Wound models for periodontal and bone regeneration: the role of biologic research

ANTON SCULEAN, IAIN L. C. CHAPPLE & WILLIAM V. GIANNOBILE

The treatment of periodontal diseases represents a significant healthcare challenge, and, since the genesis of human research endeavours in periodontology, the ultimate therapeutic goal has been to rebuild completely those tissues lost to the disease process with tissues that are structurally and functionally the same (144). Early attempts at regeneration were empirical, largely based upon clinical experience and included the utilization of scaling and root planing combined with soft-tissue curettage or the use of various flap procedures and materials for ’bone grafting’ (17, 30, 94, 100, 101, 115, 141). Such approaches occasionally resulted in clinical improvements, expressed as probing depth reductions or bone defect fill, which at the time were termed ‘new attachment’ or ‘regeneration’ (43, 99, 112, 141). However, histologic studies in animals and humans subsequently demonstrated that such methods did not predictably result in the formation of periodontal ligament, root cementum and bone (31, 32, 78, 130).

A proper understanding of the basic biologic mechanisms involved in periodontal wound repair and regeneration requires assessment of the macroscopic, microscopic, cellular and molecular components of the healing process (Fig. 1). To determine the molecular mechanisms that best guide tissue neogenesis during wound repair and regeneration, the outcomes of basic science (laboratory) studies needs to be translated to the clinical arena. The transfer of discovery research to clinical research, and eventual adoption in clinical practice, requires pre-clinical animal model studies to determine the safety and early-stage effectiveness of new technologies. This volume of Periodontology 2000 highlights advances from a myriad of approaches to determine comprehensively how wound-healing models are employed in oral, periodontal and craniofacial tissue regeneration. In this introduction we present those requirements for the use of pre-clinical research in periodontal wound repair and identify limitations of the work that would need to be addressed before human studies in pre-clinical models or directly in the human clinical trial setting (as a result of the limitations of animal models to replicate the human clinical disease scenario). Finally, the key findings of the reviews within this volume of Periodontology 2000 are summarized.

A historical perspective

The history of periodontal regeneration over the past century has recently been summarized (44). In the modern era of regenerative biology, pioneering work was carried out in the late 1960s by Tony Melcher, who demonstrated, through his studies, advances in the biology of periodontal wound healing. In 1969, Melcher distinguished the processes or ’repair’ and ’regeneration’ (82–84) and, in 1976, he postulated that ’the first cells to repopulate a root surface will dictate the nature and quality of tissue that forms there’ (82).

Half a century later, it is perhaps worthwhile reflecting upon the extent to which our current treatment concepts are biologically based, as Melcher taught, or whether we still, to some degree at least, fall into the trap of empiricism when aiming to regenerate periodontal defects.

Important questions that arise when exploring biologic approaches to periodontal regeneration include:

• is there a rationale and a role for pre-clinical (animal) models to evaluate the biologic potential of
novel regenerative technologies, and what is the clinical relevance of such models?

- are regenerative materials that have been validated using pre-clinical models also effective in the human situation?
- what are the current concepts in managing angular (intrabony), furcation and recession defects around teeth?
- what options are available for managing defects caused by infection around dental implants?
- what is the role of ‘surgical technique’, including flap management and suturing, in determining improvements in clinical outcomes?

Several decades ago, because of a paucity of knowledge regarding the etiology and pathogenesis of periodontitis, it was believed that bone was the only tissue that played a role in supporting the tooth within the alveolus, and that the ‘diseased bone’ was primarily responsible for the presence of angular (intrabony) or furcation defects arising in periodontitis patients (88, 106). Such beliefs consequently led to the development of treatment protocols that focussed primarily on filling the ‘holes’ with a great variety of bone-grafting materials. The latter were largely non-resorbable and thus effectively acted as inactive biological fillers. Moreover, the majority of studies evaluating the effects of different surgical procedures aimed at defect fill with bone grafts and only employed clinical outcome measures, such as probing pocket depth, probing attachment level, radiological analysis and direct visualization, following surgical re-entry procedures (21, 43, 52, 87, 99, 105, 107, 112). However, such approaches did not facilitate the determination of true periodontal regeneration, an outcome that requires systematic histologic investigation. Subsequently, pivotal histologic studies provided evidence that the outcomes of periodontal probing were strongly correlated with the level of soft-tissue inflammation present and, as such, may not necessarily reflect genuine tissue regeneration. For instance, if the gingiva is inflamed, the tip of the probe will easily penetrate the pocket base through the connective tissues and to a level apical to the base of the pocket (12, 77, 138).

Subsequent research, using different grafting materials in periodontal defects, led to improvements in probing pocket depths as well as radiographic bone fill, giving the impression that true regeneration had occurred. Similarly, hard-tissue infill detected during clinical re-entry procedures was interpreted as proof...
of periodontal regeneration. A greater understanding of the biology of periodontal wound healing and regeneration commenced with the publication of a series of studies employing pre-clinical models with ligature-induced periodontitis, in order to mimic chronic periodontal defects in humans. The results were somewhat disappointing and demonstrated that all treatment procedures aiming to regenerate periodontal tissues, including root planing and soft-tissue curettage, and indeed modified Widman flap surgery with or without various bone grafts, consistently resulted in the formation of a long junctional epithelium on the instrumented root surface, with no, or limited, formation of cementum with inserting periodontal ligament fibers (32). Moreover, if bone formation was evident histologically in some of the defects, an epithelial lining was always interposed between the newly formed bone and the root surface. These findings were later confirmed in several independent studies, demonstrating that the majority of approaches to periodontal regeneration remained empirical in nature and lacked a foundation in biologic science (53, 64, 102, 135).

Almost simultaneously with the experiments of Caton and co-workers were Melcher’s reports from a series of elegant experiments which had led him to hypothesize that the cells responsible for periodontal regeneration were resident within the periodontal ligament and not in the bone itself (82). Thus, Melcher proposed the periodontal ligament as the key tissue responsible for periodontal regeneration. This hypothesis was later tested in a series of animal studies, which provided evidence that only the granulation tissue originating from the periodontal ligament had the capacity to form new cementum and eventually new alveolar bone, and that the connective tissue originating from the gingival flap or the alveolar bone did not promote true periodontal regeneration, as evidenced by the formation of new cementum with inserting collagen fibers and new alveolar bone (55, 61, 63, 64, 76, 89–91).

Knowledge obtained from such pre-clinical (animal) models led to the development of the treatment concept termed ‘guided tissue regeneration’ (47, 48, 93). The guided tissue regeneration concept built upon Melcher’s thesis that the first cells to populate the root surface would dictate the tissue that formed there. Therefore, barrier membranes were developed as barriers to the downgrowth of the junctional epithelium, whilst, at the same time, occluding the ingress of fibroblasts from the gingival connective tissues lining the surgical flap. Such a dual barrier facilitated those cells arising from the intact periodontal ligament to repopulate the debrided root surfaces. This series of well-designed and reported experiments (62) is just one example of how a deeper biologic understanding of tissue structure and function may ultimately translate into a clinically relevant treatment concept, once initially tested in animal models. Furthermore, these landmark studies highlighted the need for a greater understanding of the biologic processes involved in wound healing before developing novel treatment concepts, and the potential dangers of introducing treatment methods without previously evaluating those concepts in pre-clinical models.

Periodontal regeneration: myth or reality?

A plethora of different surgical techniques involving the implantation of various types of bone graft and/or bone substitutes, root-surface demineralization procedures, guided tissue regeneration, use of growth/differentiation factors, enamel matrix proteins or various combinations thereof, have been employed in order to achieve periodontal regeneration, and have been shown to promote periodontal regeneration in animals (62, 72, 80, 105, 125, 133, 135). Despite positive observations in animal models and successful outcomes reported for many of the available regenerative techniques and materials in patients, including histologic evidence, robust information on the degree to which reported clinical improvements reflect true periodontal regeneration remains limited (19).

When analyzing the literature on human studies evaluating healing of human intrabony defects following the use of various regenerative materials, it is apparent that periodontal regeneration in humans can be achieved to a variable extent with several materials, including guided tissue regeneration, biologic factors, certain types of grafts and numerous combinations of the aforementioned (127). In contrast to the findings in animal studies, the implantation of alloplastic materials alone has demonstrated at best limited, and frequently no, periodontal regeneration in humans (19, 56, 127, 134). These findings appear to indicate that complete periodontal regeneration in humans is possible in certain clinical situations and with a limited number of regenerative materials or their combinations. Whilst studies of human defects may provide important information on the biologic potential of various regenerative protocols and biomaterials, the information obtained from such studies needs to be interpreted carefully in the light of the available evidence from pre-clinical and clinical studies.
What are the benefits and limitations of pre-clinical models for research into periodontal regeneration and wound healing?

Wound healing principles need to be carefully considered in the development of biomimetic materials that can provide an appropriate microenvironment for tissue formation (72). These materials are used as matrices for the delivery of cells, molecules or scaffolding constructs for eventual clinical use. Based on these needs, pre-clinical studies have enabled the initiation of clinical investigations that have resulted in the approval of new medical devices or drugs that promote periodontal tissue repair (46). Pre-clinical study plans must be adapted for the specific purpose of the investigation (i.e. mechanistic or outcomes that will link to a surrogate clinical measure). An example of a mechanistic goal might be to determine the role of a newly discovered cell-adhesion molecule during the induction of experimental periodontitis in a genetically modified animal (knockout or transgenic) model. By contrast, a new drug to stimulate periodontal ligament formation may be injected into the gingival tissues to examine, histologically, the soft-tissue repair processes at the microscopic level. A preclinical model is used because this approach is not readily achievable in humans (at least in a meaningful, adequately powered study). Moreover, the objectives of a pre-clinical study must relate to the efficient and effective design of a reconstructive therapy. The choice of end point is therefore a critical issue in the study design. Overall, planning a pre-clinical study to evaluate regenerative devices requires decisions on animal species, defect type, the study end point and study duration (96). In addition, end points must be defined to estimate the sample size necessary to achieve the desired power (20). Consideration of the size of the defects, as well as morphologic changes related to the anatomic defect that can occur over time, can help to estimate the appropriate study duration.

The selection of pre-clinical models considers the differing anatomy and healing characteristics of rodents and larger animals (129). A summary of preclinical models commonly used to evaluate periodontal-reconstructive therapies, highlighting those related to devices and biologics, is illustrated in Tables 1 and 2. Many new regenerative approaches that strive to demonstrate a proof-of-concept or early-stage mechanistic understanding of the wound-healing process will employ small animal models as screening tools before embarking on larger animal studies (132).

### Table 1. Advantages and disadvantages of rat defect models

<table>
<thead>
<tr>
<th>Defect type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenestration (periodontal)</td>
<td>Gives a proof-of-concept in a short interval</td>
<td>Narrow healing window</td>
</tr>
<tr>
<td></td>
<td>Well contained defects</td>
<td>Small size, surgical microscopes required</td>
</tr>
<tr>
<td></td>
<td>No gingival tissue ingrowth</td>
<td>Spontaneous healing</td>
</tr>
<tr>
<td>Capsule (vertical ridge)</td>
<td>Standardized shape-dimension</td>
<td>Not applicable to alveolar bone</td>
</tr>
<tr>
<td></td>
<td>Shielded from local environment</td>
<td>Isolation from oral environment</td>
</tr>
<tr>
<td></td>
<td>Filled solely with cells and fluids emanating from the residual bone</td>
<td>Spontaneous regeneration</td>
</tr>
<tr>
<td>Alveolar socket</td>
<td>Easy and fast to perform</td>
<td>Formation of bone outside the capsule</td>
</tr>
<tr>
<td></td>
<td>Reproduces well the events occurring in bone healing</td>
<td></td>
</tr>
<tr>
<td>Infrabony peri-implant defect</td>
<td>Surgically created</td>
<td>Surgically created</td>
</tr>
<tr>
<td></td>
<td>Short time needed to generate the defect</td>
<td>Spontaneous regeneration</td>
</tr>
<tr>
<td></td>
<td>Standard morphology-dimension</td>
<td>Narrow evaluation window</td>
</tr>
</tbody>
</table>

Reproduced from Pellegrini et al. (96), with permission.
undergo this initial phase of testing will ever reach the next phase of testing in humans (33). Assuming regulatory approval for human testing following submission of an Investigational New Drug Application, three phases of clinical trials typically follow:

- Phase I, which establishes safety of a drug.
- Phase II, which gains preliminary information relative to the efficacy of the agent.
- Phase III, which is designed to be randomized and controlled in order to determine the effectiveness of the biologic agent (98).

### Common animal models used in periodontal wound-healing research

A variety of different animal models, such as rats, dogs and nonhuman primates, have been used in periodontal-regenerative studies (96, 128). The rat periodontal model has been frequently employed for bone-regeneration studies (49, 54, 57, 67). It is valuable as a screening model for assessment of regenerative molecules because of cost-effectiveness, ease of handling, etc. However, the typical defect size is relatively small, making visualization challenging and requiring the use of surgical microscopes for defect creation (129). Large animal models, such as the canine or the nonhuman primate, are a logical next step. The canine wound-healing kinetics and tooth anatomy have many similarities to the human situation (143, 144). Nonhuman primates are highly desirable for evaluating the safety and efficacy of new molecules because their anatomic and biologic features are very similar to those of humans (68, 128). However, their high cost and handling difficulties prevent them from being

### Table 2. Advantages and disadvantages of large-animal defect models

<table>
<thead>
<tr>
<th>Defect type</th>
<th>Animal model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furcation/infrabony periodontal defect</td>
<td>Dog and monkey</td>
<td>Surgical acute/chronic</td>
<td>Surgical acute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- short time to be created – less expensive</td>
<td>- does not reproduce inflammatory/infective conditions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- standardized morphological characteristics</td>
<td>- spontaneous partial regeneration (monkey)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- does not regenerate spontaneously (chronic)</td>
<td>Surgical chronic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Class II–III furcation</td>
<td>- soft tissues compromised</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- bilateral similar defect</td>
<td>- variable amount of connective tissue regeneration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- horizontal defect allows an estimation of the origin of the newly formed tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- solid database describing healing (dog)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- minimal palatal recession (monkey)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ligature induced</td>
<td>- microbiological features similar to those of humans</td>
<td>Ligature induced</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- morphological features similar to those of humans</td>
<td>- nonpredictable disease development</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- does not regenerate spontaneously</td>
<td>- nonstandardized defect morphology (dog)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- can make similar lesions as control in contralateral defects</td>
<td>- requires time to be created and is expensive</td>
</tr>
<tr>
<td>Alveolar socket</td>
<td>Dog and monkey</td>
<td>Easy and fast to perform</td>
<td>Rapid bone repair compared with that for human (dog)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reproduces well the events occurring in bone healing</td>
<td></td>
</tr>
<tr>
<td>Infrabony peri-implant defect</td>
<td>Dog and monkey</td>
<td>Surgically created</td>
<td>Surgically created</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- short time needed to generate the defect</td>
<td>- spontaneous regeneration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- standard morphology-dimension</td>
<td>Ligature induced</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- morphological and microbiological similarities with humans</td>
<td>- spontaneous partial regeneration</td>
</tr>
<tr>
<td></td>
<td>Ligature induced</td>
<td></td>
<td>- long time required to generate the defect</td>
</tr>
<tr>
<td>Supra-alveolar peri-implant defect</td>
<td>Dog</td>
<td>Limited spontaneous regeneration</td>
<td>Requires space-providing devices</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reproducibly created</td>
<td></td>
</tr>
</tbody>
</table>

Reproduced from Pellegrini et al. (96), with permission.
THE TRANSLATIONAL CONTINUUM FOR ORAL HEALTH RESEARCH

- BASIC SCIENCE DISCOVERY
  - Promising molecule or gene target
  - Candidate protein biomarker
  - Basic epidemiologic finding

- EARLY TRANSFORMATION
  - Partnerships & collaboration (academia, government, industry)
  - Intervention development
  - Phase III trials

- LATE TRANSFORMATION
  - Phase III trials
  - Regulatory approval
  - Partnerships
  - Production & commercialization
  - Phase IV trials: approval for additional uses
  - Payment mechanism(s) established to support adoption
  - Health services research to support dissemination and adoption

- DISSEMINATION
  - To community health providers
  - To patients and public

- ADOPTION
  - Adoption of advance by providers, patients, public
  - Payment mechanism(s) in place to enable adoption
  - Surveillance

Fig. 2. The translational continuum from laboratory research discovery, through pre-clinical animal model testing and eventual acceptance to clinical practice. Reproduced from Quintessence Publishing, with permission [from Lin et al. (74), with permission].

utilized more frequently. The preferred animal model should be selected according to study requirements, such as periodontal, oral implant, sinus floor augmentation and alveolar ridge reconstruction.

A study should not be proposed if a clear end-point goal is not established that will eventually have an impact on human health or disease. Animal-based research has led to significant improvements in the quality of life for many patients (13, 95, 140). However, these advances must result from the humane use and care of animals employed in such research. The field of periodontal regenerative medicine has progressed to provide better reporting and of design animal studies with the aim of truly advancing the field. The National Centre for the Replacement, Refinement and Reduction of Animals in Research, a UK Government-sponsored organization, has documented overall marginal quality standards with respect to the documentation, design and reporting of animal studies in research (65). These findings have led to the development of guidelines for the reporting of pre-clinical studies to improve their quality and, overall, to reduce the use of animals in research. The ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines have been developed using the CONSORT (Consolidated Standards of Reporting Trials) statement as their foundation (66). These guidelines have now become a requirement for the publication of pre-clinical research in many scientific journals and support animal ethical bodies to improve the overall quality of pre-clinical research. Furthermore, organizations, such as the European Federation of Periodontology, have addressed this issue in several recent publications on periodontal and peri-implant wound healing (16, 122, 126, 139).

**Future applications of periodontal wound-healing models**

The use of pre-clinical animal models, typically under good laboratory practice settings, is an important component in the development of new biomaterials.
for clinical trials (118). *In vivo* models are, in general, superior to *in vitro* investigations in helping to understand the complex molecular, cellular and tissue reactions that occur in response to delivered regenerative molecules. Despite the limitations of pre-clinical models for human disease, *in vitro* investigations for the simulation of human disease continue to remain inappropriate before testing in humans. The increased growth of development of biologics and devices for application in periodontal-regenerative medicine requires a thorough examination of when and how the appropriate end points can be evaluated before entry into human clinical trial testing. With continued innovations in more noninvasive *ex vivo* technologies, the needs for pre-clinical testing will only decrease.

However, optimized animal models are still necessary for evaluating wound-healing promoters before human testing. Limitations include obvious differences in the host response and disease development, and anatomic differences among animals and humans. Models that can better implement risk factors of disease development known to affect periodontal wound healing, such as smoking, diabetes and microbial infection, may aid in the continued refinement of pre-clinical animal models before embarking upon expensive human trials. As the field of personalized medicine continues to evolve, host susceptibility and identification of those people who respond best to wound-healing modifiers may provide more translatable outcomes to individual patients. The ultimate result of enhanced pre-clinical testing will greatly advance patient care for the public and aid in developing a better understanding of wound-repair mechanisms.

What is the current ‘state of the art’ in periodontal wound and bone healing?

The first reviews in this volume of *Periodontology 2000* address the place for the various aforementioned models employed to evaluate novel treatment approaches for periodontal, bone and peri-implant wound healing and their potential role in informing the implementation of clinical therapy in humans (41, 60, 124, 132).

Pre-clinical studies are usually performed to evaluate proof-of-principle concepts, safety and possible unwanted effects of candidate biologic agents or bone biomaterials, before proceeding into clinical testing (45, 86, 96). Selection of the appropriate animal species and wound-healing model is largely dependent upon the research question or the disease model. For example, models involving small animals have been developed largely to screen bone biomaterials for their potential to enhance bone formation. As no single model can completely mimic the anatomic, physiologic, biomechanic and functional environment of the human mouth and jaws, the results obtained in small animals need to be validated in larger animals (e.g. dogs, mini-pigs or monkeys) whose dento–alveolar anatomy resembles more closely that of humans.

Following application of the guided tissue regeneration principle for the treatment of periodontal defects, the same principle was used in a number of pre-clinical and clinical studies for the regeneration of bone defects or for the augmentation of deficient sites. For these techniques, the term ‘guided bone regeneration’ was adopted, pointing to the biologic rationale based on the mechanical exclusion of neighboring soft tissues surrounding the bone defects, in order to allow only osteogenic cells derived from the margins of the bony defect to repopulate the wound area. This biologic principle, also based upon Melcher’s theories, was first tested in smaller animal models, such as rats and rabbits, and was confirmed later in larger animals (e.g. dogs and monkeys) (4, 37–40, 70).

An important issue when testing the guided bone regeneration principle is the use of the so-called ‘critical size defect’, indicating bone defects that heal spontaneously with fibrous connective tissue and not by osseous fill. Critical-size defect models involve healing of orthotopic bone sites, such as the mandible or the calvarium, and thus allow the evaluation of bone grafts and other bone-regenerative modalities in a standardized and discriminative manner to provide ‘proof-of-principle’ (14, 38, 58, 70, 117, 118, 119, 137). As it has been repeatedly demonstrated that guided bone regeneration can successfully regenerate critical size defects, nowadays the use of such experimental models for evaluating the effect of different treatment modalities for bone regeneration is recommended, as discussed by Donos et al. (41) in this volume of *Periodontology 2000*.

The guided bone regeneration principle has been successfully applied in the treatment of patients exhibiting different types of bone defects, leading to a substantial increase in the number of cases of dental implant therapy, including cases that were previously not feasible because of a lack of bone at the recipient site (23–26, 51, 92). Taken together, these animal experiments serve as a beautiful example of the
importance of pre-clinical models (Phase I studies) in translational regenerative dental research.

The successful integration of guided bone regeneration principles into clinical practice over the last two decades has led to a substantial increase in the number of dental implants being placed and associated bone-augmentation procedures. This has coincided with an ageing population whose expectations of tooth replacement with fixed prostheses have also increased (81, 131). However, there has been a coincident increase in the proportion of the population with age-related chronic diseases and conditions, such as diabetes and osteoporosis (50, 69, 104). Their impact upon bone-regeneration procedures related to implant rehabilitation is largely unexplored and consequently there is a pressing need to evaluate different bone-regenerative techniques in pre-clinical models, to help optimize the treatment of patients affected by such systemic conditions. Donos et al. (41) discuss these issues in their review. Interestingly, recent data from preclinical models indicate that, in osteoporotic animals, a hydrophilic barrier/implant surface may counteract the negative effect of osteoporotic-like conditions, whilst the use of guided bone regeneration appears to create an environment that facilitates bone formation in experimentally induced diabetes and osteoporosis (73, 79). Despite the fact that, at present, it is difficult to draw any conclusions regarding the extent to which systemic conditions may affect bone metabolic status, vascularization and healing capacity, the use of various pre-clinical models simulating systemic health and disease are of utmost clinical relevance for developing new concepts that enable de novo bone formation via guided bone regeneration in medically compromised patients.

The extraction socket as a wound-healing model

Tooth extraction is one of the most common surgical procedures performed on humans, yet reviewing decades of published literature it is remarkable to note that, until recently, very little was known about the biologic processes that take place during socket healing. Studying the simplest of wound-healing models (i.e. the extraction socket) has provided profound insights into the temporal sequence of healing events that follow tooth extraction (6, 10, 11, 29, 74).

Studies by Araujo et al. (8) have demonstrated that the overall wound-healing events described histologically in dogs are remarkably similar to those in humans, despite the fact that bone modeling and remodeling during socket healing are three- to five times quicker in dogs. Such findings subsequently led to the development of clinical protocols associated with tooth extraction. For example, it is currently recommended that tooth extraction is planned and performed with attention paid to the subsequent anticipated changes in ridge width and height following this intervention. As a result, there is frequently an indication to consider adjunctive therapies in order to compensate for such changes, particularly in cases in whom further treatment, such as dental implant placement, is planned (9, 75).

Pre-clinical and human models to study osseointegration and management of peri-implant infections

The use of pre-clinical (animal) and human models has been shown to provide valuable information regarding our understanding of the healing of hard and soft tissue integration around titanium dental implants (1–3, 18, 42, 71, 85). Such healing results from a complex cascade of biologic events that are initiated by the surgical intervention. The temporal sequence of healing events that culminate in osseointegration has been elucidated in animals and humans. Implant placement into alveolar bone induces a cascade of healing steps, commencing with clot formation and continuing with bone maturation in direct contact with the implant surface. Salvi et al. (113) demonstrate that osseointegration is associated with a decrease in inflammation and an increase in osteogenesis-, angiogenesis- and neurogenesis-related gene expression during the early stages of wound healing. The attachment and maturation of the soft-tissue complex to implants (i.e. epithelium and connective tissue) is established 6–8 weeks following surgery (126). Moreover, the use of pre-clinical data has provided important insights into the etiology, pathogenesis and therapeutic approaches to peri-implant diseases because established lesions in animals have shown many features in common with those analyzed in human biopsy material (5, 27, 28, 121–123).

The review of Romanos (108) provides some evidence that immediate loading of augmented ridges may improve bone stability following implant placement, and Schwarz et al. (124) discuss the utility of animal models in developing therapeutic approaches to implant failure (97, 109–111, 124). Renvert & Polyzois (103) discuss the limitations of existing approaches to managing peri-implant mucositis and peri-implantitis in humans and conclude that the
most important factor in managing implant failure is patient self-performed oral hygiene.

**What can in vitro models offer for a better understanding of periodontal wound and bone healing?**

In order to improve and further develop treatment strategies aiming to regenerate soft and hard tissues at natural teeth and dental implants, it is important to acquire a thorough understanding of the characteristics and roles of different wound-healing components (such as cells, matrices, molecules and genes) involved in the complex processes of wound healing and repair, as well as the mechanisms controlling their function. As these processes are complex, it is extremely difficult to understand and define the role of each component in determining the final outcome. Furthermore, in animal models that aim to simulate a certain clinical situation, the influence of individual components in the wound-healing cascade is difficult to assess. Such models are also expensive and frequently difficult to work with. Accordingly, in order to understand a certain mechanism, or before testing a specific biologic principle or biomaterial in animals, it makes sense to adopt a stratified approach by answering single, well-defined questions using specifically designed laboratory models.

A plethora of *in vitro* models have been described and developed to mimic the wound-healing events of soft tissues (e.g. oral epithelia or oral fibroblasts) or to measure proliferation, attachment and migration, gene expression and differentiation into a mineralizing phenotype, as well as biomineralization of periodontal ligament fibroblasts or progenitors, osteoblasts or osteoprogenitors and cementoblasts. Other *in vitro* studies have utilized various gene-transfer models, models designed to evaluate the effect of implant surface modifications on cellular behavior or models that address biocompatibility and degradation of different types of resorbable membranes (34, 142).

Despite the fact that, in general, such laboratory-based models are less expensive and easier to perform compared with animal experiments, they differ markedly from the *in vivo* situation because they typically employ single cell types without the surrounding matrix or other relevant cell types, and without the influence of external factors. Therefore, results obtained using such models often provide critical mechanistic and biologic plausibility insights. These studies lay the foundation for potential clinical application in more applicable pre-clinical and clinical studies on the disease and injury processes.

**Improved clinical outcomes through an improved understanding of biology**

There is little doubt that research conducted over the last 25 years into the biophysiology of periodontal and tooth-socket healing has informed the development of novel approaches to restoring lost periodontal tissues and bone (8, 44, 62, 125). In the laser field, comprehensively reviewed by Aoki et al. (7), a greater understanding of the role of certain wavelengths in removing soft and hard deposits, killing bacteria and improving wound healing, has led to the application of lasers and of laser-based photodynamic therapy in clinical periodontal care. Some positive effects, such as the reduction of inflammation (i.e. less bleeding on probing), following the additional application of photodynamic therapy, have been demonstrated, in randomized controlled clinical studies, to be statistically significant and clinically relevant, and consequently such approaches are frequently used in the maintenance phase (15, 35, 116, 136). By contrast, it is clear that laser therapy has thus far failed to demonstrate improved periodontal outcomes over and above those achieved by traditional instrumentation (120). It is also important to report such negative findings because they save the effort and costs involved in repeating clinical trials of the same, and open up opportunities to explore the utility of different wavelengths and protocols.

Other examples of tissue-regeneration techniques that have been developed based upon learnings from biologic research and have improved clinical outcomes, include intrabony defects, furcation lesions [see reviews by Cortellini & Tonetti (36), and Sanz et al. (114)] and recession defects [see Zucchelli & Mounssif (145)]. The novel concepts reported in these reviews have evolved following *in vitro* and animal studies, which have informed a better understanding of periodontal and bone wound healing and of the role and use of various biomaterials on periodontal ligament cells, gingival fibroblasts and bone cells. These techniques combine the use of surgical flaps and suturing techniques [see Burkhardt & Lang (22)] designed to improve blood clot protection and to enhance wound stability, and the use of biologically potent biomaterials.

Stem cell research holds great promise for the future of periodontal regeneration, but multiple challenges remain, including the development of appropriate matrices to deliver cell therapies, appropriate growth factor combinations that facilitate physiological healing processes in what is ultimately a potentially ‘infec-
tive’ healing environment. The latter is a challenge that is frequently overlooked or underestimated, but involves stimulating the body’s physiological wound-repair processes within a complex milieu of microbial and host signaling between innate and acquired immune systems and a colonizing microbiome. Similar challenges face bone-regenerative procedures around failing implants, which lack a periodontal ligament (and thus a vital source of undifferentiated stem cells) and also differ in terms of the connective tissue fiber anatomy within the investing soft-tissue cuff. Here, emerging evidence indicates that early outcomes of bone regeneration are more predictable when the implant fixture is recovered by soft tissues to facilitate healing in a ‘closed system’, in which microbial colonization is not a complication. Nevertheless, longer-term studies are needed in this emerging field in order to achieve a better understanding of the biology of healing around implants that have suffered peri-implantitis and also to understand the nature of the primary process of peri-implantitis at the tissue level. It appears that we are now at the end of the beginning of research into periodontal and bone regeneration, and understanding the pathobiology of periodontitis and peri-implantitis is fundamental to enable the design of regenerative strategies and technologies, rather than persisting with the generic approaches of today.

Acknowledgments

The collaborations from Qiming Jin, Jim Sugai, Hector Rios, Reinhard Gruber, Yang-jo Seol, Gaia Pellegrini, and Darnell Kaigler are greatly appreciated. Dr Gianobile’s work was supported, in part, by NIH/NIDCR DE 13397.

References


