

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Effects of Human Ageing on Periodontal Tissues

Eduardo Hebling

*Department of Community Dentistry, Piracicaba Dental School,
University of Campinas, Piracicaba,
Brazil*

1. Introduction

Aging and death are two natural consequences to which the human individuals are subject after their birth. Improvement in both social living conditions and health care has led to a greater life span in the world ¹, resulting in an increase in periodontal disease expectancy among the dentate elderly ². Although moderate loss of both alveolar bone and periodontal attachment is common in the elderly, severe periodontitis, defined as periodontal attachment loss of 6 mm or more and radiographic bone loss of 50% or more involving at least one tooth, is not a natural consequence of ageing. Some loss of periodontal attachment and alveolar bone may be expected in older persons, but age alone in healthy adults does not lead to a critical loss of periodontal support ³.

Human ageing induces histophysiological and clinical alterations in oral tissues ⁴. These alterations must be understood to differentiate pathological conditions from the altered physiology of oral tissues resulting from ageing ⁵. Some studies in humans and animals have demonstrated that alterations in periodontium dynamics occur with age ^{6,7}.

In spite of the fact that the periodontal disease severity is known to be associated with age, functional changes in periodontal tissue cells during the ageing process have not been well characterized ⁸. It is important to define how cellular ageing affects the progression of periodontal diseases associated with ageing ⁶. The understanding of the influence of human aging in the dynamic of the inflammatory process in patients with periodontal disease may help in the treatment planning of these patients.

2. Age-dependent changes of the periodontal tissues

The tissues that support the teeth are called the periodontium, which consists of gingiva, periodontal ligament, cementum, and alveolar bone. Anatomical and functional changes in periodontal tissues have been reported as being associated with the ageing process ⁹.

Gingiva, a tissue exposed to the oral cavity, is histologically composed by epithelium and connective tissues. Changes in the human oral epithelium caused by ageing are related to a thinning of the epithelium and diminished keratinization. Conflicting results have been reported regarding the shape of the retepegs. A flattening of retepegs and an increase in the height of the epithelial ridges associated with ageing were both demonstrated. In a morphological 3-dimensional study of the epithelium-connective tissue interface, connective

tissue ridges were observed to be more prevalent in young individuals whereas connective tissue papillae were predominant in old individuals. The change from ridges to papillae involves the formation of epithelial cross-ridges with advanced age ⁹.

Furthermore, it has been shown that the number of cellular elements decreases as age increases. The fibroblasts are the main cells in the synthesis of periodontal connective tissue. There are phenotypic subpopulations of fibroblasts with different functions in the synthesis and maintenance of extracellular matrix constituents ¹⁰. *In vivo* and *in vitro* studies have shown functional and structural alterations in fibroblasts associated with ageing ^{6, 11-13}.

Gingival fibroblasts (GF) may be constantly affected by oral bacteria and their products, such as the lipopolysaccharides (LPS), present in their cell walls. The LPS induces GF to release some inflammatory cytokines such as prostaglandin E₂ (PGE₂), interleukin (IL)-1 β , and plasminogen activator (PA) ^{6, 14}. The influence of these inflammatory mediators on both GF and periodontal ligament fibroblasts (PLF) might account for the severity of periodontal disease ⁶.

Quantitative differences in protein synthesis were found in young and old gingival fibroblasts *in vitro*. The collagen production decreased more than 5-fold as a function of increasing donor age ¹⁵. Old fibroblasts also presented an increased rate of collagen intracellular phagocytosis, which can affect the balance between synthesis and degradation of collagen in the connective tissue ¹². The ageing process in GF causes an increase in DNA structure methylation of collagen alpha 1 gene, followed by a reduction in mRNA levels and collagen type I synthesis ¹³. Alterations in the composition of extracellular matrix proteoglycans secreted by GF *in vitro* were also observed. The proteoglycans secreted by old fibroblasts might increase the rates of heparan sulfate and reduce chondroitin sulfate when compared to those secreted by young fibroblasts ¹⁶.

The periodontal ligament, which is a soft connective tissue, anchors the tooth into the alveolar bone and functions as a cushion between hard tissues to mitigate the occlusal force. It is basically constituted by fibroblasts, cementoblasts, osteoblasts, osteoclasts, Malassez epithelial rests and collagen matrix (Sharpey's fibers). The periodontal ligament cells are involved in the repair of alveolar bone, cementum and periodontal ligament itself, being able to differentiate into osteoblasts, cementoblasts and fibroblasts ¹⁷. With age, the fiber and cellular contents decrease and the structure of the periodontal ligament becomes irregular. Periodontal ligament fibroblasts (PLF) are constantly subject to mechanical stress caused by occlusal forces. Cultured PLF were observed to produce a large amount of PGE₂, IL-1 β , and PA in response to mechanical stress ⁶.

The ageing process might induce a significant reduction in chemotaxy, motility, and proliferation rate of periodontal ligament cells. The chemotaxy and differentiation of osteoclasts from the periodontal ligament induced by devitalized osseous matrix might be influenced by donor's age. A reduction in osteoblast chemotaxy and lower rate of osteoclast differentiation in the cells of elderly donors were observed ¹⁸.

The cells of the periodontal ligament from the elderly showed lower rates of chemotaxy and proliferation than those of the periodontal ligament from young patients ¹⁹. The reduced ability of senescent cells to express the *c-fos* ligand might be associated with the low rates of chemotaxy and proliferation of these cells ²⁰. The expression of osteocalcin in fibroblasts from the periodontal ligament is either reduced or ceased in senescent fibroblasts. This reduction may be directly related to the cell's difficulty in progressing in the cellular cycle (G1-S) and accomplishing cell respiration ²¹.

Cementum is a calcified connective tissue covering the roots of teeth. Its formation is a continuous process which occurs throughout the life of humans and animals. With age, the cementum increases in width. It has been demonstrated that there is a tendency towards greater cemental apposition in the apical region of the teeth. Collagen fibers are embedded in the cementum during its formation⁹. Ageing and death of cells are common characteristics of the life cycle of the cementocytes. This might be due to a rapid reduction in the accessibility of nutritive substances and poor elimination of waste products of the cementocytes. In general, cementum is cellular-except at the root apices and in the furcation areas of multirooted teeth. With age, cementum becomes acellular. Although remodeling of cementum occurs infrequently, resorption at the cementum surface followed by cementum apposition is often observed and, with age, this might result in irregular cementum surfaces^{9, 22}.

Both the alveolar bone and the periodontal ligament serve as support to the teeth. It is well known that bone formation steadily declines with age, leading to a significant reduction in bone mass²³. The alveolar bone has high plasticity and under physiological conditions it is preserved by the equilibrium between osteoblastic and osteoclastic activities. These cells are directly or indirectly influenced by the parathyroid hormone (PTH), vitamin D metabolites, calcitonin, estrogen, plasmatic concentration of calcium and phosphate, neurotransmitters, growth factors, and local cytokines²⁴.

The reduction in bone formation might be due to a decrease in osteoblast-proliferating precursors or to decreased synthesis and secretion of essential bone matrix proteins^{6, 23}. The extracellular matrix surrounding osteoblasts has been shown to play an important role in bone metabolism. A possible dysfunction of this matrix might occur concomitantly with the ageing process⁶.

Oxygen-free radicals have been reported to cause cellular damage and, consequently, contribute to the ageing process^{25, 26}. In an *in vitro* study, oxygen radical-treated fibronectin (FN) was found to inhibit bone nodule formation by osteoblasts when compared to intact FN. This finding suggested that intact FN plays an important role in osteoblast activity and that FN damaged by oxygen radicals during the ageing process might be related to less bone formation⁶.

3. Systemic ageing and periodontium

Some alterations in the organism endocrine profile influence the osseous metabolism with age. Vitamin D deficiency is a common phenomenon in persons living in elderly homes²⁷. The low levels of calcium resulting from vitamin D deficiency associated with renal insufficiency might lead to secondary hyperparathyroidism²⁸. The high levels of PTH resulting from secondary hyperparathyroidism act in the mobilization of osseous calcium, which might cause mineralization problems, bone fractures, and a reduction in osseous density²⁹. With regard to the periodontium, osteopenia and osteoporosis are considered important risk factors for alveolar bone loss in the presence of periodontal diseases^{30, 31}.

Female patients with osteoporosis or osteopenia presented greater levels of alveolar bone loss, when compared to patients with normal mineral osseous density^{32, 33}. Estrogen hormonal reposition therapy in patients with osteoporosis might reduce gingival inflammation and alveolar bone loss in relation to patients with untreated osteoporosis^{34, 35}. In a randomized double-blind study, patients with osteoporosis treated with estrogen reposition showed an increase in alveolar bone levels, when compared to the placebo group³⁶.

The supplement ingestion of calcium (1000-1200 mg/day) and vitamin D (400-600 IU/day) in elderly patients with osteoporosis has been tested. After 5 years of treatment, the results showed a reduction in tooth loss in patients who ingested calcium and vitamin D, when compared to the placebo group ³⁷. Studies evaluating periodontal status and hormonal reposition therapies, indicated for prevention and treatment of osteoporosis, have reported the reduction in alveolar bone loss and tooth loss as secondary benefits. It is worth emphasizing that some risks in hormonal reposition therapy have been recently reported; for example, an increase in the incidence of breast cancer, thromboembolic diseases, and myocardial infarction has been found ³⁸.

4. Immunosenescence and periodontal cells interaction

Immunosenescence refers to the gradual deterioration of the immune system caused by natural aging. It involves the host's capacity to respond to infections and the development of long-term immune memory. This is not a random deteriorative phenomenon; rather it appears to repeat inversely an evolutionary pattern. Most of the parameters affected by immunosenescence appear to be under genetic control. Immunosenescence can also be sometimes envisaged as the result of the continuous challenge of the unavoidable exposure of the organism to a variety of antigens such as viruses and bacteria ³⁹.

Aging is a complex, continuous, and slow process that gradually involves most if not all organs of the organism, causing their abnormal functioning of in both qualitative and quantitative terms, as well as morphological or structural changes. So, senescence is not represented by a pre-established moment, but consists of a long-lasting preparation of the organism for a morpho-functional involution, which itself is a normal part of the biological cycle ⁴⁰. In this context, periodontal tissues also are included.

The immune system also undergoes age-related modifications leading to structural changes in the lymphoid organs, and functional impairment of some types of immunocompetent cells. The most evident changes in the immune system occur in the thymus, a specialized organ of the immune system. The thymus is a primary lymphoid organ, but also an endocrine gland, responsible for T-cell production and maturation ⁴¹.

The thymus is the largest and most active during the neonatal and pre-adolescent periods. By the early teens, the thymus begins to atrophy and thymic stroma is replaced by adipose tissue. Nevertheless, residual T lymphopoiesis continues throughout adult life. Thymus involution in humans is observed until the age of 70 years ⁴². The consequences of thymic involution in the peripheral pool of T-cells are still a matter of controversy. Thus, whereas some authors report no significant decline in the total number of T-cells, but considerable shifts in the ratios of activated/memory T-cells ⁴¹, others claim that the age-related changes in the thymus and T-cells are quantitative, not qualitative ⁴³. In any case, it remains to be clarified whether changes in the immune system with age have something to do with survival ⁴⁴, although the stage of being elderly is associated with an increase in infections, tumors, and other diseases related to a decline in the immune function ⁴⁵.

T-lymphocytes are considered thymus-dependent cells of fundamental importance in the immune response. Reductions in peripheral blood T-lymphocytes, mitotic agents, anti-CD3, and monoclonal antibodies are the main alterations in the senescent phenotypes of T-lymphocytes. The proliferative phase alterations in T-lymphocytes may be induced by the reduced secretion of interleukin-2 (IL-2) and reduced expression of its high-affinity receptors. The reduced expression patterns of IL-2 and IL-2R in peripheral monocytes of

elderly patients have been reported as influencing the proliferative response of T-lymphocytes ⁴⁶. IL-2 is produced by helper T-cells and plays an important role in the proliferation and differentiation of virgin T-cells into effector T-cells (this term is used frequently for populations of T-cells with cytolytic activities and for T-helper cells, which secrete cytokines and activate directly other immune cells, to distinguish them from another class of T-cells known as regulatory T-cells) ⁴⁷.

Ageing-related immunological alterations in the leukocyte subpopulations ⁴⁸ and B-lymphocyte subpopulations ⁴⁹ have been reported. The reduction in the peripheral blood population of B-lymphocytes is associated with the decrease in the production of high-specificity antibodies as well as in the avidity of antigen-antibody legation ⁵⁰. In establishment of long-term memory of immune response, the maturation of antigen-induced CD4⁺ T-cells, which are postthymic human cell, results in the terminally differentiated CD45RA⁺. An increase in CD45RA⁺ memory cell circulation in relation to CD45RO⁺ virgin cells disturbing the response to new antigens has been noted. Other changes in immune senescence include a decline in macrophage, neutrophil, and natural killer function with ageing ⁵¹.

A more rapid and severe development of gingivitis as well as changes in inflammatory response induced by gingivitis in elderly patients have been reported ⁵². A greater presence of alpha 2-macroglobulin, IgG3, and B-lymphocytes in the crevicular fluid, and a reduction in polymorphonuclear leukocytes (PMN) have also been observed in the elderly ⁵³.

Periodontal ligament cells from the elderly showed an increase in the production of plasminogen activator (PA) ⁵⁴, prostaglandin E2 (PGE2), interleukin-1 β (IL-1 β) ^{6, 54} and interleukin-6 (IL-6) ⁵⁵ when compared to younger cells.

Lipopolysaccharide (LPS), responsible for bacterial cytotoxicity, is a component of the gram negative bacterial cell wall. It induces the activation of transcription factors in lymphocytes, promoting regions of DNA which activate genes that contribute to the adaptive response and secretion of pro-inflammatory cytokines ⁵⁶.

PA is a serine protease that acts in the activation of plasminogen into plasmin and it is secreted by many cell types, including periodontal fibroblasts. The activity of PA and the expression of tissue plasminogen activator (tPA) mRNA in fibroblasts from the periodontal ligament *in vitro* submitted to mechanical tension were evaluated. The cells from elderly individuals presented greater activity of PA and greater expression of tPA mRNA when compared to those from young individuals ⁷. The action of PA is involved in physiological and pathological mechanisms of periodontium, including host-microbiota interaction, PMN migration, and proliferation and migration of both epithelial cells and fibroblasts ⁵⁷. Analysis of PA distribution in the periodontium showed that, in healthy periodontium, PA is expressed in the superficial cells of the junctional epithelium. However, in patients with periodontitis, PA is expressed in all the epithelium lining of the periodontal pocket. The alteration in the pattern of PA distribution, according to periodontal status, suggests that PA is involved in the periodontal homeostasis ⁵⁸.

The levels of tPA and the plasminogen activator inhibitor-2 (PAI-2) are greater in gingival crevicular fluid of patients with periodontitis than in periodontally healthy patients ⁵⁹. Patients with high levels of alveolar bone loss presented higher levels of tPA and PAI-2 than those with low levels of alveolar bone loss. A greater release of PA induced by ageing might affect the gingival fibroblasts and the periodontal ligament, and aggravate the inflammation process and the degradation of the extracellular matrix from periodontal tissues in the elderly ^{7, 53}.

Fibroblasts from old mice (20 months) demonstrated a significant increase in the synthesis of PGE2 and IL-1 β when compared to the fibroblasts from young mice (6 weeks) ^{54, 60}. An increase in the production of PGE2 and Cox 2 mRNA stimulated by LPS and mechanical stress, respectively, was also observed in periodontal ligament cells from elderly donors ^{61, 62}. PGE2 is produced by the metabolism of arachidonic acid through the cyclooxygenase (COX) pathway and has a recognized role in the inflammatory process, through vascular dilatation, increased in vascular permeability, and sensibilization of nociceptors to the stimulus of histamine and bradykinin. PGE2 may have an indirect effect on alveolar bone resorption by the sensibility of osteoclasts to the action of other cytokines involved in this process ⁶³. In patients with periodontitis, high levels of PGE2 were observed to be related to the severity of periodontal disease and the increase in alveolar bone loss ^{64, 65}. The greater production of PGE2 by old periodontal ligament cells might account for the greater rate of alveolar bone resorption in elderly patients ^{54, 66}.

In vitro studies have demonstrated an increase in the synthesis of IL-1 β and in the expression of IL-1 β mRNA in fibroblasts from human elderly donors and in fibroblasts of old mice, stimulated by mechanical stress ^{60, 66}. Human gingival fibroblasts from elderly donors presented greater production of IL-6 by the stimulus of LPS from *Campylobacter rectus* when compared to young donors ⁵⁵. IL-1 β is the most active cytokine in the process of bone resorption, being 15 times more potent than IL-1 α and 1000 times more potent than TNF- β ⁶⁷. Patients with severe periodontal disease (periodontal pocket >6mm) presented with a rate of IL-1 β twice times higher than that observed for patients with moderate (<4mm) and intermediate (4-6 mm) periodontal disease ⁶⁷. In the same way, another study demonstrated that patients with periodontal bone loss had more IL-1 β in the gingival fluid when compared to periodontal patients without bone loss ($P < 0.0001$) ⁶⁸. The levels of IL-1 α , IL-1 β , plaque accumulation, gingival fluid, and gingival inflammation in young adults (20-22 years) and elderly (61-65 years) were compared with experimental periodontal disease. Levels of IL-1 β in the gingival fluid were significantly higher in elderly, with a progressive increase until the 21st day of oral hygiene suspension, while levels of IL-1 α were similar for both groups. In the elderly there was also an increase in plaque accumulation, gingival fluid, and clinical signs of inflammation ⁶⁹.

The pattern of IL-1 β and IL-6 secretion in periodontal disease in menopausal patients was analyzed ³⁴. The results demonstrated that menopausal patients that were not in hormone reposition therapy presented higher rates of IL-1 β ($p < 0.0004$) and IL-6 ($p > 0.05$) than patients under hormonal treatment. IL-6 has an important role in the osseous lyses in periodontitis. It acts stimulating the growth and proliferation of osteoclast precursors and there is evidence that it is an extracellular messenger signaling osteoblast resorption for the osteoclast ⁵⁵. A greater concentration of IL-1 β e IL-8 in patients with estrogen deficiency compared to untreated patients without estrogen deficiency was also observed ⁷⁰. The hypothesis that the greater liberation of IL-1 β by older periodontal ligament cells may represent an important factor for the greater rate of alveolar bone resorption in elderly patients has been proposed ^{54, 60, 66}.

Periodontal ligament fibroblasts also express osteoblastic phenotypes, such as the production of bone-like matrix proteins, high alkaline phosphatase activity, and the formation of calcified nodules ⁷¹. In addition, human periodontal ligament cells express and secrete osteoprotegerin (OPG) ⁷² and receptor activator of NF-kappa B ligand (RANKL) such as osteoblasts, suggesting that the periodontal ligament plays a role in alveolar bone metabolism ⁷³.

RANKL, a member of tumor necrosis factor (TNF) ligand family, is expressed on osteoblast/stromal cell membranes. This ligand binds to the receptor activator of NF-kappa B (RANK), which is a receptor on the membrane of osteoclasts and mononuclear pre-osteoclasts, and induces osteoclast differentiation and activity^{74, 75}. In contrast, OPG is known to inhibit osteoclast differentiation and activity by interrupting the interaction between RANKL and its receptor, RANK, by binding to RANKL as a decoy receptor with higher affinity than RANK⁷⁶.

RANKL expressed on gingival or synovial fibroblasts may direct macrophages present in connective tissue and monocytes recruited from blood to differentiate into osteoclasts, which leads to bone destruction in periodontitis or arthritis. RANKL is expressed also on activated T-cells^{73, 75}. RANKL promoted the survival of mature dendritic cells and enhances the ability of these cells to stimulate T-cell proliferation in a mixed leukocyte reaction. In addition, RANKL induced the production of proinflammatory cytokines (IL-1 and IL-6) and cytokines that direct differentiation of T- cells, such as IL-12 and IL-15 from dendritic cells⁷⁷. In periodontitis, LPS, which is a combination of lipid and polysaccharide, is a component of the outer membrane of gram-negative bacteria⁵⁶. It has been reported that toll-like-receptor-4 (TLR4), a protein that in humans is encoded by the TLR4 gene and it detect lipopolysaccharide from Gram-negative bacteria, is essential for the response to LPS⁷⁸. Human periodontal ligament fibroblasts express TLR4, suggesting that LPS directly acts on these cells. LPS interacts with endotoxin and CD14 (endotoxin receptor) to present LPS to TLR-4, which activates inflammatory gene expression through NF-kappa-B. CD14 is a critical receptor for LPS because monoclonal antibodies directed against CD14 can inhibit the biological effects induced both *in vitro* and *in vivo* by LPS⁷⁹.

LPS induces inflammatory cytokines, such as interleukin-1 beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α), in macrophages and neutrophilic leukocytes that infiltrate areas infected with bacteria, and in periodontal ligament fibroblasts⁸⁰. These cytokines are thought to play an important role in the pathogenesis of periodontitis because they cause inflammation and destruction of periodontal tissue and resorption of alveolar bone by various biological mechanisms. IL-1 and TNF- α induce bone resorption by acting both directly and indirectly on osteoclasts⁸¹.

The effects of LPS on OPG and RANKL expression in human periodontal ligament fibroblasts (HPLF) were reported. These suggest that LPS stimulates both OPG and RANKL expression in HPLF by up-regulating IL-1 β and TNF- α ⁸².

All of these studies related to the dynamics of the inflammatory process allow a better understanding of the interaction of immunosenescence on cells periodontal. However, continuous research on this subject should be held.

5. Ageing as a risk factor for periodontal disease

The age-related changes in the periodontal tissues show that increasing age could potentially be a risk factor for periodontal disease⁸³. Some moderate loss of periodontal attachment and alveolar bone is associated with age, but age alone in a healthy adult does not lead to a critical loss of periodontal support. Although moderate loss of alveolar bone and periodontal attachment is common in the elderly, severe periodontitis is not a natural consequence of ageing^{3, 83}. Clinical attachment level and bone loss are irreversible measures of prior disease experience. Cross-sectional studies measuring disease experience demonstrated more attachment loss and alveolar bone loss among older age groups than other groups³.

Longitudinal studies addressing potential relationships between age and attachment loss or bone loss showed statistically significant relationships between age and incidence of periodontal disease ⁸⁴⁻⁸⁶. However, this age-associated increase in risk may not be linear, since some studies show no significant differences within age groups above 65 years ⁸⁷⁻⁹¹.

The main issue related of this fact is the magnitude of any increase in risk. Studies that demonstrate statistically significant associations do not necessarily indicated that these will lead to severe clinical outcome for older adults ⁹². A 28-year follow-up study reported an odds ratio of 10.4 for people aged 36-50 years compared with people aged 5-15 years. While this result is comparable in magnitude with other clinically important risk factors (smoking odds ratio in the same study was 14), it corresponds to a mean increase in clinical attachment level of only 1.34 mm over 28 years ⁸⁵.

This level of increased risk probably is not sufficient, alone, to cause tooth loss. Consequently, periodontal disease may be considered as time-associated, and ageing itself appears to be responsible for some attachment and bone loss, it is of a magnitude that is unlikely to have a clinical significance ^{83, 92}. This fact is influenced by multiple factors that have been found to be associated with the prevalence and incidence of the periodontal disease ⁸⁸.

6. Clinical Implications on ageing

Periodontal disease is not thought to be the major cause of tooth loss among adults, the loss of teeth in epidemiological studies usually induce an underestimate of periodontal disease incidence, since attachment loss tends to be greater in teeth that are extracted (whether or not the extraction is due to periodontal disease), and such teeth cannot be measured in follow-up studies ⁹².

Although longitudinal studies showed moderate levels of attachment loss in a high percentage of middle-aged and elderly subjects, severe loss is confined to a minority. Approximately one-fifth of older patients have experienced more generalized severe loss. The rate is higher in the oldest subjects ⁹². The habitual observation in elderly populations is some loss of periodontal attachment and alveolar bone, but age alone in a healthy adult does not lead to a critical loss of periodontal support ³.

The most important clinical conclusion to draw from the longitudinal studies concerning the effects of aging is that increased age poses some increased risk for periodontal loss, but the amount of loss due to age alone is probably consistent with "successful aging" rather than accelerated pathological processes ⁹². Really, it is probably appropriate to view the aging-periodontitis association as a rationale for redefining appropriate endpoints of periodontal therapy, such that the objective of treatment is to maintain a functioning dentition rather than a perfect level of periodontal attachment. Models for decision making regarding periodontal treatment needs in elderly have been proposed. Age-related thresholds could be used to decide on appropriate levels of therapy ^{92, 93}.

Recognition of the dental needs of this special category of the population compels us to bear the responsibility of treating them now and in the future. One of the major criteria of successful ageing is to maintain a natural, healthy, functional dentition throughout life, including all the social and biological benefits, such as aesthetics and comfort, and the ability to chew, taste and speak. However, the oral health of elderly people is far from optimal. The demand for treatment is much lower than the need. In the future the elderly will retain their natural dentition and more teeth per individual will be present ⁹⁴. This fact can result in an increase in periodontal disease expectancy among the dentate elderly ².

Certainly, in a short time, the trends for oral health care will be change. The new elderly populations will be more critical and more demanding for oral health care services than current elderly population. The dental profession must be aware of these trends which should be reflected in undergraduate and postgraduate dental education ⁹⁴. In worldwide, some countries recognized the Geriatric Dentistry as a new specialty into the dentistry ⁹⁵ or included specific dental programs for elderly treatment and research ⁹⁶. If as an specific specialty or developed by a established specialty, such as the Periodontics, periodontal care services for elderly people must show proficient dental professionals, including knowledge about the ageing interactions on periodontal treatment.

7. Conclusions

In conclusion, ageing alone leads to no critical loss of the periodontal attachment in the healthy elderly. The effects of ageing human on periodontal tissues were based on biomolecular changes of the cells of periodontium that exacerbate bone loss in elderly patients with periodontitis ⁸³.

These effects may be associated with: 1) alterations in differentiation and proliferation of osteoblasts and osteoclasts; 2) an increase in periodontal cell response to the oral microbiota and mechanical stress leading to the secretion of cytokines involved in bone loss; and 3) systemic endocrine alterations in the elderly ⁸³.

8. References

- [1] Sternberg SA, Gordon M. Who are older adults? Demographics and major health problems. *Periodontol* 2000. 1998; 16: 9-15.
- [2] Ellen RP. Periodontal disease among older adults: what is the issue? *Periodontol* 2000. 1998; 16: 7-8.
- [3] Burt BA. Periodontitis and ageing: reviewing recent evidence. *J Am Dent Assoc*. 1994; 125: 273-9.
- [4] Mombelli A. Ageing and the periodontal and peri-implant microbiota. *Periodontol* 2000. 1998; 16: 44-52.
- [5] Savitt ED, Kent RL. Distribution of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* by subject age. *J Periodontol*. 1991; 62: 490-494.
- [6] Abiko Y, Shimizu N, Yamaguchi M, Suzuki H, Takiguchi H. Effect of ageing on functional changes of periodontal tissue cells. *Ann Periodontol*. 1998; 3: 350-369.
- [7] Miura S, Yamaguchi M, Shimizu N, Abiko Y. Mechanical stress enhances expression and production of plasminogen activator in ageing human periodontal ligament cells. *Mech Ageing Dev*. 2000; 112: 217-231.
- [8] Beck JD, Koch G, Rozier RG, Tudor GE. Prevalence and risk indicators for periodontal attachment loss in a population of older community-dwelling blacks and whites. *J Periodontol*. 1990; 61: 521-528.
- [9] Van der Velden U. Effect of age on the periodontium. *J Clin Periodontol*. 1984; 11: 281-294.
- [10] Hou LT, Yaeger JA. Cloning and characterization of human gingival and periodontal ligament fibroblasts. *J Periodontol*. 1993; 64: 1209-1218.
- [11] Dumas M, Chaudagne C, Bont F, Meybeck A. In vitro biosynthesis of type I and III collagens human dermal fibroblasts from donors of increasing age. *Mech Ageing Dev*. 1994; 73: 179-187.

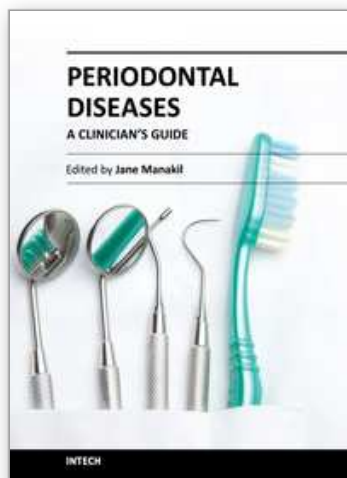
- [12] Lee W, McCulloch CA. Deregulation of collagen phagocytosis in ageing human fibroblasts: effects of integrin expression and cell cycle. *Exp Cell Res.* 1997; 237: 383-393.
- [13] Takatsu M, Uyeno S, Komura J, Watanabe M, Ono T. Age-dependent alterations in mRNA level and promoter methylation of collagen alpha1(I) gene in human periodontal ligament. *Mech Ageing Dev.* 1999; 110: 37-48.
- [14] Sismey-Durrant HJ, Hopps RM. Effect of lipopolysaccharide from *Porphyromonas gingivalis* on prostaglandin E2 and interleukin-1 β release from rat periosteal and human gingival fibroblasts in vitro. *Oral Microbiol Immunol.* 1991; 6: 378-380.
- [15] Johnson BD, Page RC, Narayanan AS, Pieters HP. Effects of donor age on protein and collagen synthesis in vitro by human diploid fibroblasts. *Lab Invest.* 1986; 55: 490-496.
- [16] Bartold PM, Boyd RR, Page RC. Proteoglycans synthesized by gingival fibroblasts derived from human donors of different ages. *Aust N Z J Med.* 2000; 30: 209-214.
- [17] Somerman MJ, Young MF, Foster RA, Moehring JM, Imm G, Sauk JJ. Characteristics of human periodontal ligament cells in vitro. *Arch Oral Biol.* 1990; 35: 241-247.
- [18] Groessner-Schreiber B, Neubert A, Muller WD, Hopp M, Griepentrog M, Lang KP. Osteoclast recruitment in response to human bone matrix is age related. *Mech Ageing Dev.* 1992; 62: 143-154.
- [19] Nishimura F, Terranova VP, Braithwaite M, Orman R, Ohyama H, Mineshiba J, et al. Comparison of in vitro proliferative capacity of human periodontal ligament cells in juvenile and aged donors. *Oral Dis.* 1997; 3: 162-166.
- [20] Asahara Y, Nishimura F, Arai H, Kurihara H, Takashiba S, Murayama Y. Chemotactic response of periodontal ligament cells decreases with donor age: association with reduced expression of c-fos. *Oral Dis.* 1999; 5: 337-343.
- [21] Sawa Y, Phillips A, Hollard J, Yoshida S, Braithwaite MW. Impairment of osteocalcin production in senescent periodontal ligament fibroblasts. *Tissue Cell.* 2000; 32: 198-204.
- [22] Tonna EA. Factors (ageing) affecting bone and cementum. *J Periodontol* 1976; 47: 267-280.
- [23] Roholl PJM, Blauw E, Zurcher C, Dormans J, Theuns HM. Evidence for a diminished maturation of pre-osteoblasts into osteoblast during ageing in rats: an ultrastructural analysis. *J Bone Miner Res.* 1994; 9: 355-366.
- [24] Sodek J, McKee MD. Molecular and cellular biology of alveolar bone. *Periodontol* 2000. 2000; 24: 99-126.
- [25] Selkoe DJ. Deciphering Alzheimer's disease: the amyloid precursor protein yields new clues. *Science* 1990; 248: 1058-1060.
- [26] McCord JM. Free radicals and inflammation: protection of synovial fluid by superoxide dismutase. *Science* 1974; 185: 529-531.
- [27] Inderjeeth CA, Nicklason F, Al-Lahham Y, Greenaway TM, Jones G, Parameswaran VV, et al. Vitamin D deficiency and secondary hyperparathyroidism: clinical and biochemical associations in older non-institutionalised Southern Tasmanians. *Aust N Z J Med* 2000; 30: 209-214.
- [28] Freaney R, McBrinn Y, McKenna MJ. Secondary hyperparathyroidism in elderly people: combined effect of renal insufficiency and vitamin D deficiency. *Am J Clin Nutr.* 1993; 58: 187-91.

- [29] Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* 2001; 22: 477-501.
- [30] Mohammad AR, Hooper DA, Vermilyea SG, Mariotti A, Preshaw PM. An investigation of the relationship between systemic bone density and clinical periodontal status in post-menopausal Asian-American women. *Int Dent J*. 2003; 53: 121-125.
- [31] Yoshihara A, Seida Y, Hanada N, Miyazaki H. A longitudinal study of the relationship between periodontal disease and bone mineral density in community-dwelling older adults. *J Clin Periodontol*. 2004; 31: 680-684.
- [32] Payne JB, Reinhardt RA, Nummikoski PV, Patil KD. Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. *Osteoporos Int*. 1999; 10: 34-40.
- [33] Tezal M, Wactawski-Wende J, Grossi SG, Ho AW, Dunford R, Genco RJ. The relationship between bone mineral density and periodontitis in postmenopausal women. *J Periodontol*. 2000; 71: 1492-1498.
- [34] Reinhardt RA, Payne JB, Maze CA, Patil KD, Gallagher SJ, Mattson JS. Influence of estrogen and osteopenia/osteoporosis on clinical periodontitis in postmenopausal women. *J Periodontol* 1999; 70: 823-828.
- [35] Ronderos M, Jacobs DR, Himes JH, Pihlstrom BL. Associations of periodontal disease with femoral bone mineral density and estrogen replacement therapy: cross-sectional evaluation of US adults from NHANES III. *J Clin Periodontol*. 2000; 27: 778-786.
- [36] Civitelli R, Pilgram TK, Dotson M, Muckerman J, Lewandowski N, Armamento-Villareal R, et al. Alveolar and postcranial bone density in postmenopausal women receiving hormone/estrogen replacement therapy: a randomized, double-blind, placebo-controlled trial. *Arch Intern Med*. 2002; 162: 1409-1415.
- [37] Krall EA, Wehler C, Garcia RI, Harris SS, Dawson-Hughes B. Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am J Med*. 2001; 111: 452-456.
- [38] Biscup P. Risks and benefits of long-term hormone replacement therapy. *Am J Health Syst Pharm*. 2003; 60: 1419-1425.
- [39] Franceschi C, Valensin S, Fagnoni F, Barbi C, Bonafè M. Biomarkers of immunosenescence within an evolutionary perspective: the challenge of heterogeneity and the role of antigenic load. *Exp Gerontol*. 1999; 34: 911-921.
- [40] Malaguarnera L, Ferlito L, Imbesi RM, Gulizia GS, Di Mauro S, Maugeri D, Malaguarnera M, Messina A. Immunosenescence: a review. *Arch Gerontol Geriatr* 2001; 32:1-14.
- [41] Aspinall R, Andrew D. Thymic involution in aging. *J Clin Immunol* 2000; 20:250-256.
- [42] Tosi P, Kraft R, Luzi P, Cintonino M, Fankhauser G, Hess MW, et al. Involution patterns of the human thymus. I Size of the cortical area as a function of age. *Clin Exp Immunol*. 1982; 47: 497-504.
- [43] Douek DC, Koup RA. Evidence for thymus function in the elderly. *Vaccine* 2000; 18: 1638-1641.
- [44] Straub RH, Cutolo M, Zietz B, Scholmerich J. The process of aging change interplay of the immune, endocrine and nervous systems. *Mech Ageing Dev*. 2001; 122: 1591-1611.
- [45] Gavazzi G, Krause KH. Aging and infection. *Lancet Infect Dis*. 2002; 2: 659-666.

- [46] Caruso C, Di Lorenzo G, Modica MA, Candore G, Portelli MR, Crescimanno G, Ingrassia A, Sangiorgi GB, Salerno A. Soluble interleukin-2 receptor release defect in vitro in elderly Subjects. *Mech Ageing Dev.* 1991; 59: 27-35.
- [47] McArthur WP. Effect of ageing on immunocompetent and inflammatory cells. *Periodontol 2000.* 1998; 16: 53-79.
- [48] Rink L, Cakman I, Kirchner H. Altered cytokine production in the elderly. *Mech Ageing Dev.* 1998; 102: 199-209.
- [49] Pawelec G, Barnett Y, Forsey R, Frasca D, Globerson A, McLeod J, et al. T cells and ageing. *Front Biosci.* 2002; 7: 1056-1183.
- [50] Burns EA, Leventhal EA. Ageing, immunity, and cancer. *Cancer Control.* 2000; 7: 513.
- [51] Fransson C, Berglundh T, Lindhe J. The effect of age on the development of gingivitis. Clinical, microbiological and histological findings. *J Clin Periodontol.* 1996; 23: 379-385.
- [52] Fransson C, Mooney J, Kinane DF, Berglundh T. Differences in the inflammatory response in young and old human subjects during the course of experimental gingivitis. *J Clin Periodontol.* 1999; 26: 453-460.
- [53] Mochizuki K, Yamaguchi M, Abiko Y. Enhancement of LPS-stimulated plasminogen activator production in aged gingival fibroblasts. *J Periodontal Res.* 1999; 34: 251-260.
- [54] Okamura H, Yamaguchi M, Abiko Y. Enhancement of ipopolysaccharides-stimulated PGE2 and IL-1 β production in gingival fibroblast cells from old rats. *Exp Gerontol.* 1999; 34: 379-392.
- [55] Ogura N, Matsuda U, Tanaka F, Shibata Y, Takiguchi H, Abiko Y. In vitro senescence enhances IL-6 production in human gingival fibroblasts induced by ipopolysaccharides from *Campylobacter rectus*. *Mech Ageing Dev.* 1996; 87: 47-59.
- [56] Ogawa T, Uchida H, Amino K. Immunobiological activities of chemically defined lipid A from lipopolysaccharides of *Porphyromonas gingivalis*. *Microbiology.* 1994; 140: 1209-1216.
- [57] Kinnby B. The plasminogen activating system in periodontal health and disease. *Biol Chem.* 2002; 383: 85-92.
- [58] Schmid J, Cohen RL, Chambers DA. Plasminogen activator in human periodontal health and disease. *Arch Oral Biol.* 1991; 36: 245-50.
- [59] Yin X, Bunn CL, Bartold PM. Detection of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 2(PAI-2) in gingival crevicular fluid from healthy, gingivitis and periodontitis patients. *J Clin Periodontol.* 2000; 27: 149-156.
- [60] Shimizu N, Goseki T, Yamaguchi M, Iwasawa T, Takiguchi H, Abiko Y. In vitro cellular ageing stimulates interleukin-1 β production in stretched human periodontal-ligament-derived cells. *J Dent Res.* 1997; 76: 1367-1375.
- [61] Takiguchi H, Yamaguchi M, Mochizuki K, Abiko Y. Effect of in vitro ageing on *Campylobacter rectus* lipopolysaccharide-stimulated PGE2 release from human gingival fibroblasts. *Oral Dis.* 1996; 2: 202-209.
- [62] Ohzeki K, Yamaguchi M, Shimizu N, Abiko Y. Effect of cellular ageing on the induction of cyclooxygenase-2 by mechanical stress in human periodontal ligament cells. *Mech Ageing Dev.* 1999; 108: 151-163.
- [63] Lader CS, Flanagan AM. Prostaglandin E2, interleukin 1 α , and tumor necrosis factor- α increase human osteoclast formation and bone resorption in vitro. *Endocrinology.* 1998; 139: 3157-3164.

- [64] Nakashima K, Roehrich N, Cimasoni G, Gazi MI, Cox SW, Clark DT, et al. Characterization of protease activities in *Capnocytophaga* spp., *Porphyromonas gingivalis*, *Prevotella* spp., *Treponema denticola* and *Actinobacillus actinomycetemcomitans*. *Oral Microbiol Immunol*. 1997; 12: 240-248.
- [65] Tsai CC, Hong YC, Chen CC, Wu YM. Measurement of prostaglandin E2 and leukotriene B4 in the gingival crevicular fluid. *J Dent*. 1998; 26: 97-103.
- [66] Shimizu N, Yamaguchi M, Uesu K, Goseki T, Abiko Y. Stimulation of prostaglandin E2 and interleukin-1beta production from old rat periodontal ligament cells subjected to mechanical stress. *J Gerontol A Biol Sci Med Sci*. 2000; 55: B489-95.
- [67] Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature*. 1986; 319: 516-518.
- [68] Faizuddin M, Bharathi SH, Rohini NV. Estimation of interleukin-1beta levels in the gingival crevicular fluid in health and in inflammatory periodontal disease. *J Periodontol Res*. 2003; 38: 111-114.
- [69] Tsalikis L, Parapanisiou E, Bata-Kyrkou A, Polymenides Z, Konstantinidis A. Crevicular fluid levels of interleukin-1alpha and interleukin-1beta during experimental gingivitis in young and old adults. *J Int Acad Periodontol*. 2002; 4: 5-11.
- [70] Payne JB, Reinhardt RA, Masada MP, DuBois LM, Allison AC. Gingival crevicular fluid IL-8: correlation with local IL-1 beta levels and patient estrogen status. *J Periodontol Res*. 1993; 28: 451-453.
- [71] Chien HH, Lin WL, Cho MI. Expression of TGF-beta isoforms and their receptors during mineralized nodule formation by rat periodontal ligament cells in vitro. *J Periodontol Res*. 1999; 34: 301-309.
- [72] Wada N, Maeda H, Tanabe K, Tsuda E, Yano K, Nakamuta H, et al. Periodontal ligament cells secrete the factor that inhibits osteoclastic differentiation and function: the factor is osteoprotegerin/osteoclastogenesis inhibitory factor. *J Periodontol Res*. 2001; 36: 56 - 63.
- [73] Kanzaki H, Chiba M, Shimizu Y, Mitani H. Dual regulation of osteoclast differentiation by periodontal ligament cells through RANKL stimulation and OPG inhibition. *J Dent Res*. 2001; 80: 887- 891.
- [74] Lacey DL, Timms E, Tan H-L, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; 93: 165-176.
- [75] Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, et al. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. *Biochem Biophys Res Commun* 1998; 253: 395- 400.
- [76] Yasuda H, Shima N, Nakagawa N, Mochizuki S, Yano K, Fujise N, et al. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology* 1998; 139: 1329-1337.
- [77] Park HJ, Park OJ, Shin J. Receptor activator of NF-kappaB ligand enhances the activity of macrophages as antigen presenting cells. *Exp Mol Med*. 2005; 37: 524-532.

- [78] Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. *Immunity* 1999; 11: 443-51.
- [79] Hatakeyama J, Tamai R, Sugiyama A, Akashi S, Sugawara S, Takada H. Contrasting responses of human gingival and periodontal ligament fibroblasts to bacterial cell-surface components through the CD14/Toll-like receptor system. *Oral Microbiol Immunol* 2003; 18: 14-23.
- [80] Miyauchi M, Sato S, Kitagawa S, Hiraoka M, Kudo Y, Ogawa I, et al. Cytokine expression in rat molar gingival periodontal tissues after topical application of lipopolysaccharide. *Histochem Cell Biol* 2001; 116: 57-62.
- [81] Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, et al. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J Exp Med* 2000; 191: 275- 86.
- [82] Wada N, Maeda H, Yoshimine Y, Akamine A. Lipopolysaccharide stimulates expression of osteoprotegerin and receptor activator of NF-kappa B ligand in periodontal ligament fibroblasts through the induction of interleukin-1 beta and tumor necrosis factor-alpha. *Bone* 2004; 35: 629-35.
- [83] Huttner EA, Machado DC, de Oliveira RB, Antunes AG, Hebling E. Effects of human aging on periodontal tissues. *Spec Care Dentist*. 2009; 29:149-55.
- [84] Papapanou PN; Wennström JL; Grondahl K. A 10-year retrospective study of periodontal disease progression. *J Clin Periodontol* 1989; 16: 403-411.
- [85] Ismail AI; Morrison EC; Burt BA; Cafesse RG; Kavanagh MT. Natural history of periodontal disease in adults: findings from the Tecumseh Periodontal Study, 1959-87. *J Dent Res* 1990; 69: 430-435.
- [86] Haffajee A; Socransky SS; Lindhe J; Kent RL; Okamoto H; Yoneyama T. Clinical risk indicators for periodontal attachment loss. *J Clin Periodontol* 1991; 18: 117-125.
- [87] Albandar JM. A 6-year study on the pattern of periodontal disease progression. *J Clin Periodontol* 1990; 17: 467-471.
- [88] Grbic JT; Lamster IB; Celenti RS; Fine JB. Risk indicators for future clinical attachment loss in adult periodontitis: patient variables. *J Periodontol* 1991; 62: 322-329.
- [89] Brown LF; Beck JD; Rozier RG. Incidence of attachment loss in community-dwelling older adults. *J Periodontol* 1994; 65: 316-323.
- [90] Beck JD; Koch GG. Incidence of attachment loss over 3 years in older adults - new and progressing lesions. *Community Dent Oral Epidemiol* 1995; 23: 291-296.
- [91] Ship JA; Beck JD. Ten-year longitudinal study of periodontal attachment loss in healthy adults. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; 81: 281-290.
- [92] Locker D, Slade GD, Murray H. Epidemiology of periodontal disease among older adults: a review. *Periodontol* 2000. 1998; 16: 16-33.
- [93] Wennstrom JL, Papapanou PN, Grondahl K. A model for decision making regarding periodontal treatment needs. *J Clin Periodontol* 1990; 17: 217-222.
- [94] Kalk W, de Baat C, Meeuwissen JH. Is there a need for gerodontology? *Int Den J*. 1992; 42: 209-16.
- [95] Hebling E, Mugayar L, Dias PV. Geriatric dentistry: a new specialty in Brazil. *Gerodontol*. 2007; 24: 177-80.
- [96] Ettinger RL. The development of geriatric dental education programs in Canada: an update. *J Can Dent Assoc* 2010; 76:a1.



Periodontal Diseases - A Clinician's Guide

Edited by Dr. Jane Manakil

ISBN 978-953-307-818-2

Hard cover, 368 pages

Publisher InTech

Published online 03, February, 2012

Published in print edition February, 2012

"Periodontal diseases" is a web-based resource intended to reach the contemporary practitioners as well as educators and students in the field of periodontology. It is fully searchable and designed to enhance the learning experience. Within the book a description is presented of the current concepts presenting the complex interactions of microbial fingerprint, multiple genotypes, and host modulations. In addition, an overview is given of the clinical outcome of the disease's progression, as influenced by the epigenetic factors. Emerging concepts on periodontitis as a risk factor for various systemic diseases and as a bilateral modulating factor have been elucidated in detail as well.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Eduardo Hebling (2012). Effects of Human Ageing on Periodontal Tissues, Periodontal Diseases - A Clinician's Guide, Dr. Jane Manakil (Ed.), ISBN: 978-953-307-818-2, InTech, Available from:
<http://www.intechopen.com/books/periodontal-diseases-a-clinician-s-guide/effects-of-human-ageing-on-periodontal-tissues>

INTech
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen