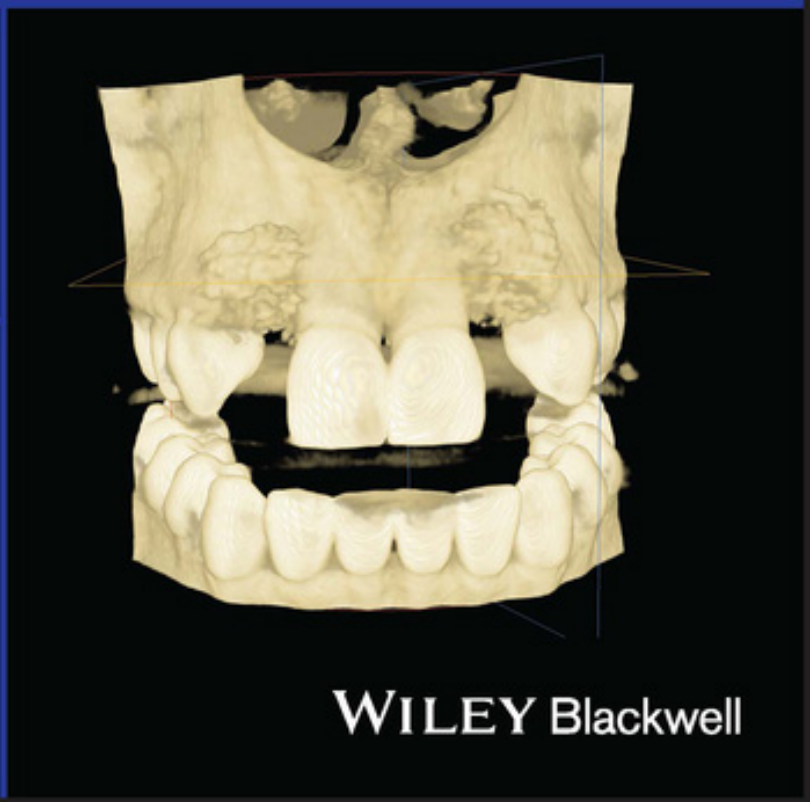
Essential Techniques of Alveolar Bone Augmentation in Implant Dentistry

A Surgical Manual

with website

Edited by **Len Tolstunov**





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Essential Techniques of Alveolar Bone Augmentation in Implant Dentistry

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A Surgical Manual

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Contents

Preface

Education is not a learning of facts but training of the mind to think.

Albert Einstein

Implant dentistry, like any medical or surgical discipline, is a constantly evolving science and practice. Old, complex, and traumatic surgical techniques slowly become obsolete and newer, simplified, less traumatic, and minimally invasive techniques come to the forefront. New medical books and surgical manuals come out to reflect new technological advances and techniques, new materials and methods, with the goal of helping to train a new generation of doctors who will be treating our patients with a higher degree of safety, precision, and efficiency.

The first edition of the book, published in 2016, consisted of two volumes titled *Horizontal Alveolar Ridge Augmentation in Implant Dentistry: A Surgical Manual* and *Vertical Ridge Augmentation in Implant Dentistry: A Surgical Manual*. This is the second edition of the book in a single volume. This second edition is different in two key points:

- As a single volume, it combines the best material from the previous two books with new material and techniques in a concise manner.
- It is geared towards a younger professional audience and written in a more didactic and step-by-step teaching approach. Another words, the material is presented in the easy to follow logical manner, similar to courses that are taught in dental and medical universities and schools. The chapters has plenty of illustrations, tables, photographs and radiographs, providing an ideal learning experience.

This surgical manual is a collaborative effort of more than 40 authors from around the world. They were hand-picked by the editor and, in order to qualify to be contributors, they had to possess three important professional qualities: be an authority in a particular surgical technique; be a full- or part-time professor with long teaching credentials at a university or dental school; and be a clinical scientist with an extensive publishing history (some of the authors have published more than 100 articles and several book chapters). So, the chosen clinical professors, teachers, and scientists from the start had the upper hand in contributing good-quality and high-value material for this book.

There was also another important feature of this multi-contributor approach to writing a professional medical book. Having more than 40 writers from numerous countries automatically gave this book a unique quality – it is *unbiased*. We believe that this impartial ("no agenda") approach of a plurality of material, written by several authors, leads to this book comparing positively to other books written by one or two authors who share a singular, and therefore somewhat partial and subjective, surgical experience.

This book therefore is an international, comprehensive, and balanced source of knowledge presented by experts for a wide audience, including oral surgeons, periodontists, periodontal and oral surgery residents in training, as well as dental students at dental and medical universities and schools around the world. We hope you like it.

BOOK ORGANIZATION

The book has two components: the written (book) version and the web version, which includes video clips prepared by contributors and related to specific chapters. The written text is divided into sections covering different aspects of bone preservation and bone and soft tissue augmentation in implant dentistry. The sections contain chapters demonstrating a variety of surgical techniques. There follows a short description of each section to help readers to orient themselves and choose sections or chapters of interest (see Video 1).

SECTION I

We had to start the story from the beginning. Section 1 begins with an important chapter on bone biology and wound healing. Chapter 1 should help a reader to develop understanding of the parameters of success of a surgical procedure based on the physiology of the hard and soft tissues of the jaws. This chapter is followed by Chapter 2 on the topographic anatomy of the stomatognathic system and Chapter 3 on radiographic evaluation of a dental patient. "Anatomy is destiny," said Albert Einstein. The physics genius was correct. When you choose a path to become a surgeon, anatomy would and should automatically become the second nature to you. You cannot proceed to and succeed in surgery if you do not know, understand, and respect human anatomy and physiology.

Still in Section I, we continue our journey and in Chapter 4 discuss the prosthetic evaluation of a patient to understand the need for a prosthetically guided approach in alveolar ridge augmentation in implant dentistry. Bone augmentation procedures are not a surgical exercise and only make sense if dental implants can be fully utilized for the function of mastication.

The section continues with Chapter 5 on a surgical classification or algorithm of bone augmentation techniques to demonstrate the decision tree that can help you to arrive at a certain horizontal (H), vertical (V), or both (3D) bone augmentation technique, required in each particular case. Chapter 6 is on key surgical maneuvers: incision designs, soft tissue flaps, and wound closures, imperative knowledge for any successful surgeon. Then comes Chapter 7, which discusses the biological rationale of a surgical procedure to help each surgeon to select a surgical technique with the best possible outcome.

SECTIONS II, III, AND IV

These sections describe the biomechanics of bone augmentation as it relates to implant dentistry, discussing two reconstructive options: ridge preservation and ridge augmentation.

Ridge preservation, the prophylactic approach to bone grafting discussed in Section II, demonstrates surgical techniques of prevention of bone loss after tooth extraction. It proposes bone grafting of a fresh extraction socket to prevent ridge collapse and bone atrophy.

Ridge augmentation, the therapeutic approach to bone grafting discussed in Sections III and IV, will showcase surgical techniques for existing and collapsed edentulous alveolar bone. The resulting bone deficiency, as discussed above, can be horizontal, which is more common and easier to treat; vertical, which is less common and more complex; or both, horizontal and vertical, which is the most difficult to manage. Often, bone loss has both width and height components. Sections III and IV demonstrate the techniques of (mainly) horizontal and (mainly) vertical bone augmentation, respectively.

SECTION V

It has been shown that the quality and quantity of soft tissue envelope covering the bone are another essential factor in implant dentistry. That is why this book presents a separate section, Section V, describing soft tissue grafting techniques for implant site development.

SECTION VI

In some complex cases of severe bone atrophy, a surgeon has to be inventive and think "outside the box." Complicated and traumatic cases can dictate a different approach for implant placement by planning an implant placement without bone grafting in areas where bone is available or using special implant designs. Section VI describes these innovative techniques: the "all-on-four" approach that utilizes a combination of four or more tilted and straight implants to immediately load a full-arch fixed and rigidly connected prosthesis; zygomatic and pterygoid implants that can be placed in less common bone regions, like the zygomatic bone and pterygoid plates of maxillary bone, providing rigid cross-arch support for the maxillary implant prosthesis; and short implants that can be placed in severely atrophic bone regions and prevent nerve injury and sinus complications, providing a viable restorative solution.

SECTION VII

"The best way to predict your future is to create it," as Abraham Lincoln said. Repair and regeneration of missing human tissue by harnessing patients' growth factors that attract mesenchymal (embryonic) stem cells into the surgical wound are bioengineering methods that are designed today, but lead medical and dental scientists and surgical practitioners into the future. This method is performed through the osteoinductive (bone-forming) process of cell proliferation and differentiation. Section VII is the last in the book, but one that a thoughtful reader should not miss. Chapter 30 on tissue bioengineering will teach the surgeon about these surgical techniques by explaining their biological rationale, encouraging choice of the one that has the highest regenerative value.

This book focuses on key bone reconstructive surgical techniques for deficient alveolar bone in implant dentistry. As an editor and one of more than 40 international contributors of this book, I encourage you to open the first page and start your learning journey, which should benefit both you, as a clinician, and your patients, the fortunate recipients of your acquired knowledge and skills.

Len Tolstunov

To access the videos for this preface section, please go to
www.wiley.com/go/tolstunov/essential

VIDEO 1 Introduction to the Book by the Author and Editor Dr. Len Tolstunov.

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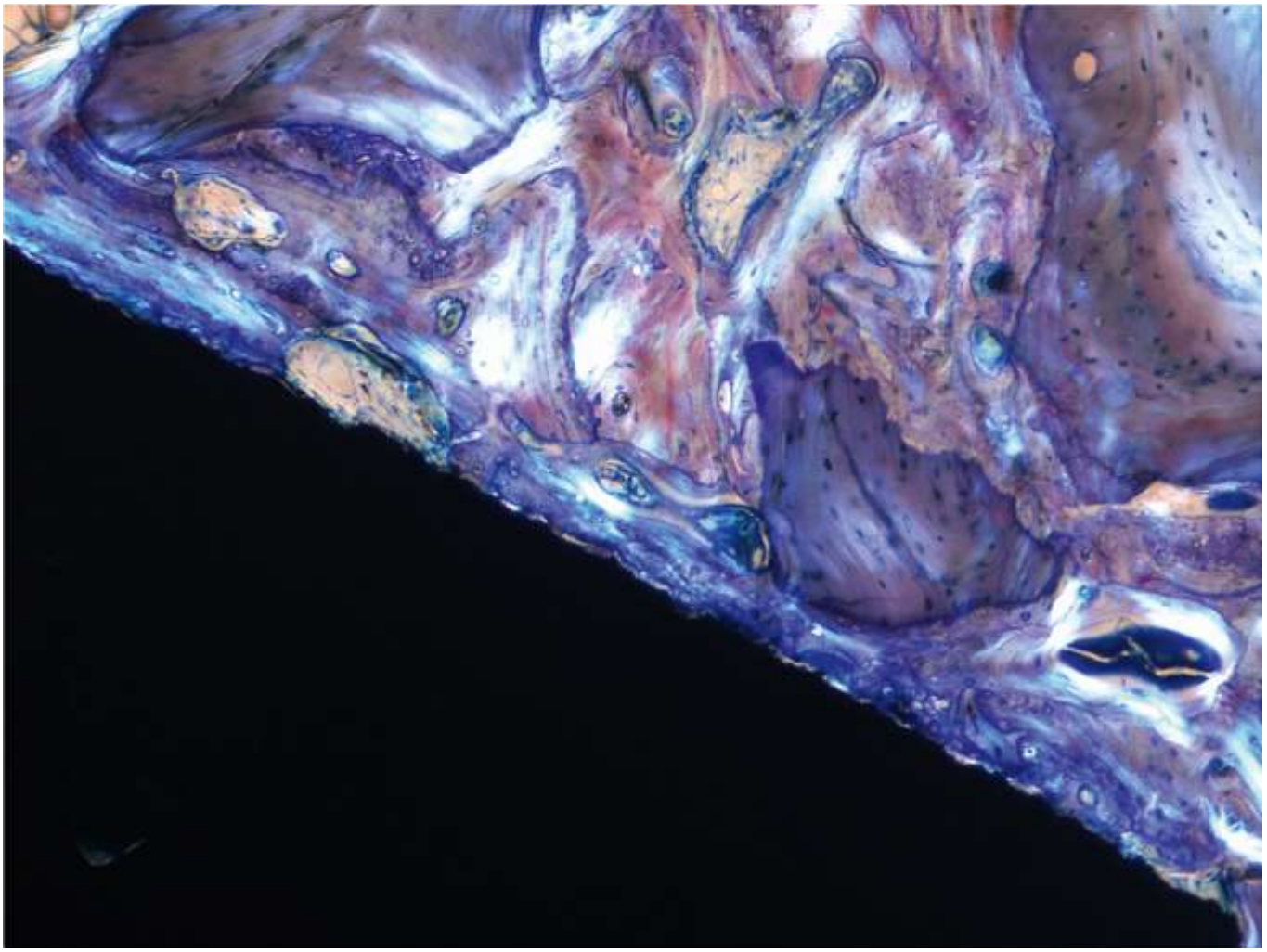
Introduction: Essential Clinical Knowledge

SECTION I

Bone Biology and Wound Healing

Although the bone is a rigid structure with an inert component, it is a living and breathing complex structure that maintains its micro-macroscopic assembly through human life and responds to external stimuli by absorbing or dissipating impacts, healing when fractured or growing. The continuous and adaptive metabolic response of this tissue is accomplished by a specific cellular apparatus that includes osteoblasts, osteoclasts, and osteocytes (see Video 1.1).



Intimate and functional contact established by the newly formed bone with the implant surface. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 100×.

CHAPTER 1.1

Bone Biology

Claudia Dellavia[1], Gaia Pellegrini[1], and Luiz Guilherme Fiorin[2]

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BONE TISSUE ANATOMICAL ASPECTS

Bone is a specialized mineralized connective tissue with the functions of protection, mechanical support, insertion of muscles, and reserve of ions for the maintenance of mineral homeostasis in the organism.

Although bone is a rigid structure with an inert component, it is a living complex that maintains its micro-macroscopic structure through the organism's life and responds to external stimuli by absorbing or dissipating impacts, healing when cracked or fractured, and growing. The continuous and adaptative metabolic response of this tissue is performed by a specific cellular apparatus that includes osteoblasts, osteoclasts and osteocytes, and lining cells.

MAIN CELLULAR COMPONENTS

Osteoblasts are bone-forming cells, differentiated from mesenchymal stem cells, that migrate to the local region, secreting proteins in the non-mineralized matrix, called osteoid, that later become mineralized from the accumulation of calcium phosphate as hydroxyapatite. After this process, osteoblasts have different fates: they may become trapped in the bone, remain as cell lining the bone surface, or suffer apoptosis (Figure 1.1.1).

Osteoblasts are responsible for the production of important cytokines including osteocalcin, osteopontin, osteonectin, bone sialoprotein, alkaline phosphatase, and a large amount of type I collagen, insulin-like growth factor I and II, transforming growth factor-beta (TGF-β), and bone morphogenetic proteins (BMPs) that are involved in bone matrix deposition and organization as well as its mineralization. For this reason, some of these proteins, in combination with bone substitute materials [1], cells, or scaffolds, have been proposed with encouraging results to promote bone regeneration when the volume of alveolar crest is insufficient for the correct three-dimensional (3D) implant placement.

Osteocytes are mature osteoblasts entrapped within the matrix; they represent most of the cell population in the bone. During their formation, the cellular body is reduced, organelles are lost, and dendrite-like processes form. These processes are localized in canals filled with fluid, are responsible for communication between other osteocytes, and are able to detect local changes in structure, stress, and microfractures as metabolic changes. These cells act as mechanosensors and release signaling molecules promoting anabolic/catabolic bone activities such as RANKL (receptor activator of nuclear factor kappa-B ligand), responsible for osteoclast recruitment [2, 3]. Furthermore, they assume a specific morphology and align following the collagen fiber orientation, which corresponds to the direction of the tensile strain through the bone tissue [4]. This 3D organization improves the mechanical properties of this tissue when it undergoes masticatory load.

Osteoclasts are multinuclear giant cells formed from the fusion of monocyte/macrophage precursor cells into the bone marrow area adjacent to the bone surface (Figures 1.1.2 and 1.1.3). The osteoclastogenesis is mainly dependent on RANKL, which binds its receptor (RANK) on the surface of the precursor cells, inducing their polarization into the clastic lineage [5].

In periodontal and peri-implant diseases, osteoclast differentiation and activity are stimulated by the presence of bacterial lipopolysaccharides. These molecules increase the secretion of RANKL and pro-inflammatory cytokines by resident cells and the expression of RANK on macrophages, which then upregulates bone resorption activity.

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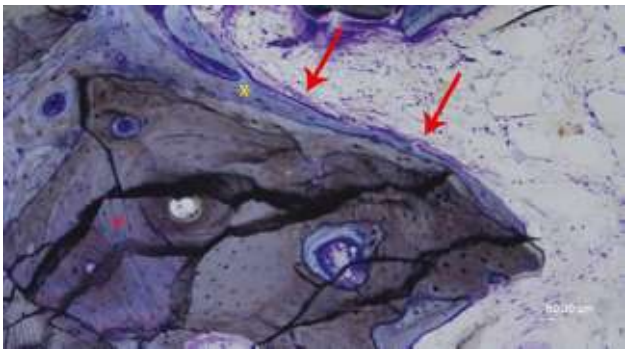

FIGURE 1.1.1 Biomateral particles (red asterisk) surrounded by woven bone (yellow asterisk) and bone lining cells (red arrow). Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 200×.

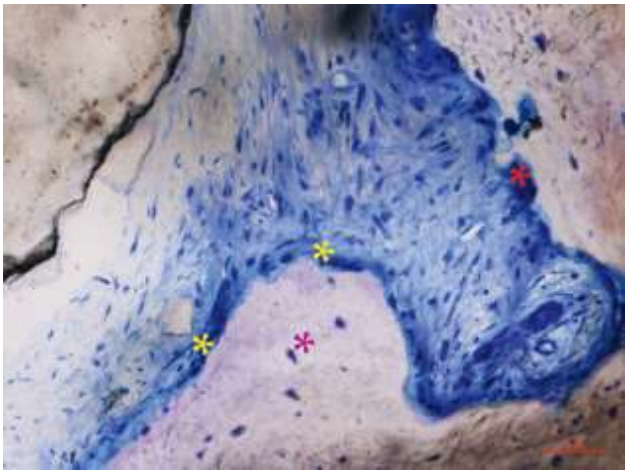

FIGURE 1.1.2 Osteoclast-like cells (red asterisk), osteoblast-like cells (yellow asterisk), and osteocite-like cells (purple asterisk). Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 400×.

COMPOSITION

The bone tissue is mainly composed of mineral, water, and organic matrix. The mineral part is composed of one analog to hydroxyapatite with a calcium deficiency, with a basic formulation of $Ca_{10}(PO_4)_6OH_2$. Other ions are also found associated with the bone hydroxyapatite, such as phosphorus, potassium, sodium, magnesium, carbonate, chloride, and fluorine. Mineralization begins with the deposit of mineral crystals in the vesicles of the organic matrix, which progressively increases with the maturation of the tissue (Figure 1.1.4).

The organic part is composed of 90% collagen type 1, with mechanical and structural functions being responsible for the elastic stiffness and bending strength. The remaining part is composed of non-collagenous proteins, with structural signaling and mechanical roles.

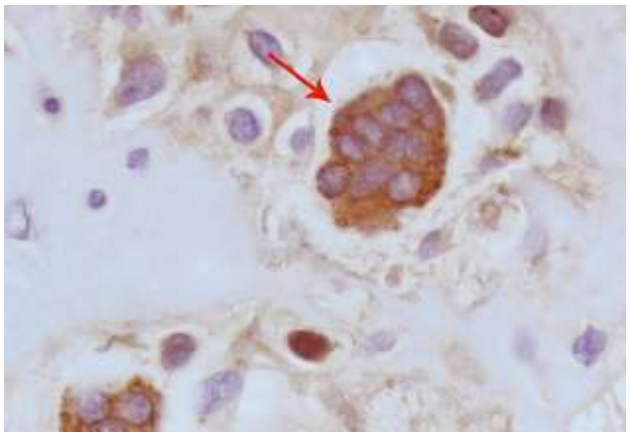

FIGURE 1.1.3 Immunohistological staining for tartrate-resistant acid phosphatase (TRAP) and multinuclear TRAP-positive cells (red arrow). Osteoclasts (red arrow) are responsible for the bone resorption by secreting hydrogen ions and lytic enzymes (including TRAP) that dissolve the mineral content and then degrade the remaining organic structure (red arrow). Light micrography, decalcified sample, Harris hematoxylin counterstaining, total magnification 1000×.

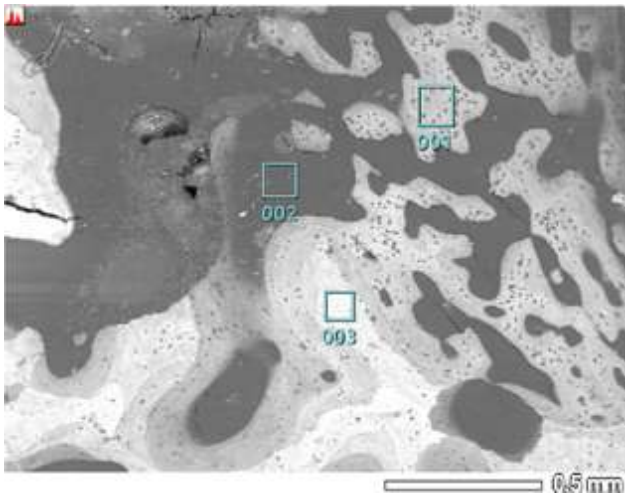

FIGURE 1.1.4 Alveolar bone in post-extraction socket at 4 months of healing: interface between pristine bone characterized by high mineral content (003) and hypomineralized woven bone (001). Dark areas (002) represent the medullary spaces. Sections were observed using a BSE-SEM system without additional fixation and previous coating of carbon film to assess level of mineralization of regenerated tissue.

The intense and constant anabolic and catabolic activity of bone may be altered by age, and by primary or secondary conditions that induce changes to the microstructure, mineralization, and cellular compound. Hypermineralization, changes in composition of the collagen matrix, and accumulation of micro damages affecting the resistance to

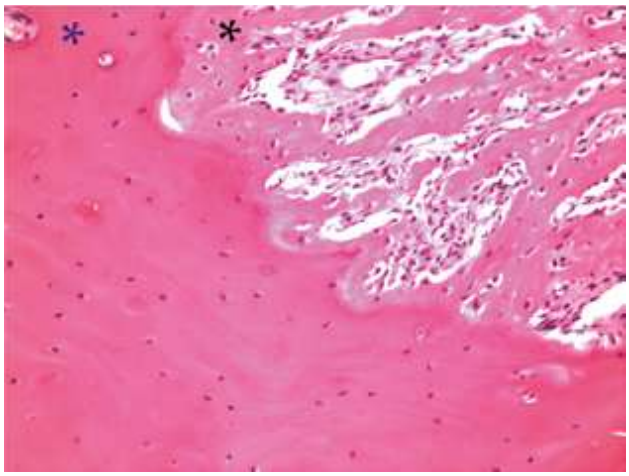

FIGURE 1.1.5 Cancellous (black asterisk) and cortical bone (blue asterisk) structure in rat. Light micrography, decalcified sample, hematoxylin and eosin staining, total magnification 100×.

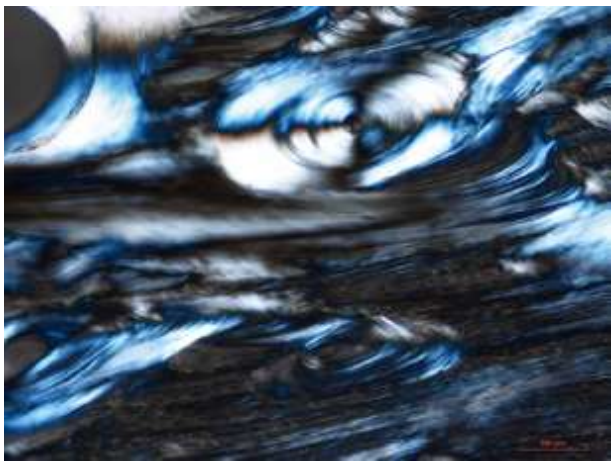

FIGURE 1.1.6 Osteons of the compact bone. Photomicrograph at polarized light, total magnification 200×.

tensile and compressive strain, as well as the fracture toughness, have been observed in aged cortical bone. Even systemic diseases, including diabetes, osteoporosis, and hematological diseases, and drugs such as anticoagulants, antacids, bisphosphonates, and corticosteroids, profoundly affect bone metabolism and structure [6], although the association between osteoporosis and jawbones is still not well understood [7].

STRUCTURE

Bone has a cancellous or cortical macrostructure; the microstructure includes the Haversian systems, osteons, and trabeculae; the sub-microstructure is the single lamella.

Anatomically, cortical bone is essentially compact and suffers less remodeling than cancellous bone. The latter is formed by trabeculae that define bone marrow spaces: a mesh of rods and plates 3D oriented to sustain stress and mechanical forces [8–10].

Both compact and cancellous bone are constituted by lamellae (Figure 1.1.5); in the compact bone these are organized in concentric rings around the central Haversian canal forming osteons (the structural compound of the bone; Figure 1.1.6). These systems communicate with each other via Volkmann canals.

BONE REMODELING AND HEALING

BONE REMODELING

Bone tissue physiologically undergoes a continuous remodeling process where two opposite activities coexist: tissue degradation and reabsorption by the osteoclasts and deposition of new bone by the osteoblasts (Figures 1.1.7–1.1.9). In this process, the osteoclasts form lacunae, called Howship's lacunae

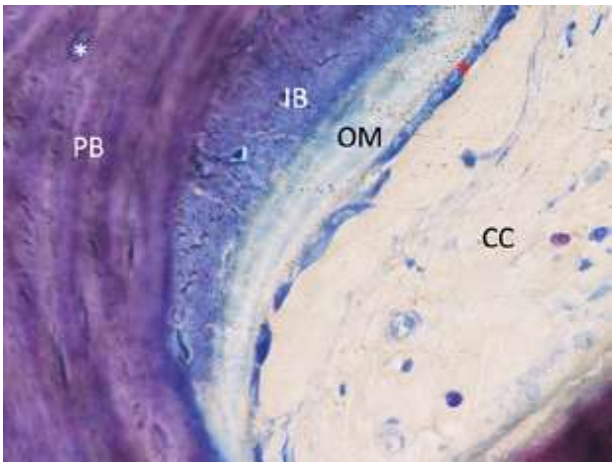

FIGURE 1.1.7 Particular of a cutting bone. White asterisk: osteocyte; red asterisk: osteoblast lining the matrix. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 600×. CC, cutting cone; IB, immature bone; OM, osteoid matrix; PB, primitive/pristine bone.

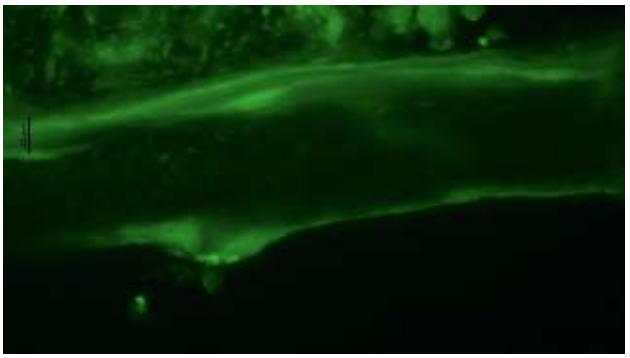

FIGURE 1.1.8 Bone apposition rate detected by tetracycline double labeling technique for the measurement of bone growth *in vivo* assessed at fluorescence microscopy, total magnification 400×.

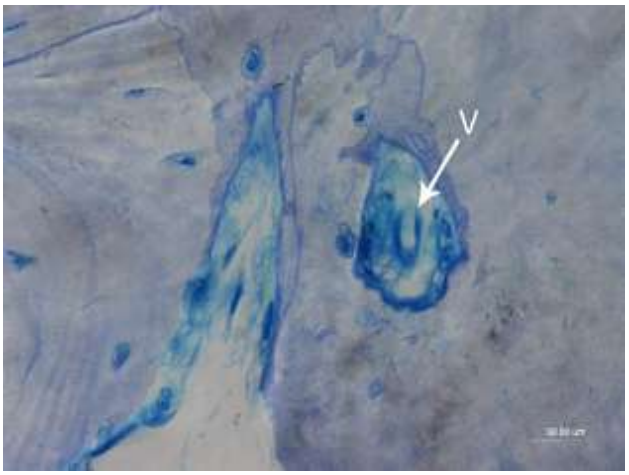

FIGURE 1.1.9 Cutting cones in longitudinal and transversal sections. During the remodeling activity, osteoclasts at the top of the cutting cone are followed by osteoblasts, which depone the osteoid matrix on the walls of the cone, and by newly formed blood vessels in the center of the resorption area. All these components constitute bone multicellular units (BMU). Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 600×. V, vessel in the center of the cutting cone and surrounded by the resorption cavity.

(Figure 1.1.10), where they pour out the acid ions, and by lowering of the pH the mineral matrix is decalcified, exposing the organic matrix [11]. Then the osteoclasts secrete the collagenases responsible for the degradation of the organic matrix. After completion of the reabsorption, the osteoclast undergoes apoptosis.

But how does the organism know where and when to start the remodeling process? Studies indicate that the osteocyte has a vital function in signaling the start of bone remodeling. Through sensing changes in tissue integrity, associated with microdamage and fatigue load, the cell enter apoptosis. The death of the cells itself does not produce the cytokines responsible for recruiting the osteoclasts, but the neighbor osteocytes that remain viable secrete cytokines as RANKL, directly enhance osteoclast formation, and start the remodeling [12–14]. Not only does the organism have a targeted remodeling process, but the osteocytes are responsible for metabolic signaling. Bone remodeling seems to be triggered also by the presence of metal particles/ions that derive from the degradation of dental implants [15]. This debris may result from corrosion of the implant surface when it is exposed to bacteria from the oral cavity, saliva, and chemical agents from food, mouthwash, and toothpaste, or even when implantoplasty is performed to reduce the surface roughness.

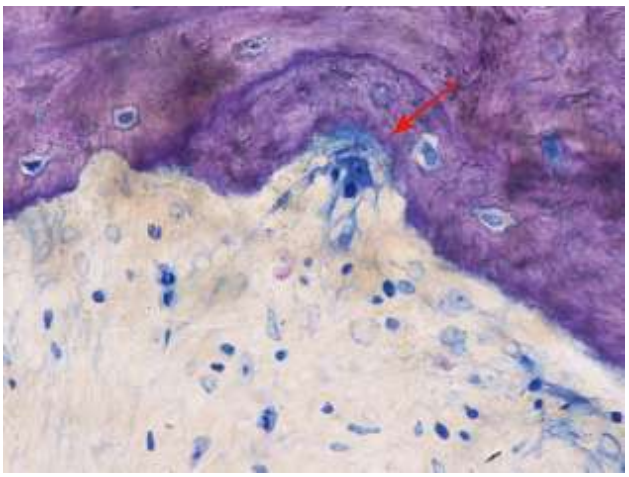

FIGURE 1.1.10 Howship's lacunae (red arrow). An osteoclast-like cell secretes the collagenases responsible for the degradation of the organic matrix. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 600×.

BONE HEALING

Bone formation can occur in two ways: via intramembranous ossification, where the bone is formed directly from the connective tissue, and via endochondral ossification, where a skeleton of hyaline cartilage shapes the bone formation. Maxillary and mandibular bone formation and healing proceed via intramembranous osteogenesis, through the deposition of immature woven bone that is remodeled in parallel-fibered and lamellar mature bone [16]. The process of alveolar bone formation that spontaneously occurs within the socket after tooth extraction has been well studied in humans and can be reported as a representative model.

Even though most volume changes occur within a year following the extraction [17], most of the structural changes happen within 180 days. During the first weeks of healing, the blood clot is infiltrated by inflammatory cells and vessels migrating from the walls of the pristine bone and is progressively substituted by a provisional matrix (2–4 weeks; Figure 1.1.11). In the subsequent weeks, cancellous bone with centripetal growth occupies most of the alveolus (6–8 weeks; Figure 1.1.12). Maturation of this bone intensifies in the following days and occurs from the periphery to the center. At 12–24 weeks lamellar bone and medullary spaces can be observed and a bone bridge closes the alveolus coronally (Figure 1.1.13) [18].

In clinical practice, an alveolar socket preservation procedure can be performed to preserve the alveolar bone volume for subsequent implant placement. The effect of the grafted biomaterial on socket healing will be discussed in the section on biomaterials.

WHAT CAN COMPROMISE BONE HEALING?

Several factors can influence bone healing; systemic conditions and drugs are common factors that change the physiology of bone healing, alongside aging. In the elderly, with the

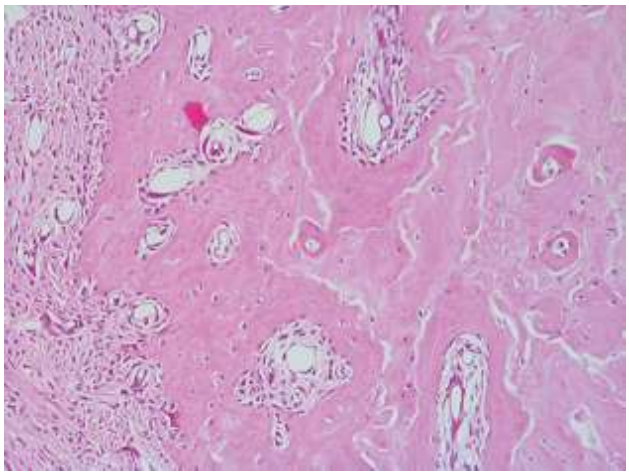

FIGURE 1.1.11 Healing socket after 10 days of extraction (rat). Immature woven bone with newly formed osteons can be observed. Light micrography, decalcified sample, hematoxylin and eosin staining, total magnification 100×. *Source*: Courtesy of Professor Edilson Ervolino, Department of Basic Sciences, School of Dentistry, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil.

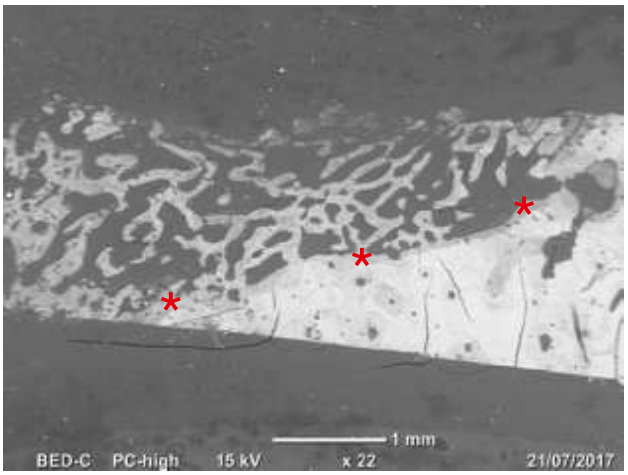

FIGURE 1.1.13 Alveolar socket healing at 4 months after extraction. The highly mineralized pristine bone is lined by newly formed tissue with lower mineral content (red asterisks). Trabecular bone is still visible within the area of the extraction. Photomicrograph at BSE-SEM system without additional fixation and previous coating of carbon film.

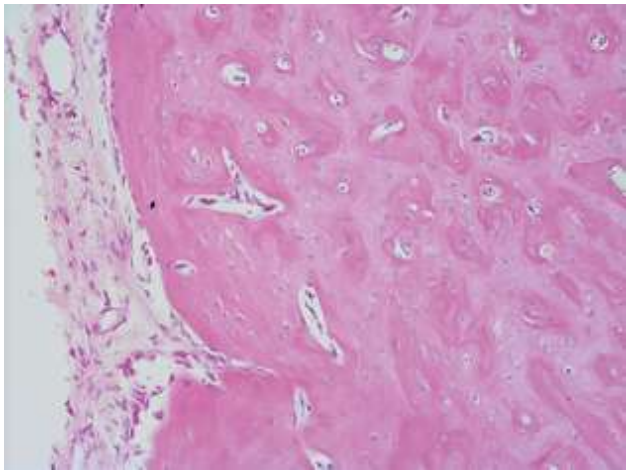

FIGURE 1.1.12 Healing socket after 20 days of extraction (rat). The structure of bone tissue appears more mature as a consequence of the remodeling activity. Light micrography, decalcified sample, hematoxylin and eosin staining, total magnification 100×. *Source*: Courtesy of Professor Edilson Ervolino, Department of Basic Sciences, School of Dentistry, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil.

decrease of the sex hormones, reabsorption surpasses the formation of new bone, lowering the bone mass and bone strength [19].

Alcohol and smoking habits also impair healing. Alcohol consumption decreases bone remodeling, causing osteopenia by suppression of bone marrow cell differentiation [20]. Smoking leads to lower vascularization, impairing the blood flow essential to bone healing, and also causes damage to the DNA of the cell, leading to somatic changes and increasing the possibility of cancer. Antineoplastic agents are known to exacerbate the inflammation, reducing the expression of osteoblastic lineage cells while increasing osteoclastic expression markers [21, 22].

Diabetes also causes changes in bone strength, leading to deterioration of the organic matrix and accumulation of microdamage, lowering the tensile resistance and elevating its fragility [23].

BONE ANGIOGENESIS

As for all tissues, bone needs a blood supply for its homeostasis and healing activity. The terms angiogenesis and vasculogenesis both refer to the formation of a vascular network, but with a different mechanism. Vasculogenesis is *in situ* differentiation and growth derived from multipotent precursor cells, while angiogenesis is the formation of a vascular network derived from pre-existing endothelial cells. In bone tissue, angiogenesis and osteogenesis are closely related and angiogenesis–osteogenesis coupling activity is referred to. After tooth extraction, bone regenerative procedures, or implant placement, the deposition of immature matrix and its mineralization occur along the newly formed blood vessels that branch out into the granulation tissue [24]. In this chapter, we will focus on factors that influence angiogenesis, stimulating natural bone healing, and that have been most often researched and described.

Compact vs. Cancellous Bone

The blood supply occurs differently in compact/lamellar and cancellous (medullary) bone. In the former, the blood vessels are located in the Haversian and Volkmann canals; in the

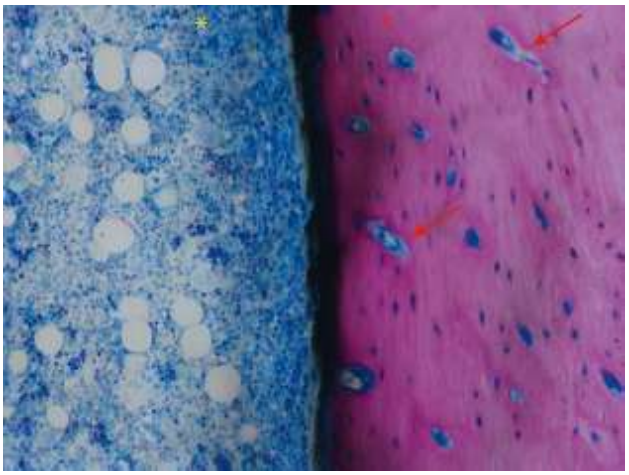

FIGURE 1.1.14 Havers and Volkmann canals (red arrow) in compact/lamellar bone (red asterisk), where the blood vessels are in intimate contact with its walls. Their caliber is limited by the size of the canal. Bone medulla (yellow asterisk) composed mainly of stem cells and adipocytes in rat. Light micrography, calcified sample, Toluidine blue and eosin staining, total magnification 200×. *Source*: Courtesy of Professor Juliano Milanezi de Almeida, Department of Diagnosis and Surgery-Periodontics Division, São Paulo State University (UNESP), Brazil.

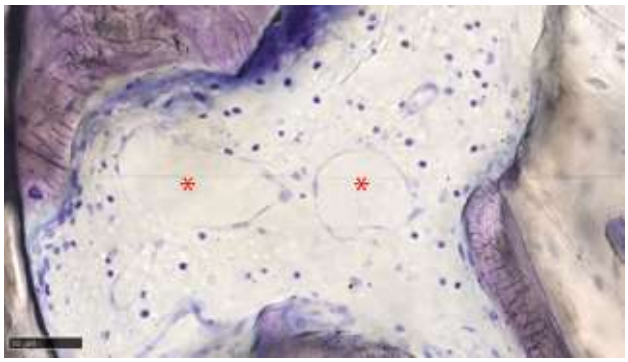

FIGURE 1.1.15 Vessels (red asterisks) running in the center of the medullary area. Compared to the compact bone, the cancellous bone is vascularized by a higher number of vessels with larger caliber located in the bone marrow. Due to these anatomical differences, the angiogenesis that begins in response to a trauma (as implant placement) or to an inflammatory stimulus occurs differently in compact and cancellous bone. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 400×.

latter, vessels run within the medullary area surrounded by connective tissue (Figures 1.1.14 and 1.1.15).

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) has been extensively studied and is described as affecting endothelial cell proliferation during both vasculogenesis and angiogenesis. It is responsible for coupling angiogenesis with osteogenesis, and also for controlling the differentiation of osteoblasts and osteoclasts. Its isoform VEGF-A, produced by osteoblasts, regulates bone defect regeneration and stimulates the resorptive activity of osteoclasts; the administration of drugs that possess anti-VEGF effects can impair osseointegration and the remodeling of bone [25–27]. In a preclinical model, this protein has been associated with bone morphogenetic protein-2 (BMP-2) to increase the vertical regeneration of alveolar bone around implants, with encouraging results [28].

Hypoxia as an Angiogenetic Determinant

After bone damage or fracture occurs and the blood vessels rupture, blood flow decreases, thus reducing tissue oxygenation. As a consequence, hypoxia induced factor (HIF) is expressed in areas with low oxygen and is responsible for increasing VEGF gene expression.

Type H Vessels

These types of vessels are located along the periosteum and endosteum of the cortical and trabecular bone adjacent to the growth plate. They are highly specialized, since they guide osteogenic activity by expressing proteins that promote the proliferation and differentiation of osteoprogenitors in the tissue such as VEGF and HIF. Osteoprogenitor cells and osteoblasts were found arranged around type H endothelial cells, in which they have high oxygen availability. Furthermore, when hypoxia signaling is activated, these endothelial cells respond, increasing their number [29, 30]. These vessels were also found to promote osteogenesis during alveolar bone remodeling in the healing of post-extraction sockets [31].

BIOMATERIALS FOR BONE REGENERATION

Bone defects may result from tooth extraction, trauma, infection, or genetic problems. Procedures for the replacement of pre-existing or missing bone come from the necessity to remake contour, mainly for implant placement. For this purpose, several biomaterials have been proposed in clinical practice.

Biomaterials are organic or inorganic substances for biomedical use, including in regenerative medicine. They can be classified by their interaction with the surrounding tissues after grafting or by their chemical structure/composition. When taking into consideration the interaction with the surrounding tissues, the biomaterials can be:

- *Biocompatible*: after grafting they do not elicit an adverse immune reaction and are safe for the patient.
- *Bioinert*: when placed in the human body they have little to no interaction with the surrounding tissue.
- *Bioresorbable*: upon placement within the human body they dissolve and are slowly replaced by another tissue.

Bone grafts and bone substitutes may also be osteoinductive, osteoconductive, and osteogenic. Osteoinductive material induces the recruitment, proliferation, and differentiation of undifferentiated stem cells in the grafted area. Osteoconduction is a process in which the material acts as a scaffold and enhances bone formation. The bone graft is osteogenic when it contains vital osteoblasts that induce bone formation.

Regarding their composition, materials can be divided into bone grafts, synthetic hydroxyapatite-based biomaterials, polymers, ceramics, metals, and composites.

BONE GRAFTS

Bone grafts are largely used in clinical practice and can be classified according to their origin. Bone harvested from the patient, often from the iliac crest or the mandibular ramus, is termed autologous bone. Although this is considered the gold standard in bone grafting due to its osteoinductive, osteoconductive, and osteogenic properties and its biocompatibility and biological safety, autologous bone needs a second surgical area for harvesting and presents a high resorption rate. For this reason, bone grafts harvested from the human cadaver (allogeneic) and from animal sources (xenogeneic) have been proposed. Allogenic bone contains bioactive proteins and presents a 3D structure similar to that of the patient's bone. However, potential immune reaction and disease transmission limit its use in clinical practice. Xenogenic bone grafts are deproteinized, and thus are safer than allografts even if they are less bioactive. These materials are largely used for bone regeneration due to their practicality and manageability. Furthermore, their low resorption rate offers dimensional stability to the regenerated area (Figure 1.1.16) [32].

SYNTHETIC HYDROXYAPATITE-BASED BIOMATERIALS

The need for biomaterials that are even more safe, resorbable, and osteoconductive encouraged research into synthetic substances. Hydroxyapatite (HA) is biocompatible and osteoconductive. For this reason, several HA-based synthetic materials, functionalized and loaded with bioactive molecules and therapeutic agents, have been proposed in the orthopedic and dental fields, such as a nanostructured magnesium (Mg)-enriched HA and a vegetable-originated HA [33, 34], with encouraging data (Figures 1.1.17 and 1.1.18).

POLYMERS

Polymers are materials made by long and repeating chains of natural or synthetic molecules [35]. Natural polymers, such as collagen, chitosan, and hyaluronic acid, are bioactive, biodegradable, and present a biomimetic surface. However, due to their physical and mechanical properties, natural polymers have limited application in bone regeneration.

Synthetic polymers, such as polycaprolactone (PCL), polylactide (PDLA, PLLA), and polylactide-co-glycolide (PLGA), are produced by a range of techniques that determine their 3D spatial and molecular arrangement. These characteristics are useful for the design of tailored scaffold for tissue engineering. Otherwise, synthetic polymers have shown reduced bioactivity, low osteoconductivity, and cellular

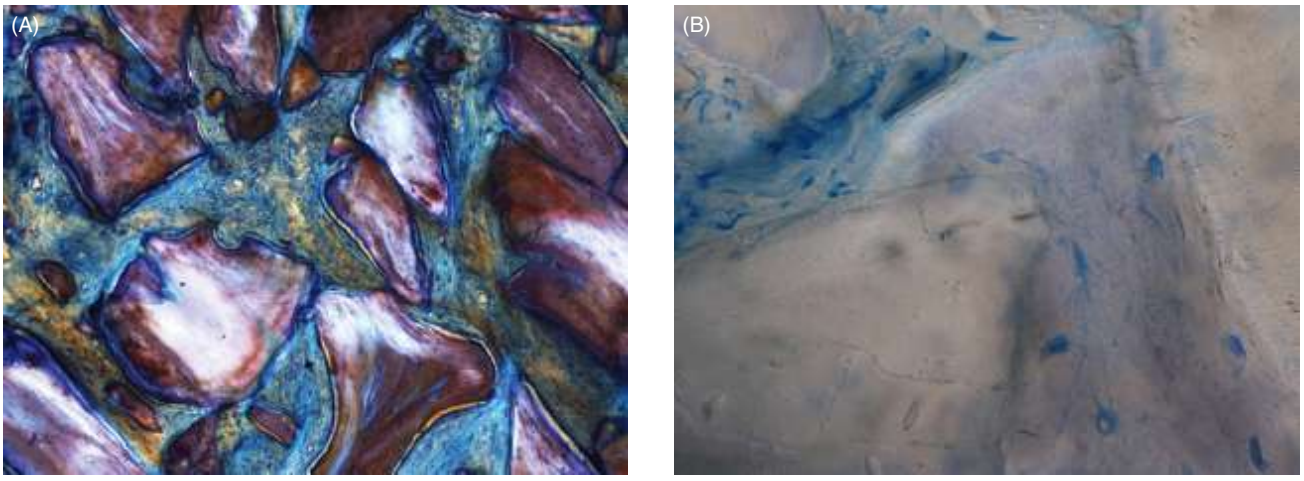

FIGURE 1.1.16 Xenogenic particles at 3 (A) and 9 (B) months of healing in a human extraction socket. The host response after grafting involves the enrollment of inflammatory cells as well as of the overall bone multicellular unit. Despite this activity, the biomaterial remains even after several months of healing. At 3 months, particles appear partially resorbed and embedded in osteoid matrix. At 9 months, remnants of biomaterial are still visible osteointegrated in the newly formed mature bone tissue. Toluidine blue and pyronin yellow staining, photos from an optical microscope (B) equipped with polarized light (A). Total magnification 100× (A), 600× (B).

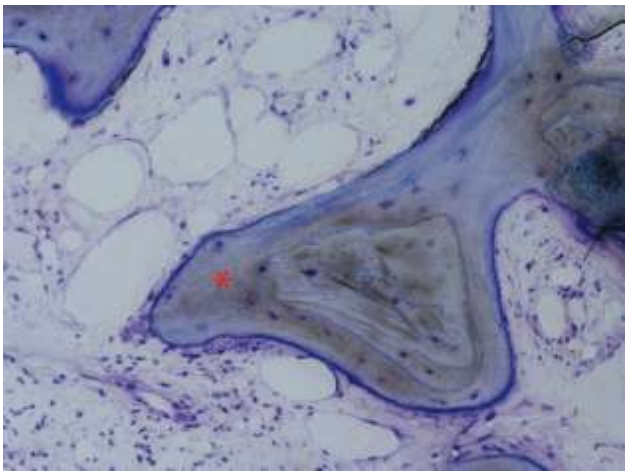

FIGURE 1.1.17 A histological study conducted on humans observed the biocompatibility, osteoconductivity, and resorbable capability of hyaluronic acid (HA)-based biomaterials. In this figure, newly formed bone surrounding the particle of nanostructured magnesium (Mg)-enriched HA (red asterisk) can be observed. This biomaterial presents the partial substitution of HA with Mg for increased solubility. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 200×.

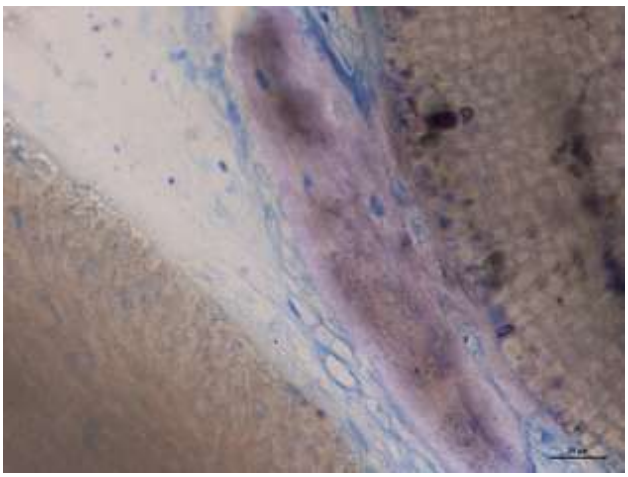

FIGURE 1.1.18 Newly formed bone in tight contact with a particle of vegetable-originated hyaluronic acid. Light microscope, toluidine blue and pyronin yellow staining, total magnification 400×.

adhesion. To overcome these limits, they have been associated with bioceramic particles, forming composite materials with improved biological properties.

BONE MORPHOGENETIC PROTEIN-2

BMPs are growth factors with multiple functions that belong to TGF-β family. Up to 20 different kinds of BMPs have been

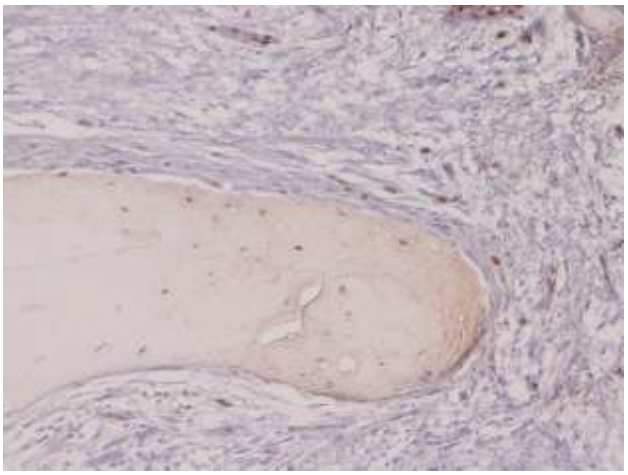

FIGURE 1.1.19 Bone morphogenetic protein (BMP) promotes osteogenesis by inducing the differentiation of mesenchymal cells in osteoblasts, as well as the proliferation of bone mesenchymal stem cells. Immunohistochemical staining with Ab anti-BMP2. Total magnification 200×.

identified and BMP-2 seems to be one of the most important factors in post-natal ossification [36, 37] by inducing the differentiation of mesenchymal cells in osteoblasts, as well as the proliferation of bone mesenchymal stem cells (Figure 1.1.19) [38, 39]. BMP-2 is available for clinical practice through recombinant gene technology [40, 41]. Clinical trials evaluated the association of this protein with metallic and ceramic scaffolds or biomaterials such as absorbable collagen sponges or hydrogels acting as a drug-delivery system, for the treatment of fractures and bone defects, with encouraging results [42]. In dental practice, absorbable collagen sponges embedded with BMP-2 solution have been approved only for sinus augmentation and alveolar ridge preservation by the US Food and Drug Administration, but not by the European Medicines Agency [43, 44].

PLATELET CONCENTRATES

Platelet concentrates consist of autologous blood concentrates containing active molecules, mainly derived from platelets and circulating cells. They include platelet-rich plasma and platelet-rich fibrin and have been introduced in oral surgery because of their potential to stimulate the regeneration of bone tissue as well as oral mucosa. Due to the lack of mechanical properties and space-maintaining activity, these compounds are usually associated with a mixture of particulate autogenous and xenogenous graft when used for vertical and horizontal bone augmentation procedures. However, based on data from clinical and histological studies, the efficacy of these compounds to improve bone regeneration as well as the vitality of the newly formed tissue is still controversial [45–47].

OSSEOINTEGRATION

Osseointegration is the direct structural and functional connection between live bone tissue and implant, and is considered the primary outcome to determine the success of implants. Molecular and cellular events that occur after the preparation and placement of the implant lead to intimate contact between the newly formed bone and the implant surface. Apposition of the newly formed bone into the implant mimics the pattern of the bone deposited in the healing of bone fractures.

Patient systemic disease or conditions and implant features, including the micro and macro topography of the surface, may affect osseointegration (for factors related to the implant surface, see Table 1.1.1). Conditions such as smoking, non-controlled diabetes, and chemotherapy may impair bone and soft tissue healing or cause loss of peri-implant bone, leading to implant failure [48, 49]. Likewise, periodontally compromised patients are prone to a higher risk of complications and implant loss than periodontally healthy patients [50]. The development of surface treatments aiming at reliable osseointegration and faster healing is a recurrent topic in biomedical research.

PHASES OF OSSEOINTEGRATION

The events of osseointegration are reviewed below as described in animal models [51–53]. The osseointegration phase has a similar pattern in different animal species; however, the healing time changes drastically when considering humans and other animals.

Table 1.1.1 Factors related to the implant surface that can affect osseointegration during implant osseointegration stages.

Healing phases	Implant surface influence on osseointegration
Immediate post-operatory healing and early healing phase	The formation of the titanium oxide, adsorption, adhesion of plasma proteins, and the coagulum suffers the influence of the microtomography and physical-chemical properties of the implant surface. Implant treatment may improve the affinity with the clot, leading to a higher and faster deposition of osteoid matrix.
3–4 days after surgery	Higher number of fibroblast-like cells and an improved tissue metabolism on rough implant surface compared to the machined one. Higher expression of bone-inducing formation and resorption genes was also found when comparing active surfaces with machined implants, while machined implants presented higher expression of pro-inflammatory markers.
1 week after surgery	Fibroblast-like cells observed on machined implants. On active surfaces, cuboid osteoblastic-like cells were observed producing osteoid matrix.
2–4 weeks after surgery	Higher amount of newly formed bone on active titanium surfaces than the machined one.

Immediately after implant installation (0–4 hours) the blood clot forms. Ions (i.e. calcium, Ca) and plasma proteins, like albumin and fibrin, adhere to the implant surface. This protein coat is fundamental to the attachment of inflammatory and tissue-forming cells. After exposure to oxygen in the atmosphere, titanium (Ti) is coated by an oxide layer that plays an important role in biocompatibility and osseointegration [54], since Ca and phosphate (P) ions are gradually included in the Ti oxide layer, forming a 3D structure composed of Ca, P, and oxygen (O) ions, in which the concentration decreases as the Ti ions increase, thus supporting the theory of ingrowth of bone into implant surface nanostructures [55].

During the first days after surgery, the blood clot is progressively organized in granulation tissue through the angiogenesis that comes from the pristine bone. A layer of fibroblast-like cells encircles the implant and elongates through its surface [51–53].

One week after surgery, a thin layer of osteoid matrix begins to be deposited on the implant surface and in the perivascular area (angiogenesis–osteogenesis coupling; Figures 1.1.20 and 1.1.21). Genes related to extracellular matrix formation and organization of collagen fibrils are found to be upregulated in cells in the implant surface [56].

Two weeks after surgery, immature bone is found along the implant surface advancing from the pristine bone. Progressively intramembranous ossification occurs and the provisional matrix with lining osteoblasts is remodeled into lamellar bone. Early signs of bone remodeling appear in the osteons, as a result of osteoclast activity (Figures 1.1.22–1.1.25).

To summarize:

- The formation of bone in the implant surface is a consequence of contact with the old pristine bone, yet the deposition of new bone results from the differentiation of mesenchymal cells into osteoblastic lineage cells.

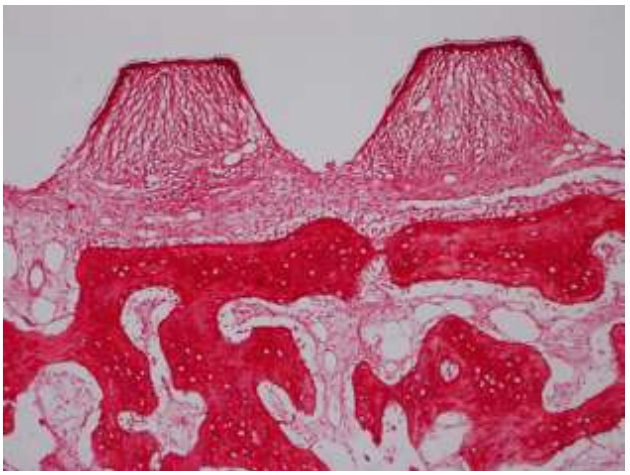

FIGURE 1.1.20 Bone at the implant interface 10 days after surgery (in a dog model). Sample obtained with fracture technique, sirius red staining for collagen fibers, total magnification 100×.

FIGURE 1.1.21 Bone around an implant thread 7 days after installation (rat). Light micrography, decalcified sample, hematoxylin and eosin staining, total magnification 100×.

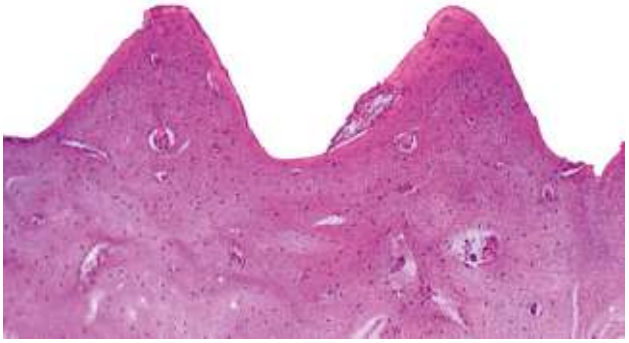

FIGURE 1.1.22 Bone around titanium implant threads 90 days after installation (rat). Light micrography, hematoxylin and eosin staining, total magnification 100×.

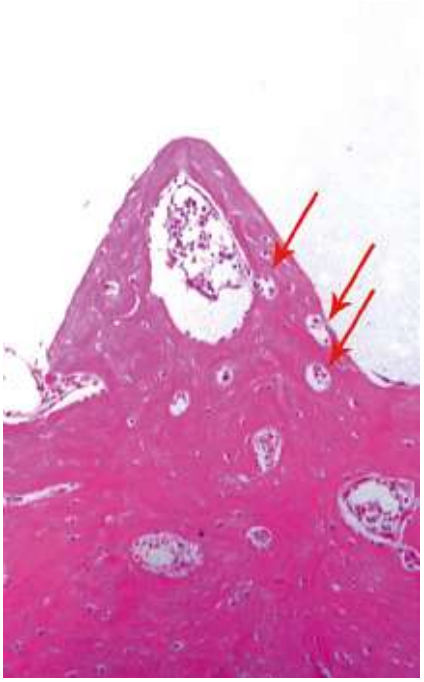

FIGURE 1.1.23 Single implant thread. The arrows indicate blood vessels inside the thread. Light micrography, hematoxylin and eosin staining, total magnification 200×.

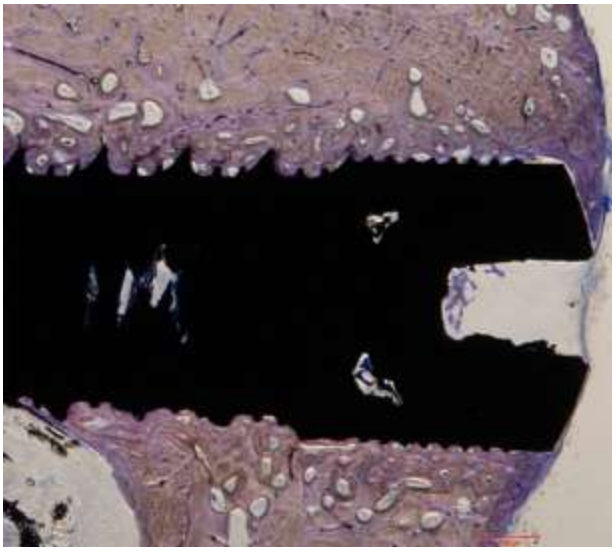

FIGURE 1.1.24 Two weeks after surgery (dog model). Immature bone along the implant surface advancing from the pristine bone. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 40×.

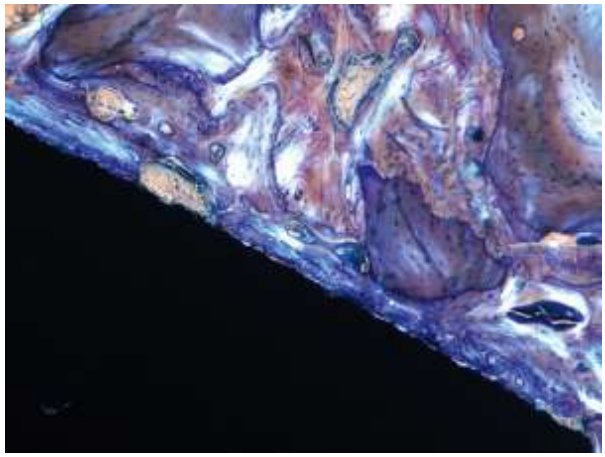

FIGURE 1.1.25 Intimate and functional contact established by the newly formed bone with the implant surface. Toluidine blue and pyronin yellow staining, acquisition by scanner at high resolution, total magnification 100×.

- Properties of the implant surface influence the cellular behavior.
- Treated and rough surfaces appear to osseointegrate faster and at a higher rate by the increased contact with osteogenic cells.
- Ti ions are released from the implant and are incorporated by the bone.

Table 1.1.1 reviews the main factors related to the implant surface that could affect osseointegration during different healing phases.

FIGURE 1.2.1 Primary intention healing wounds on papilla and attached gingiva.

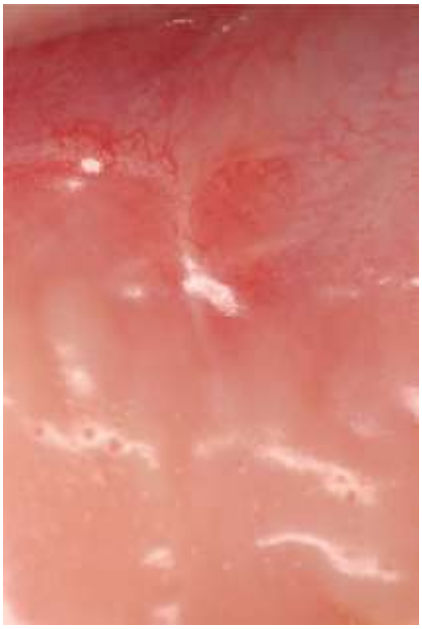

FIGURE 1.2.2 Same area of vertical incision as in Figure 1.2.1 at 20 days.

It is typically seen in the case of donor sites, such as the palate, when tissue grafts are harvested for mucogingival procedures (Figure 1.2.3).

ORAL SOFT TISSUE WOUND HEALING

Most researchers commonly considered that oral wounds can heal better and faster than other wounds such as dermal ones (Figures 1.2.1–1.2.4). When compared to cutaneous healing, mucosal wounds seem to proceed through the same stages as mentioned above [1], although an accelerated healing pattern of mucosal wounds is clearly demonstrated [9, 10]. Moreover, mucosal wounds also generally heal with minimal scar formation and hypertrophic scars are rarely seen in the oral cavity [11]. Until recently, it was reasonable to assume that the basic cell biological and molecular biological events of wound

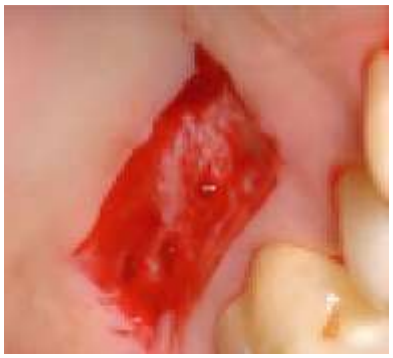

FIGURE 1.2.3 Palate heals by secondary intention after harvesting of a free gingival graft.

healing would follow the same principles as at the tooth–gingiva interphase, at least at the supracrestal locations [12].

ORAL SOFT TISSUE SPECIAL FEATURES: INTRINSIC DIFFERENCES BETWEEN ORAL MUCOSA AND SKIN

Healthy skin and oral mucosa share many features, but also present several intrinsic histological differences:

- The epidermis is entirely keratinized. Within the oral cavity, there is a difference between the keratinized epithelium of the hard palate and of gingiva (with a major function in resisting mechanical forces during mastication) versus the non-keratinized epithelium of the buccal mucosa that has the flexibility to stretch and withstand compression [13].
- The oral epithelium is generally thicker compared to skin. In particular, palatal and buccal gingiva presents a higher proliferation rate in the basal lamina and more cell layers compared to skin [14].
- More blood vessels are present in the oral mucosa compared to skin [10, 15].
- ECM in oral mucosa contains more elastin compared to skin, hard palate, or gingiva [16, 17].

From a biomolecular standpoint, several studies have clearly demonstrated that the differences between skin and oral mucosa can clearly be seen at only 24 h after wounding and explain the intrinsic and peculiar potential of oral wounds to heal without the necessary tools for the inflammatory response typical in skin wounds (Table 1.2.1) [10, 18].

SCAR AND SCARLESS WOUND HEALING IN ORAL SOFT TISSUES

The oral cavity is lined with what is generally called the "oral mucosa," which, in turn, is made up of the following three types or zones:

Table 1.2.1 Differences between oral mucosa and skin wound repair.

Re-epithelialization at 24 h	40%	100%
Inflammatory infiltrates		
MPO (U/mg protein) at 24 h	2.82 ± 0.2	1.26 ± 0.2
Mφ (HPF) at 72h	10.8 ± 0.3	6.7 ± 0.4
T cells (HPF) at day 7	15.1 ± 1.5	5.6 ± 0.3
Cytokine/chemokine		
IL-6	↑↑	↑
IL-a	↑↑	↑
IL-1β	↑↑	↑
TNF-α	↑↑	↑
TGF-β1	↑↑	↑
TGF-β3	↑	↑
KC	↑↑	↑
Angiogenesis		
Vessel density compared to normal tissue at day 5 (fold increase)	11.5	3.4
VEGF	↑↑	No marked change
Collagen fibril diameter in wound	Decreased	No marked change

Source: Chen et al. 2010 [19].

- The *masticatory mucosa*, comprising the covering of the hard palate and the gingiva, i.e. the part of the oral mucosa that covers the alveolar processes of both mandible and maxilla and surrounds the cervical part of the teeth.
- The *specialized mucosa*, lining the dorsum of the tongue.
- The *oral mucous membrane*, lining the rest of the oral cavity.

Differential outcomes in terms of scarring between oral mucosa and attached gingiva are a common finding in clinical practice, with gingival repair resulting in a clinically scarless repair (Figure 1.2.4) [20–22]. Larjava et al. [22] reported that palatal wounds heal with minimal scars and rapid resolution of inflammation, showing the main changes in gene expression during the first day after wounding. In 2017, a human comparative study, analyzing biopsies obtained 24 h after injury, showed how, in oral mucosa, characterized by partially fibrotic outcome during repair, the activation of autophagy determined an increase in alpha-smooth muscle actin (αSMA) and collagen Ia1 production.

Conversely, wound healing did not stimulate autophagy in buccal-attached gingiva, and subsequently no increase in myofibroblast differentiation and collagen deposition could be seen, thus justifying the scarless outcome [22]. The same tendency was observed for palatal tissue, showing the faster clinical wound healing response [23].

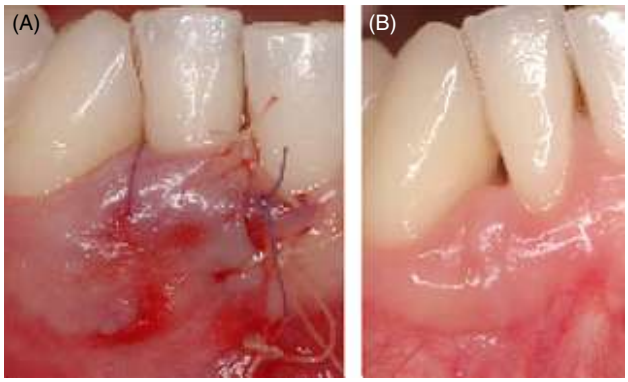

FIGURE 1.2.4 (A) Periodontal flap with vertical releasing incision sutured in place. (B) Scarless healing of the same area at 1 year post op.

EARLY WOUND HEALING SCORE: A METHOD TO ASSESS PRIMARY INTENTION WOUND HEALING

Several qualitative and semi-quantitative assessment methods have been proposed in the last 30 years to evaluate early wound healing of periodontal soft tissues. In 2018, Marini et al. [24] proposed the Early Healing Score (EHS), composed of three parameters: clinical signs of re-epithelialization (CSR), clinical signs of hemostasis (CSH), and clinical signs of inflammation (CSI). To evaluate CSR 0, 3, or 6 points were used, whereas 0, 1, or 2 points were used for both CSH and CSI (Table 1.2.2 and Figure 1.2.5).

Table 1.2.2 The Early Healing Score (EHS).

Parameter	Description	Points
CSR	Merged incision margins	6
	Incision margins in contact	3
	Visible distance between incision margins	0
CSH	Absence of fibrin on the incision margins	2
	Presence of fibrin on the incision margins	1
	Bleeding at the incision margins	0
CSI	Absence of redness along the incision length	2
	Redness involving <50% of the incision length	1
	Redness involving >50% of the incision length and/or pronounced swelling	0
Maximum total score: 10		

EHS: Early Wound Healing Score, CSR: clinical signs of re-epithelialization, CSH: clinical signs of haemostasis, CSI: clinical signs of inflammation.
Source: Marini et al. 2018 [24].

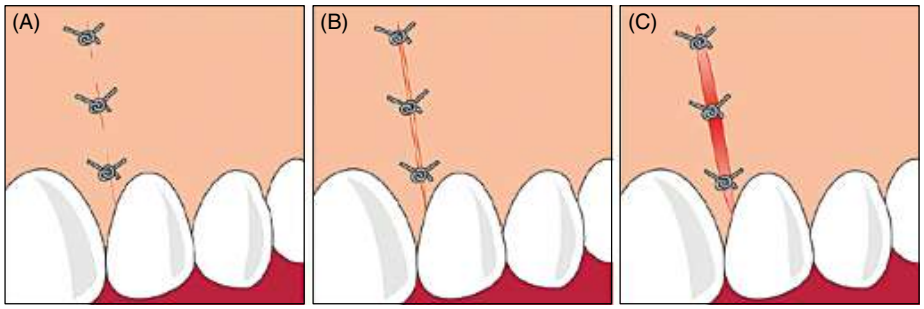

FIGURE 1.2.5 Graphic examples of clinical signs of re-epithelialization (CSR). (A) Incision margins considered "merged" (6 points for CSR); (B) margins in contact (3 points for CSR); (C) distance between incision margins considered "visible" (0 points for CSR). *Source*: Marini et al. 2018 [24].

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VIDEO 1.1 Introduction to Chapters 1.1 and 1.2 by the Authors Drs. Claudia Dellavia, Gaia Pellegrini, Luiz Guilherme Fiorin, and Andrea Pilloni.

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