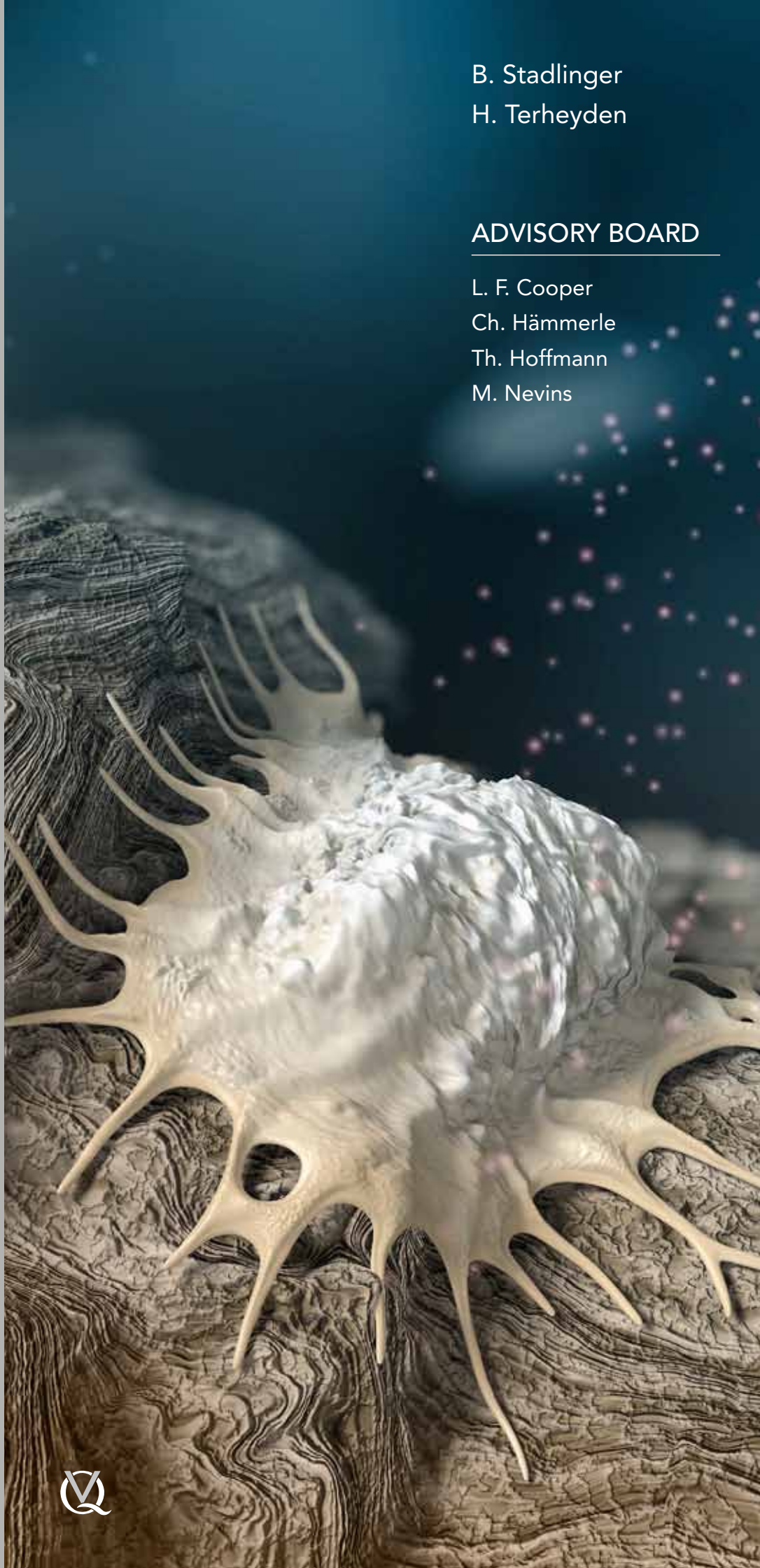
CELL-TO-CELL COMMUNICATION

OSSEOINTEGRATION



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PREFACE

A picture is worth a thousand words, they say. A video is worth much more, and is much more effective, than a thousand words.

The idea for the "Osseointegration" project came from Alexander Ammann of Quintessence Publishing ten years ago. At the time, much research was being conducted into bone morphogenetic proteins (BMP), and there was frequent mention of bone healing processes in the slide presentations of the time. But the conference slides lacked the third dimension – spatial depth – and especially the fourth dimension – time. The more complicated the content shown in terms of space and time, the faster and more effectively it can be transported by video instead of by a two-dimensional slide.

The project received a lift at the 60th anniversary congress of Quintessence in Berlin in January 2009, through a lecture on wound healing – "Cell-To-Cell Communication." With that, the key had been identified: communication was the name of the game. And biology was added to the didactic concept. Then, at the EAO Congress in October 2009, the time was ripe: a team of authors had been identified, with the undersigned and Christoph Hämmerle and Thomas Hoffmann as expert advisors.

The sequence of the various phases of wound healing in the bone is the topic of the present film. As in any good thriller, there are special characters: the cells. They communicate via messenger molecules, they call each other, they fulfill their roles, and then they disappear again. Through their efforts, order is gradually being restored after the initial trauma. What is more: the dental implant actively promotes the healing process and assumes a new function. – And this, in a nutshell, is the dramatic concept of the film.

It was fortunate that Quintessence Publishing was able to enlist the assistance of InterActive Systems, Berlin, a group of video animation artists with a biological background – Dr. Marco Reschke, Matthias Gauer and Thomas Kramer could be named here as representatives of the entire team. This group possesses the know-how and technical infrastructure required for virtual 3D animations. Meetings were held and a storyboard was written that covered every single second of every scene, and the spoken texts were added as well. The essence of a film lies in the art of omission, in the instrumentalization of empty space. It was important to limit the number of facts to the bare minimum necessary and to respect the appropriate size and scale, as it is not feasible in the world of 3D video to jump back and forth between the macroscopic, cellular, and molecular levels. In parallel with these efforts, the scientific authors contributed the results of their research, which were documented in a review article (in English). The group of authors included Dr. Susanne Bierbaum, biochemist at the Technische Universität Dresden, and Prof. Klaus Lang, University of Hong Kong, emeritus of the University of Bern.

With the resulting scientific film, *Cell-To-Cell Communication – Osseointegration*, our working group wanted to introduce innovative video animation technologies into dental education. Oral implantology plays an important role in graduate and postgraduate dental training at our universities. Our goal is none less than to instill in students and other viewers a fascination with science. This film is a first step in our efforts to create a series of 3D video training aids visualizing biological topics in dentistry with state-of-the-art tools and methods.

The scientific achievement of this film and its accompanying documentation is that it transfers the concept of the four didactic phases of wound healing – hemostasis, inflammation, proliferation, remodeling – from the soft tissue to the bone. We have learned a tremendous amount since osseointegration was discovered by P. I. Brånemark in the 1950s, and the most recent findings – especially at the protein level – were incorporated. The film is intended to stimulate interest in the complex biological processes that facilitate osseointegration, with special attention to the cell types and mediators (cytokines) implicated.

We, the authors, would like to extend our thanks to all those who participated in the production of this film, whether or not we were able to name them here. The English edition would not have been possible without the excellent support from Lyndon F. Cooper and Myron Nevins. Special thanks are due to our project manager, Alexander Ammann, whose outstanding coordination and motivation abilities made this project possible. Finally, we thank Quintessence Publishing and Astra Tech Dental for supporting this project and for contributing to the transfer of knowledge between the research, education, and clinical realms.

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OSSEOINTEGRATION – COMMUNICATION OF CELLS

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Abstract

The article provides the scientific documentation for the three-dimensional (3D) animated film, *Cell-to-Cell Communication – Osseointegration*. As depicted in the film, the article describes the molecular and cellular events during the healing of an osseous wound after placement of a dental implant with a special emphasis on the process of osseointegration. According to the concept of soft tissue healing, wound healing is divided into four phases: *(1)* hemostasis, *(2)* inflammation, *(3)* proliferation, and *(4)* remodeling. This article proposes to transfer the concept of the four phases of wound healing to a bone wound. Wound healing throughout these phases is the result of a coordinated action of different cell types that communicate with each other via interaction with regulatory molecules like cytokines, extracellular matrix proteins, small molecules, and hormone-like substances. A regulated sequence of cell types and regulatory molecules results in undisturbed healing. Disturbed healing is associated with a continuation of the early inflammatory phase and the development of a toxic wound environment. Understanding this cascade of molecular and cellular events may provide the clinician with new targets to improve therapy with dental implants.

Keywords

osseointegration, wound healing, osteoblast, osteoclastogenesis, growth factors, cytokines, chemokines, bone morphogenetic proteins, angiogenesis, extracellular matrix

Introduction

Wound healing, and in particular the healing of an osseous wound around a dental implant, is a coordinated and sequentially organized repair mechanism of the organism (Nguyen et al 2009). The main players in this process are cells. These cells communicate with each other via exchange of molecules that are read by specific receptors on the cell surface. The different cell types appear in a chronological sequence with a certain overlap. This sequence is known as the four phases of wound healing, a concept that originates from the scientific observation of soft tissue healing (Stadelmann et al 1998). However, this concept can be transferred to bone healing and in particular to

intraoral bone healing of an implant wound – hemostasis, the inflammatory phase, the proliferative phase, and finally the remodeling phase. In a physiologic soft tissue wound, the hemostasis takes minutes to hours, the inflammatory phase hours to days, the proliferative phase days to weeks, and the remodeling phase begins at approximately 3 weeks and lasts for years (Stadelmann et al 1998).

The temporal sequence of bone healing around dental implants has been investigated histologically in dogs (Berglundh et al 2003, Abrahamssson et al 2004) and in human volunteers **(Figs 1 to 4)** (Lang et al 2011, Bosshardt et al 2011). In the dog study, the first biopsy, showing erythrocytes and inflammatory cells, was taken after 2 hours at the transition between hemostasis and inflammatory phase. The second biopsy was taken after 4 days and showed new vessels as well as fibroblasts and osteoclasts on the old bone (early proliferative phase). After 1 week, woven bone had appeared (late proliferative phase). After 2 weeks, a load-oriented remodeling of the woven bone by osteoclasts was noted in the areas of the tips of the threads (early remodeling phase). After 4 weeks, the remodeling at the tips of the threads was most intense. After 6 weeks, woven bone formation continued and remodeling also took place in the grooves of the implant threads. After 8 and 12 weeks, most woven bone was replaced by lamellar bone. In the human volunteer study, four time points were examined: after 1, 2, 4, and 6 weeks. After 1 week, new bone was observed occasionally on the implant surface in humans, which was comparable to what had been seen in the dog study. After 2 weeks, woven bone formation had increased but only in the grooves. In contrast to the dog study, no marked osteoclastic activity was observed in humans (proliferative phase). After 4 weeks, bridging between the parent bone and the implant took place in humans. After 6 weeks, first signs of transition to the remodeling phase were noted, 2 weeks later than in the dog. The direct comparison of the bone-implant contact rates revealed a delay of at least 2 weeks for humans compared to dogs (Abrahamssson et al 2004). A microarray analysis of the transcriptome of the material of the human volunteer study showed genes associated with inflammation upregulated at day 4, for angiogenesis at day 7, and for skeletogenesis at day 14 (Ivanowsi et al 2011, Donos et al 2011). Thus, the duration of the phases of bone healing around dental implants in humans approximates the duration of the same phases in physiological soft tissue healing as a biological constant.

The key players in this process are the different cell types. We observe coordinated action of several cell types and numerous individual cells in the defect. The action of cells is controlled by sequential activation of typical genes, which in turn are activated by soluble cytokines, small molecules, and molecules from the extracellular matrix (Midwood et al 2004). These messenger molecules interact with specific receptors on the surface of the cells. Usually, this causes a change of the conformation of transmembrane receptor proteins, which become enzymatically active and start an intracellular second messenger system that amplifies or modifies the information and transports it through the nuclear membrane to the DNA. The cellular response is then initiated by activation of genes and expression of certain proteins, either secretory products or intracellular regulatory proteins.

Adjacent cells can communicate with each other through direct membrane channels. However, over distances the cells communicate through chemical messenger molecules. The most important classes of messenger molecules are cytokines and hormones. Cytokines are proteins (interleukins [ILs], growth and differentiation factors). Hormones are subdivided into peptide hormones (eg, bradykinin), lipid hormones (eg, prostaglandins or steroid hormones), and amine hormones (eg, histamine). Although there is an overlap between the definitions of cytokines and hormones, hormones are usually active in nanomolar concentrations and longer ranges, whereas cytokines can be active in femtomolar concentrations through very specific protein receptors within a more restricted area. In addition, cells receive information through interaction with the extracellular matrix to which they attach with specific receptors (Schultz et al 2005). On a very local level, small molecules like nitric oxide or even ions like calcium play a role in signalling.

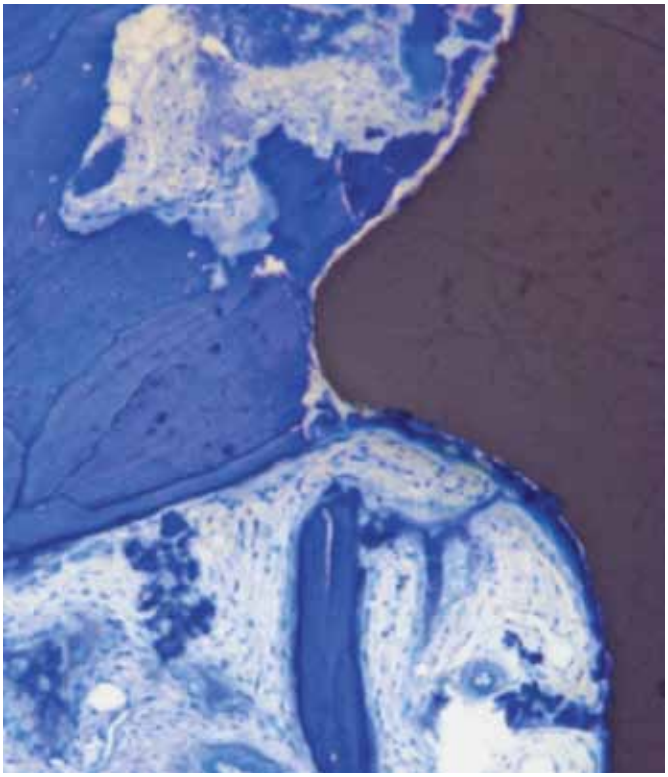

Fig 1 Human histology (toluidine blue) at 1 week, early proliferative phase. Note a moderately rough surface with initial bone formation on the surface toward the grooves, and bone debris without signs of osteoclastic degradation. (Reprinted from Lang et al 2011 with permission.)

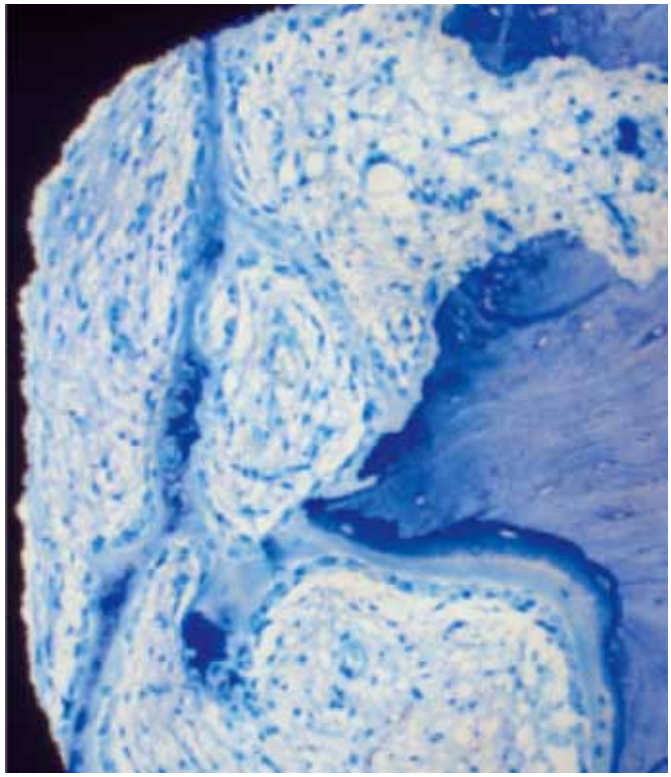

Fig 2 Human histology (toluidine blue) at 2 weeks, proliferative phase. Note a moderately rough surface with new bone starting to bridge between parent bone and implant. Bone debris particles are incorporated into immature new bone trabeculae, and no osteoclastic degradation of bone debris is seen. (Reprinted from Bosshardt et al 2011 with permission.)

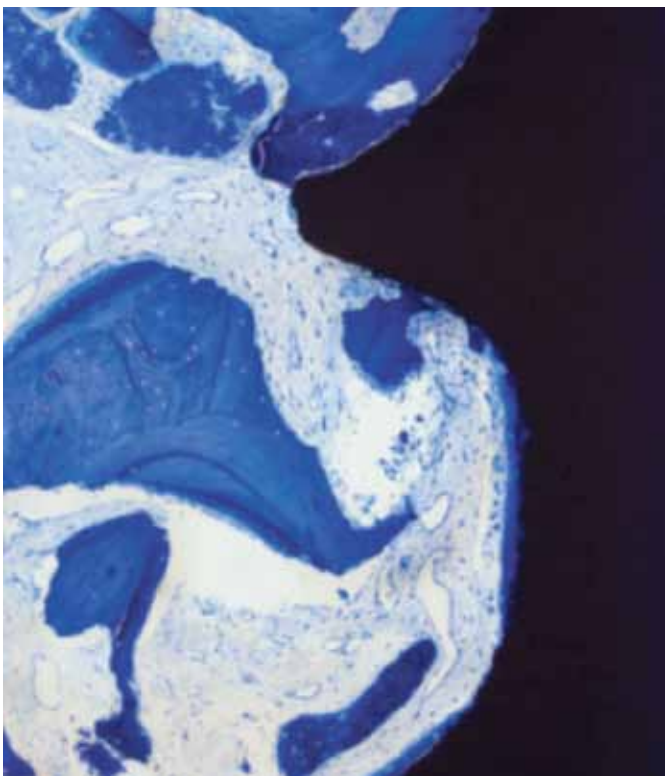

Fig 3 Human histology (toluidine blue) at 4 weeks, transition to remodeling phase. Note a moderately rough surface, and parent bone has been degraded. (Reprinted from Lang et al 2011 with permission.)

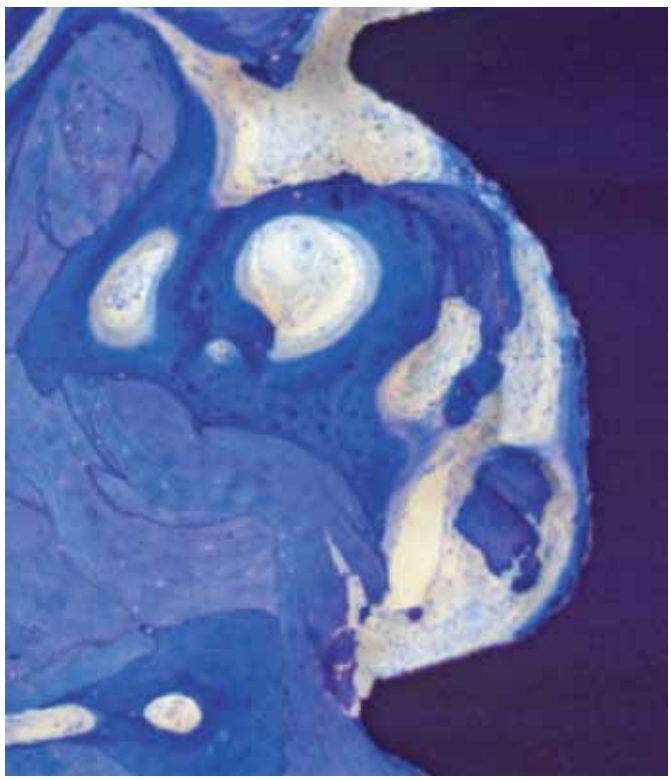

Fig 4 Human histology (toluidine blue) at 6 weeks, remodeling phase. Note a moderately rough surface and remodeling with formation of new primary and secondary osteons. (Reprinted from Lang et al 2011 with permission.)