Review Article

Extracellular Matrix Degradation and Tissue Remodeling in Periprosthetic Loosening and Osteolysis: Focus on Matrix Metalloproteinases, Their Endogenous Tissue Inhibitors, and the Proteasome

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The leading complication of total joint replacement is periprosthetic osteolysis, which often results in aseptic loosening of the implant, leading to revision surgery. Extracellular matrix degradation and connective tissue remodeling around implants have been considered as major biological events in the periprosthetic loosening. Critical mediators of wear particle-induced inflammatory osteolysis released by periprosthetic synovial cells (mainly macrophages) are inflammatory cytokines, chemokines, and proteolytic enzymes, mainly matrix metalloproteinases (MMPs). Numerous studies reveal a strong interdependence of MMP expression and activity with the molecular mechanisms that control the composition and turnover of periprosthetic matrices. MMPs can either actively modulate or be modulated by the molecular mechanisms that determine the debris-induced remodeling of the periprosthetic microenvironment. In the present study, the molecular mechanisms that control the composition, turnover, and activity of matrix macromolecules within the periprosthetic microenvironment exposed to wear debris are summarized and presented. Special emphasis is given to MMPs and their endogenous tissue inhibitors (TIMPs), as well as to the proteasome pathway, which appears to be an elegant molecular regulator of specific matrix macromolecules (including specific MMPs and TIMPs). Furthermore, strong rationale for potential clinical applications of the described molecular mechanisms to the treatment of periprosthetic loosening and osteolysis is provided.

1. Pathobiology of Periprosthetic Loosening Process

The total hip or knee replacement is an operation whereby the damaged cartilage and the subchondral sclerotic bone of the hip or knee joint are surgically replaced with artificial materials. The continuous improvement of the materials and the surgical techniques have given comfort to patients suffering from painful diseases of the joints, such as primary osteoarthritis and secondary ones caused by rheumatoid arthritis, posttraumatic conditions, congenital dysplasia or dislocation, and aseptic necrosis of the femoral head. After the improvement in prophylaxis against infection, aseptic loosening of endoprostheses represents the predominant

complication of this operation, which usually occurs during the second decade, after the primary arthroplasty. Although many reports have been published on the pathogenesis of periprosthetic loosening, the precise biological mechanisms responsible for this process have not yet been completely elucidated.

Wear-generated particular debris at the interface between implant components is associated with chronic inflammation and osteolysis, limits the lifespan of the implants, and is the main cause of initiating this destructive process. However, many other factors, such as cyclic loading or micromotion of the implants and hydrostatic fluid pressure, have also been implicated revealing the high heterogeneity in the histology of the tissue around the prosthesis [1]. Evidence in support

FIGURE 1: Schematic representation of periprosthetic loosening and osteolysis. Implant-derived wear debris induces an early inflammatory response from the resident or infiltrating macrophages in the periprosthetic tissue. Small particles are phagocytosed, whereas the larger induce fusion of macrophages and giant cell formation. Activated macrophages release proinflammatory cytokines, chemokines, and enzymes that recruit multiple cell types within periprosthetic tissue, which are further activated by the particles resulting in sustained inflammation, increased secretion of cytokines/chemokines/osteoclastogenic factors/MMPs/TIMPs, and osteolysis.

of the central role of wear debris in periprosthetic loosening and osteolysis includes the observations that osteolysis is correlated with higher wear rates [2] and that vast numbers of wear particles are found associated with the periprosthetic interfacial membrane removed during revision surgery [3–5]. Furthermore, experimental systems have demonstrated that particulate debris can induce osteolysis in a variety of animal models [6–12] and inflammatory responses in cultured macrophages [8, 13–17]. Wear debris may include particles from all the various components of the prosthesis (such as polyethylene, metal, and ceramic) as well as bone cement [18]. Since cellular responses are highly dependent upon the composition, size, and shape of particles, the type of prosthesis and bearing surface used may have a significant impact on the potential for development of osteolysis [19].

The release of implant-derived particles induces a cellular host response, which initially is taking place in the pseudocapsular tissue (PCT). This membranous tissue is formed postoperatively around the artificial joint and practically replaces the normal joint capsular tissue, which is usually removed during the primary joint replacement procedure. The most important and active cells in this tissue are macrophages and fibroblasts, which after their interaction with the wear debris produce most of the soluble chemical factors and mediators, which are going to be analyzed below. Additionally these soluble factors migrate through the joint fluid (pseudosynovial fluid, PSSF) in the layer between the implant and the bone (interface), where they continue their action, mainly affecting the bony tissue. Finally the fibrous interface tissue (IFT), between the prosthesis and the bone, is formed and this leads to failure of the implant, which becomes loose. The communication

of the interface layer with the space of the initial foreign body reaction is described as effective joint space, may result an early micromotion of the implant, and could be related to the surgical technique [20]. The interface tissue is heavily infiltrated with several different cell types, mainly macrophages, lymphocytes, fibroblasts, endothelial cells, and osteoclast precursors (OCPs)/osteoclasts. Beside enhanced and chronic inflammatory reactions in the periprosthetic region, the cellular recruitment to this region is promoted by induced chemokine expression [21–25]. Macrophages activation by phagocytosis of the wear debris particles, which are impervious to enzymatic degradation, has been shown to be the principle pathophysiologic mechanism in particle-induced periprosthetic osteolysis. Activated macrophages secrete proinflammatory and osteoclastogenic cytokines as well as proteolytic enzymes exacerbating the inflammatory response leading to activation of a periprosthetic osteolytic cascade (Figure 1). It is known that particles smaller than 8–10 μm are phagocytosed by macrophages, while bigger particles induce giant cell reaction and are associated with such cells [26]. However, it has been reported that contact between wear particles and macrophages without phagocytosis is also important for the signal transduction of cytokines and activation of macrophages [14].

The identity of the macrophage surface receptors responsible for recognition of the particles and the full repertoire of signaling cascades initiated or modified by particle binding remain poorly understood although macrophages are the best-characterized cellular target for particle action. One important consideration in determining the involvement of specific cell surface receptors is the extent to which different particles become opsonized with host serum proteins prior

to phagocytosis. There is evidence that polyethylene activates complement [27], and this would argue in favor of a role for complement receptors, such as complement receptor 3 (CR3), in particle uptake. Indeed, CR3 expressing phagocytes have been detected in granulomatous lesions associated with hip replacement [28]. An involvement of CR3 in particle action is also supported by observations that antibodies against CR3 reduce particle uptake [14] and that activation of this receptor mimics several aspects of downstream signaling by particles in that MAP kinases [29] and the transcription factors nuclear factor κ B (NF- κ B) and activator protein-1 (AP-1) are activated [14, 30], and production of proinflammatory cytokines [14] and chemokines [30] is elevated. By contrast, research on alveolar macrophage response to environmental particulate matter has implicated scavenger receptors (SRs), such as scavenger receptor A (macrophage receptor with collagenous structure; MARCO), in opsonin-independent uptake of titanium particles [31], suggesting that different particles may use different surface receptors. Accordingly, Rakshit and coworkers suggested the involvement of opsonization, complement, and integrin receptors, including CR3 and fibronectin receptors, in polymethylmethacrylate action, and an involvement of scavenger receptors (scavenger receptor A) in macrophages responses to titanium [32]. This would provide an intriguing explanation of the abilities of different types of wear debris to elicit particle type-specific responses in cultured macrophages. The concept that opsonization may differentially regulate uptake of different compositions of wear debris is also supported by observations that the spectra of adherent human serum proteins demonstrate a level of particle specificity [33].

Other cell types that are abundant within the periprosthetic tissue are fibroblasts and osteoclasts. Frequently, a proliferation of periprosthetic fibroblasts, which constitute a major source of proinflammatory and osteoclastogenic mediators [34–37], is accompanied by tissue hypervascularization. Periprosthetic fibroblasts exposed to wear and/or proinflammatory mediators are a major source of the receptor activator for nuclear factor κ B ligand (RANKL) required to drive osteoclastogenesis in patients with osteolysis (discussed below). Particles can also induce production in cultured fibroblasts of proinflammatory mediators, collagenases, and stromelysins [36, 37], which contribute to the development of osteolysis and chemokines, which promote the recruitment of increased numbers of osteoclast precursors to periprosthetic tissues. The final cellular consequence of particle action is an excess of osteoclast activity, which results in uncontrolled bone erosion. Osteoclasts, which are the unique cell type capable to resorb bone, are derived from circulating hematopoietic cells of the monocyte/macrophage lineage. Therefore, wear particles might increase osteoclast activity either by generation of functional osteoclasts from osteoclast precursor cells within the periprosthetic space or recruitment of osteoclast precursor cells from the blood or both [19]. However, it is not only an increased osteoclastic bone resorption due to particle exposure that can disrupt the balance in the bone remodeling process, but also a reduced bone formation caused by a direct negative impact of particles on osteoblasts [38]. As shown by Lochner and coworkers, wear particles

can alter the metabolism of human primary osteoblasts [39]. In particular, metallic particles in the wear debris of cemented hip endoprostheses can compromise the vitality and activity of bone cells and bone matrix. In consequence, this may lead to a reduction of implant integration strength. Osteoblasts are rather responsible for bone formation but can indirectly participate in bone degeneration by changing cell viability and expression of specific chemokines as well as directly by the secretion of preosteolytic mediators and specific proteinases.

Collectively, the extensive body of research on *in vitro* cellular responses to wear debris suggests that while an inflammatory response by macrophages is central to the development of periprosthetic osteolysis, the detailed nature of this response will vary based upon several parameters, including prosthetic type, patterns of wear, cellular cross-talk, host factors, and cell-associated/extracellular molecular effectors.

2. Matrix Metalloproteinases, Their Endogenous Tissue Inhibitors, and Cytokines/Chemokines in the Periprosthetic Extracellular Matrix

Extracellular matrix (ECM) degradation and connective tissue remodeling around implants have been considered as major biological events in the periprosthetic loosening. Critical mediators of wear particle-induced inflammatory osteolysis released by periprosthetic synovial cells (mainly macrophages) are inflammatory cytokines (such as tumor necrosis factor- α (TNF- α), interleukin- (IL-) 1β , IL-6, and IL-10), chemokines (monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α)), inflammatory enzymes (inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2)), and proteolytic enzymes, mainly matrix metalloproteinases (MMPs).

TNF- α , IL- 1β , and IL-6 are known to be important molecules involved in the foreign body reaction process, and their upregulation is considered to be a marker of inflammation. They are well recognized as key proinflammatory cytokines that provoke cellular proliferation, stimulate osteoclast formation, and increase bone resorption around prostheses [40–44]. In particular, TNF- α has a catabolic effect on bone. It can upregulate bone resorption in cultured mouse calvaria by a prostaglandin-independent mechanism and stimulates osteoblasts to produce osteoresorptive factors such as IL-6 and prostaglandin E_2 (PGE $_2$) [45, 46]. High levels of TNF- α have been detected in periprosthetic tissues of loose endoprostheses with focal osteolysis [47]. It has also been shown to exhibit a synergistic effect with titanium particles, when added in osteoblast culture [48]. IL- 1β induces differentiation and proliferation of osteoclasts as well as the production of MMPs and PGE $_2$ from fibroblasts and synovial cells [49, 50]. It also reduces the osteocalcin production by the osteoblasts [51]. According to Jiranek and coworkers, IL- 1β might play a significant role in the formation of IFT, because of its stimulatory activity on fibroblasts [52]. Kusano and coworkers have shown that IL- 1β augments bone

resorption in mouse calvaria culture *in vitro*, by inducing MMP-2, MMP-3, MMP-9, and MMP-13 production [53]. IL-6 is strongly implicated in bone catabolism. It is produced by the osteoblasts and induces bone resorption [54]. It also stimulates the formation of osteoclast-like cells in long-term human marrow cultures [55]. In periprosthetic tissues from loose orthopaedic implants with osteolysis, IL-6 levels are much higher than in tissues from loose implants without bone loss [47]. The role of prostaglandins in mediating pseudomembrane-associated bone resorption remains questionable. It is proposed, from *in vitro* studies, that prostaglandins play an important role in bone resorption [56]. Periprosthetic tissue, cultured in the presence of indomethacin, showed less bone resorptive capacity. Other investigators have shown that conditioned media from predialysed periprosthetic tissue cultures maintained their ability to cause bone resorption, indicating that the prostaglandins, removed by dialysis, had no effect whatsoever upon bone resorption [57]. Therefore, prostaglandins may be implicated in the loosening process through complex mechanisms involving interactions with MMPs and cytokines. On the other hand, IL-10 is synthesized by activated immune cells, in particular monocytes/macrophages, and has profound anti-inflammatory and immunoregulatory effects. This anti-inflammatory cytokine diminishes the expression of inflammatory mediators, inhibits antigen presentation, and induces expression of endogenous TNF- α inhibitors (soluble TNF receptors) to suppress the effects of proinflammatory cytokines in periprosthetic tissues [47, 58].

Chemokines play pivotal roles in the recruitment of inflammatory and immune cells subsequent to the development of periprosthetic inflammation following wear particle generation. MCP-1 and MIP-1 α are two chemokines involved in this adverse process by recruiting monocytes/macrophages and lymphocytes to the site around prostheses and play important roles in periprosthetic osteolysis [59–61]. Previous studies suggest that high levels of inflammatory enzymes, such as iNOS and COX-2, are also present in the tissues around prostheses and therefore may account for periprosthetic bone resorption [62]. Macrophages are the major inflammatory cells accounting for this response. iNOS is closely involved in regulating inflammatory responses and COX-2 is induced by many cytokines, such as TNF- α and IL-1 β , and the overexpression of these two enzymes plays a key role in chronic inflammatory diseases [63]. Furthermore, iNOS and COX-2, as well as TNF- α and IL-6, are inductive regulators of osteoclastogenesis [64].

A key role in periprosthetic ECM remodeling and destruction belongs to MMPs because of their ability to degrade in concert most extracellular matrix components, such as collagens, gelatin, elastin, laminin, fibronectin, or proteoglycan core proteins. MMPs contain four well-defined domains: a signal peptide, a propeptide with a conserved cysteine residue, a catalytic domain with a Zn-binding site, and a hemopexin-like domain at the COOH-terminal region, and they are frequently subgrouped based on substrate specificities and sequence characteristics. There are six main families of MMPs: collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3,

MMP-10, and MMP-11), matrilysins (MMP-7 and MMP-26), membrane-type MMPs (MT-MMPs: MMP-14, -15, -16, -17, -24, and -25), and other MMPs, which are not categorized in any of the previous groups (MMP-12, -19, -20, -21, -23, -27, and -28). The expression of MMPs is under tight control at the transcription level and their proteolytic activity is regulated posttranslationally in several ways [65]. MMPs are synthesized as zymogens, which are then activated extracellularly, with the exception of MMP-11 (stromelysin 3), MT-MMPs, MMP-21, MMP-23, and MMP-28. Although pro-MMPs can be activated *in vitro* by various proteolytic and nonproteolytic means, the *in vivo* activation mechanisms have not yet been completely clarified. Further, the proteolytic activity of MMPs is regulated by specific tissue inhibitors of metalloproteinases (TIMPs). Four TIMPs have been identified (named TIMP-1 to -4), which form high-affinity 1:1 noncovalent complexes with all active MMPs, thereby inhibiting their action. TIMPs inhibit all MMPs tested so far, but TIMP-1 is a poor inhibitor for MT3-MMP, MT5-MMP, and MMP-19. TIMP-3 has been shown to inhibit members of the ADAM (a disintegrin and metalloproteinase) family (ADAM-10, -12, and -17) and ADAMTSs (ADAM with thrombospondin motifs) (ADAMTS-1, -4, and -5). TIMP-1 inhibits ADAM-10. While TIMP-1-null mice and TIMP-2-null mice do not exhibit obvious abnormalities, TIMP-3 ablation in mice causes lung emphysema-like alveolar damage [66] and faster apoptosis of mammary epithelial cells after weaning [67], indicating that TIMP-3 is a major regulator of metalloproteinase activities *in vivo*. However, the functions of TIMPs go beyond the inhibition of MMPs and are also partakers in the activation and coactivation of others [68]. The balance between the levels of activated MMPs and free TIMPs determines in part the net MMP activity. In addition to regulating the MMPs, TIMPs have also been shown to have angiogenic and growth factor-like activities [69].

Numerous studies have demonstrated that specific MMPs and TIMPs are expressed in periprosthetic tissues and are critically involved in the bone resorption and subsequent implant failure (Tables 1 and 2). In a study conducted by Takei and coworkers, the mRNA expression patterns of 16 different types of MMPs in synovium-like interface tissues between bone and prosthesis of loose artificial hip joints were analyzed to evaluate which MMPs were present at the mRNA level and possibly contributed to periprosthetic loosening [70]. It was shown that periprosthetic tissues were characterized by highly elevated expression of MMP-1, MMP-9, MMP-10, MMP-12, and MMP-13; moderate expression of MMP-2, MMP-7, MMP-8, MMP-11, MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT4-MMP (MMP-17), and MMP-19; lower expression of MMP-3; and little significance of MMP-20. Quantitative analysis of mRNA expression of their endogenous inhibitors (TIMPs) in periprosthetic tissues showed a significant upregulation of TIMP-1, -2, and -3 mRNA expressions in contrast to the decreased levels of TIMP-4 [71]. On protein level, strong immunoreactivity was observed for the extracellular matrix metalloproteinase inducer (EMMPRIN/CD147) in the lining-like layers, sublining area, and vascular endothelium of synovium-like interface tissue around loosened prostheses.

TABLE 1: Matrix metalloproteinases (MMPs) in periprosthetic microenvironment (expression and/or activity: ↑ with bold data: high; ↑ without bold data: moderate).

MMPs	Substrates	Expression and/or activity in periprosthetic microenvironment [References]
<i>Collagenases</i>		
Contain hemopexin domain and peptide linking with catalytic domain	MMP-1 (interstitial collagenase; collagenase 1) MMP-8 (neutrophil collagenase; collagenase 2) MMP-13 (collagenase 3)	Collagen type I, III, V, VII, VIII, X, gelatin, IL-1 β , MMP-2, -9, fibronectin ↑MMP-1 [39, 58, 70, 72, 80, 82, 90] ↑MMP-8 [70] ↑MMP-13 [70, 82, 89]
<i>Gelatinases</i>		
High substrate specificity to native collagen and gelatin	MMP-2 (gelatinase A; 72 kDa metalloproteinase) MMP-9 (gelatinase B; 92 kDa metalloproteinase)	Collagen type IV, V, VII, X, proteoglycans, gelatin, elastin, laminin ↑MMP-2 [58, 70, 73, 74, 76, 77, 80, 90] ↑MMP-9 [58, 70, 76, 77, 80, 90, 91]
<i>Stromelysins</i>		
Metalloproteinases of stroma	MMP-3 (stromelysin 1) MMP-10 (stromelysin 2) MMP-11 (stromelysin 3)	Proteoglycans, fibronectin, laminin, elastin, gelatin, plasminogen, vitronectin, fibrinogen, fibrin, collagen type III, IV, V, antithrombin III, MMP-1, -2, -8, -9, -13 ↑MMP-3 [58, 70, 74, 80] ↑MMP-10 [70] ↑MMP-11 [70]
<i>Matrilysins</i>		
The smallest among MMPs, lack of hemopexin domain	MMP-7 (matrilysin, metalloendopeptidase) MMP-26 (matrilysin-2, endometase)	Collagen type IV, proteoglycans, glycoproteins, gelatin ↑MMP-7 [70]
<i>Membrane-type MMPs</i>		
(A) Transmembrane-type MMPs	MMP-14 (MT1-MMP) MMP-15 (MT2-MMP) MMP-16 (MT3-MMP) MMP-24 (MT5-MMP)	Collagen type I, II, III, gelatin, elastin, laminin, fibronectin, fibrin, proteoglycans, proMMP-2, proMMP-13 ↑MMP-14 [70, 73] ↑MMP-15 [70] ↑MMP-16 [70]
(B) GPI-anchored MMPs	MMP-17 (MT4-MMP) MMP-25 (MT6-MMP)	 ↑MMP-17 [70]
<i>Other MMPs</i>		
MMPs that are not categorized in any of the previous groups	MMP-12 (macrophage metalloelastase) MMP-19 MMP-20 (enamelysin) MMP-21, MMP-23 MMP-27, MMP-28	 ↑MMP-12 [70] ↑MMP-19 [70]

Moreover, double immunofluorescence labeling revealed EMMPRIN/MMP-1 double-positive cells in lining-like areas and the sublining area of interface tissue. These data indicated that EMMPRIN expression was upregulated in interface tissues, and that locally accumulated EMMPRIN may modulate MMP-1 expression [72].

In another study, Nawrocki and coworkers used immunohistochemistry (IHC) to identify the cells responsible for the synthesis of MMPs in the periprosthetic microenvironment [73]. MMP-2 (gelatinase A) and its activator MT1-MMP were strongly detected in macrophages and multinucleated giant cells in contact with polyethylene wear debris. Similar results have been also obtained by other IHC studies on MMP-2 in

this pathological process [58, 74, 75]. Indeed, these studies reported the expression of MMP-2, as well as those of other MMPs, such as MMP-9 and MMP-1 and, in a more restricted pattern, MMP-3, in macrophages, fibroblasts, and endothelial cells. The strong expression of MMP-2 and its activator MT1-MMP in phagocytic cells of periprosthetic samples suggests their contribution to aseptic loosening of prosthetic components. These data are supported by the observation that high levels of gelatinolytic activities were also previously detected in the same type of lesion [76–79]. Of particular interest was the colocalization of MMP-2, MT1-MMP, and TIMP-2 in the same cells [73]. The strong expression of TIMP-2 in interface tissue around implants was also reported by Ishiguro and

TABLE 2: Tissue inhibitors of metalloproteinases (TIMPs) in periprosthetic microenvironment (expression and/or activity: ↑ with bold data: high; ↓ without bold data: low).

TIMPs	Preferred MMP/ADAM/ADAMTS	Expression and/or activity in periprosthetic microenvironment [References]
TIMP-1	Most MMPs, ADAM-10 (inhibition). MT3-MMP, MT5-MMP, MMP-19 (weak inhibition)	↑TIMP-1 [71, 74, 76, 78, 80, 82]
TIMP-2	Most MMPs (inhibition). MMP-2 (activation)	↑TIMP-2 [71, 73, 76, 80, 82]
TIMP-3	Most MMPs, ADAM-10, -12, -17, and ADAMTS-1, -4, -5 (inhibition). MMP-2, MT3-MMP (activation)	↑TIMP-3 [71]
TIMP-4	Most MMPs (inhibition)	↓TIMP-4 [71]

coworkers [80]. These data support the concept of Strongin and coworkers, who postulated that proMMP-2 activation could be mediated by a trimolecular stoichiometric complex involving MMP-2, TIMP-2, and MT1-MMP [81]. More specifically, these authors demonstrated that the activated form of MT1-MMP acts as a cell surface TIMP-2 receptor. The MT1-MMP/TIMP-2 complex may in turn serve as a receptor for proMMP-2, leading to its processing into the active enzyme. Interestingly, the detection of a soluble type of MT1-MMP (~56 kDa) in synovial and pseudosynovial fluid of patients with rheumatoid arthritis, osteoarthritis, and loose arthroplasty endoprostheses has been previously reported, without clarifying the origin of this type or its activation state. It was proposed that this form was probably processed proteolytically from the transmembrane type of MT1-MMP [75]. A protein band of ~56 kDa was also detected in periprosthetic tissues extracts and pseudosynovial fluids from loose arthroplasty endoprostheses that was ascribed to a soluble form of MT1-MMP [82].

The contribution of different members of the MMP family in gelatinolytic and collagenolytic potential was evaluated by gelatin zymography, and the degradation of synthetic dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg (DNP-S) together with reverse phase high performance liquid chromatography, respectively [82]. Activated species of both MMP-1 and MMP-13 were identified in most periprosthetic tissues, which could be responsible for the detected DNP-S-degrading activity, while the gelatinases MMP-2 and MMP-9 did not contribute in this potential, since they mainly existed in complex with TIMP-2 and TIMP-1, respectively. These data indicated that MMP-1 and MMP-13 may play a key role in the degradation of periprosthetic ECM, since they degrade native type-I and type-III collagens. Moreover, they may directly contribute to bone resorption, by removing the osteoid layer from calcified bone, facilitating the osteoclastic bone resorption [83–85]. Accordingly, it has been previously reported that periprosthetic tissue extracts exhibited high TIMP-free collagenolytic activity although TIMP-1 and

TIMP-2 have been detected in periprosthetic tissues [78, 79]. TIMPs produced by pseudosynoviocytes may be released into synovial fluid to limit MMP proteolysis, but their localization far from local degradation sites leads to the hypothesis of a disruption of the MMP-TIMP balance in favor of MMPs surrounding wear particles.

Immunohistochemical study of the plasminogen activation system, which is closely associated with MMP activities, disclosed localization in periprosthetic tissues of urokinase plasminogen activator (uPA), uPA-receptor (uPAR), and tissue type plasminogen activator (tPA) in macrophages with phagocytosed metal, polyethylene, cement particles, or accompanying pieces of necrotic bone [86]. Plasminogen activator inhibitor-1 (PAI-1) staining was present in the neighboring areas that stained for uPA or tPA, but PAI-1 staining was also found overlapping and outside these areas. These findings suggest a role for the uPA/uPAR and PAI-1 in activation and focalization of extracellular matrix degradation in periprosthetic tissues. The expression of the plasminogen activation system by macrophages containing phagocytosed material suggests undegradable microdebris as a possible initiating and perpetuating stimulus for a proteolytic activation cascade, which may contribute to loosening of the prosthesis. In contrast to most ECM-degrading proteases, uPA has restricted substrate specificity. Although uPA best-documented proteolytic action is the conversion of inactive plasminogen to active plasmin, it has been also reported that it is able to activate the cell surface MT1-MMP proenzyme [87]. Like uPA, plasmin is also a serine protease but, in contrast to uPA, has broad substrate specificity. Apart from native collagen, plasmin can degrade most proteins present in the ECM. It can also activate the precursor forms of a number of MMPs, such as MMP-3, MMP-9, MMP-12, and MMP-13 [88]. Elevated protein levels of MMP-13, together with uPA and PAI-1 in periprosthetic pseudocapsular and interface tissues were also reported by Diehl and coworkers [89]. However, no significant correlation between the protein expression of these factors and years from arthroplasty to revision or to type of fixation (cemented versus cementless) was observed.

It should be noted that the physical characteristics of wear particles (size, shape, and sintering temperature) as well as their amounts in the periprosthetic tissues can modify the toxicity of the biomaterials and the production of cytokines, MMPs, and TIMPs by various cell types. For example, macrophages seemed to release MMPs (MMP-1, -2, and -9) in proportion to the amount of particulate debris at the prosthetic interface [90]. Laquerriere and coworkers demonstrated that sintering temperature (that modify crystal size and surface area) had little effect on MMPs and TIMPs production. Nonphagocytatable particles induced more MMP-9, although phagocytatable particles induced more IL-1 β release. The shape of the particles was the most important factor since needle-shaped particles induced the most significant upregulated expression of MMPs (mostly MMP-9) and IL-1 β [91]. In another study, human osteoblasts were incubated with particles experimentally generated in the interface between hip stems with rough and smooth surface finishings as well as different material compositions [39].

The results revealed distinct effects on the cytokine release of human osteoblasts towards particulate debris. Thereby, human osteoblasts released increased levels of IL-6 and IL-8 after treatment with metallic wear particles. The expression of VEGF was slightly induced by all particle entities at lower concentrations. Apoptotic rates were enhanced for osteoblasts exposed to all the tested particles. Furthermore, the *de novo* synthesis of type I collagen was reduced and the expression of MMP-1 was considerably increased. Therefore, by the secretion of degrading effectors, osteoblasts may actively contribute to matrix weakening.

3. Molecular Mechanisms Controlling the Periprosthetic Microenvironment: Implication of MMPs/TIMPs and an Emerging Role for Proteasome

A large body of studies reveals a strong interdependence of MMP expression and activity with the molecular mechanisms that control the composition and turnover of periprosthetic ECMs. MMPs can either actively modulate or be modulated by the molecular mechanisms that determine the debris-induced remodeling of the periprosthetic microenvironment (summarized in Figure 2). One likely mechanism whereby particulate debris may induce osteoclast generation and activation is an indirect one, mediated through the actions of proinflammatory mediators that can act on osteoclast precursors and, most importantly, modulate the RANKL/osteoprotegerin (OPG) ratio through actions on cells within the periprosthetic tissue. RANKL is a type II homotrimeric transmembrane protein, which is normally expressed on osteoblastic cell membrane but is also expressed by fibroblasts and activated T cells [92]. Binding of RANKL to RANK on preosteoclasts (OCPs) activates NF- κ B and Jun N-terminal kinases (JNKs) pathways to induce cell differentiation [93]. NF- κ B is likely the most notable transcription factor implicated in wear debris action. This protein complex, long known as a key regulator of inflammatory gene expression, is also emerging as an important player during osteoclastogenesis. Supporting evidence for a role of NF- κ B in periprosthetic osteolysis comes from observations that deficiency of NF- κ B in mice protects against titanium-induced calvarial osteolysis [94], and that inhibition of NF- κ B blocks wear debris induction of osteoclastogenesis *in vitro* [95, 96]. Osteoblasts also secrete OPG, a soluble decoy receptor for RANKL, which strongly binds to RANKL and effectively inhibits its activity on preosteoclasts differentiation and maturation [97]. OPG is a glycoprotein possessing 4 cysteine-rich domains at its Nterminus by which it binds to RANKL, whereas its C-terminus contains 22 homologous death domains of unknown function and a heparin binding domain by which the glycoprotein interacts with matrix macromolecules, such as glycosaminoglycans and proteoglycans. Importantly, any imbalance in the RANKL/OPG ratio impairs normal bone remodeling and evidence suggests a role of RANKL/OPG ratio in wear debris-induced osteolysis. In particular, it has been shown that RANKL blockade with OPG [98, 99] or RANK:Fc (RANKL antagonist consisting of the extracellular

region of RANK fused to the Fc portion of human IgG1), or by using mice genetically deficient in RANK prevents wear debris-induced osteolysis in the murine calvarial model [100]. Moreover, wear debris can increase the RANKL/OPG ratio in murine calvarial tissues [101], and several reports have identified elevated RANKL expression in IFTs [102–105]. However, the fact that several different cell types within the periprosthetic tissue are capable of RANKL expression, including osteoblasts, fibroblasts, T lymphocytes, and also macrophages and giant cells [102–106], makes the cellular basis for elevated RANKL expression very complicated.

Several MMPs are overexpressed and correlated with osteoclast differentiation, maturation and activation by interfering with the RANK/RANKL/OPG system in inflammation and cancer [107]. MMP-9 is likely to play an important role in the recruitment of osteoclasts at inflammatory and metastatic sites since the use of chemical inhibitors or antisense oligonucleotides against MMP-9 abrogated the recruitment of osteoclasts [108]. Franco and coworkers showed that doxycycline (Dox), which can suppress the enzyme activity of MMP-9 [109], as well as MMP-9 inhibitor (MMP-9 inhibitor I), downregulated the expression of RANKL-induced osteoclast maturation genes in conjunction with the suppression of RANKL-induced osteoclastogenesis [110]. These findings indicated that MMP-9 induced by RANKL plays a role as an upstream effector of osteoclast gene expression, and, as such, it may also be a regulator of osteoclastogenesis. Previous studies reported that MMP inhibitor (RP59794) [111] or MMP-9 gene knockout [112] reduced osteoclast migration, which results in reduction of the resorption process in the growth plate and, as a consequence, attenuated development of bone marrow cavity. However, since the latter studies treated the aspect of osteoclast migration, but not differentiation, the study by Franco and coworkers is the first to report the involvement of MMP-9 activity in RANKL-induced osteoclastogenesis. In another report, MMP-7 could solubilize RANKL in mouse models of prostate and breast cancer promoting osteoclast activation and osteolysis [113]. The limiting step in RANKL-dependent osteoclastogenesis is the contact of RANKL-expressing osteoblasts with RANK on the cell surface of osteoclasts. This limitation is prohibited by proteolytic cleavage of RANKL from the cell surface through the action of MMP-7 and cathepsin G. Importantly, it has been shown in tumor-induced bone disease that soluble RANKL retains its activity and is liberated at the tumor-bone interface promoting osteoclastogenesis without the necessity of direct interaction of osteoblast with osteoclasts [113–115].

Possible accumulation of cell membrane and matrix proteoglycans at the inflammatory periprosthetic ECM may also modulate the RANK/RANKL/OPG system through both MMP-independent and -dependent manners. For example, it has been shown that myeloma cells decrease OPG availability by internalizing it through binding to glycosaminoglycan side chains of surface syndecan-1 and degradation to lysosomes, thereby regulating its inhibitory effect on RANKL [116]. Moreover, shed syndecan-1 secreted by myeloma cells may also bind OPG [117] and block its inhibitory activity to RANKL triggering further osteoclast differentiation and activation. Syndecan ectodomain shedding is an important

regulatory mechanism, because it rapidly changes cell surface receptor dynamics and generates soluble ectodomains that can function as paracrine or autocrine effectors or competitive inhibitors. Strong evidence indicates the involvement of several MMPs in syndecan cleavage *in vitro* and *in vivo* [118]. Matrilysin (MMP-7) cleaves syndecan-1 [119], gelatinases MMP-2 and MMP-9 can cleave syndecans-1, -2, and -4 [120, 121], whereas the membrane-associated metalloproteinases MT1-MMP and MT3-MMP are known to cleave syndecan-1 [122]. Taken together, these data suggest a critical role of certain members of MMPs in interfering with the RANK/RANKL signaling axis by directly and/or indirectly regulating OPG and RANKL availability, thereby modulating osteoclast generation and activation within the periprosthetic tissue (Figure 2).

An important observation in several studies was that specific gene responses were induced in different cell types of the periprosthetic microenvironment by an initial and early particulate biomaterial-cell interaction. The differential gene expression indicated that particle-cell interactions activated specific signaling events and transcription factors. Vermes and coworkers have found that particles rapidly activated protein tyrosine phosphorylation and induced the nuclear transcription factor NF- κ B in osteoblasts [123]. The rapid kinetics of the activation suggested that the particles elicited signals before the phagocytosis process. Importantly, inhibition of NF- κ B function by either tyrosine kinase inhibitors or antioxidants reversed the suppressive effect of titanium particles on procollagen α [I] gene expression suggesting a functional relationship in osteoblasts between tyrosine phosphorylation, NF- κ B activation, and collagen gene expression. Thus, particle-cell interactions before their phagocytosis appear to initiate an intracellular tyrosine phosphorylation cascade that targets the nuclear activation of the inducible transcription factor NF- κ B.

A role for protein tyrosine kinases (PTKs) in regulation of the activation of MMPs/TIMPs in ion-induced activation of macrophages was suggested by Luo and coworkers [124]. In particular, cobalt (Co) and chromium (Cr) ions, two corrosion products found in the periprosthetic environment of metal-on-metal prostheses, were shown to upregulate MMP-1, TIMP-1, and cytokines (such as TNF- α) in cultures of human U937 macrophages. The inhibitory effect of genistein suggested the implication of PTKs in the induction of MMP-1 and TIMP-1 expressions by Co²⁺ and Cr³⁺ ions in macrophages, the most important cellular target of wear debris. Genistein, a soy isoflavonoid that is a natural broad spectrum PTK (such as EGFR, PDGFR, IGFR, Src) inhibitor, has been shown to regulate the transcription of several MMPs and their endogenous inhibitors (TIMPs) by breast cancer cells [125, 126]. Moreover, previous *in vitro* studies demonstrated that genistein downregulates the expression of vascular endothelial growth factor (VEGF), which is a major signaling protein that contributes to angiogenesis [127]. VEGF is produced by multiple cell types, including macrophages and osteoblasts [128, 129]. It exerts its biological activity by binding to two TK receptors, VEGF receptor-1 (VEGFR-1; Flt-1) and VEGFR-2 (Flk-1/KDR) [130]. VEGF

is actively involved in the process of inflammation, osteoclastogenesis, and bone resorption [131–133] and probably plays an important role in wear debris-induced inflammatory osteolysis since the periprosthetic tissues at the bone-implant interface show a high degree of vascularization [134]. Notably, Luo and coworkers showed a more potent effect of herbimycin A, Src kinase-specific inhibitor, on the expression of MMP-1 and TIMP-1 compared to genistein [124], providing strong evidence for a critical role of Src kinases in modulating the expression levels of MMP-1 and TIMP-1 in macrophages in the presence of Co²⁺ and Cr³⁺ ions (Figure 2). Other ions released from hip prostheses, such as titanium [135] and nickel [136], have been shown to stimulate TNF- α in a manner similar to Co and Cr, suggesting that other ions may also modulate tyrosine kinase activity probably affecting the amounts and activities of MMPs/TIMPs in periprosthetic ECMs.

Moreover, the adhesion of macrophages to phosphorylcholine-polymer coated surfaces stimulated the expression of MMP-1 and TIMP-1, suggesting that cell adhesion induced a remodeling of the macrophage ECM. The inhibition of the expression of these genes by genistein and herbimycin A suggested that PTKs were also implicated in this remodeling [124]. Interestingly, this kind of materials-stimulated expression of genes implicated in ECM remodeling was also observed in fibroblasts induced by three-dimensional collagen [137, 138]. In these studies, α 1 β 1 and α 2 β 1 integrins mediated the signals inducing downregulation of collagen gene expression and upregulation of MMP-1, respectively. Therefore, the potential impact of macrophage surface integrins-evoked signals on the periprosthetic microenvironment should be further investigated to better understand the cellular effects of particles liberated from the articular surface of prostheses (Figure 2).

One major part of the organism's first line of defense against infection is a family of pattern recognition receptors (PRRs) called the Toll-like receptors (TLRs). TLRs are transmembrane proteins found in various cells and recognize infectious and endogenous threats, so-called danger signals, which evoke inflammation and assist adaptive immune reactions. It has been suggested that TLRs play a role in periprosthetic tissues and arthritic synovium. Tamaki and coworkers found that peri-implant tissues were well equipped with TLRs and, in aseptic loosening, monocytes/macrophages were the main TLR-expressing cells [139]. This could lead to production of inflammatory cytokines and MMPs after phagocytosis of wear debris derived from an implant. A major conclusion of the study was that inflammatory cells in both aseptic and septic tissues were equipped with TLRs, providing them with responsiveness to both endogenous and exogenous TLR ligands. In this line, the high expression of TLRs in the periprosthetic tissues could be potentially important, as they can reflect occurrence of subclinical biofilms on the prosthetic surfaces. Activation of TLRs has been suggested to modulate the expression levels of certain MMPs but not TIMPs. In a recent study, Lisboa and coworkers showed that activation of TLR-2 and TLR-4, two TLR members expressed by a variety of human cells that participate in the recognition

the periprosthetic microenvironment. Therefore, the possibility of potentiation of MMPs activation concomitant with TLR activation in periprosthetic tissues needs to be further investigated (Figure 2).

Genetic variation may determine individual responses in terms of susceptibility to osteolysis and recovery. Expression

levels of the MMPs at both the mRNA and protein levels can be affected by the introduction or loss of transcription binding sites by single nucleotide polymorphisms (SNPs). SNPs are the most common sequence variation in the human genome and can affect coding sequences, splicing, or transcription regulation. In a case control, it was shown that a single-nucleotide polymorphism (SNP) of MMP-1 was highly associated with total hip replacement aseptic failure [142]. This SNP existed within a promoter region of the gene and as such may have a direct effect on the amount of gene expression (Figure 2). However, the mechanisms of MMP gene regulation are still not fully delineated, and it is likely that many more functionally important elements in their promoter regions are yet to be identified [143–145]. Moreover, investigation of SNPs in the TIMP genes would be a necessary complement for any study of MMP SNPs, given the evidence that the MMP-to-TIMP ratio plays a role in defining overall MMP activity.

In another line of research, Ortiz-Lazareno and coworkers found that the proteasome inhibitor MG-132 significantly diminished proinflammatory cytokines (TNF- α , IL-1 β , IL-6) release by U937 macrophages, whereas, induced a decrease in the membrane receptors TNF-R1 and IL-1R1 and an increase in the soluble receptors sTNF-R1 and sIL-1R1. However, MG-132 increased the IL-6R and decreased sIL-6R [146]. In another report, Mao and coworkers investigated the effects of Ti particles and the specific proteasome inhibitor bortezomib on the secretory profile of inflammatory cytokines, chemokines, and inflammatory enzymes in a murine macrophage cell line [147]. It was shown that Ti particles increased the production of TNF- α , IL-1 β , IL-6, IL-10, MCP-1, MIP-1 α , iNOS, and COX-2 in this cell line, while bortezomib inhibited the expression of all factors, except IL-10, in a time-dependent manner. Bortezomib, a potent, reversible and selective inhibitor of the chymotryptic activity of the proteasome, prevents the degradation of I κ B proteins, which mask the nuclear localization sequence of NF- κ B, therefore inhibiting the translocation of NF- κ B into the nucleus and further inhibiting the transcription and secretion of inflammatory mediators. It is known that NF- κ B regulates the transcription of a variety of genes of inflammatory cytokines (TNF- α , IL-1 β , IL-2, IL-6, and GM-CSF (granulocyte macrophage colony-stimulating factor)), chemokines (IL-8, MCP-1, and MIP-1 α), and inflammatory enzymes (iNOS and COX-2) [148]. Therefore, bortezomib may inhibit the proinflammatory factors mentioned earlier through inhibition of NF- κ B activity. Moreover, proteasome inhibition with bortezomib alters the binding of other transcription factors to the promoter region of several molecular effectors, thus modulating their expression levels [149]. The anti-inflammatory cytokine IL-10 induced by bortezomib inhibits TNF- α gene expression via inhibiting NF- κ B activity or by directly inhibiting TNF- α itself [150, 151]. It should be noted that bortezomib (as well as other proteasome inhibitors) exhibits beneficial effect on bone metabolism as it inhibits osteoclastic function and promotes osteoblastic activity by inhibiting NF- κ B activation induced by the RANK-RANKL signaling axis, which is the master regulator of differentiation and activation of osteoclasts [152–155].

The inhibitory effects of bortezomib and MG-132 on the secretion of inflammatory cytokines and their receptors by macrophages suggested the potential involvement of the proteasome pathway in periprosthetic loosening and osteolysis process. The proteasome is a major cellular protease complex that functions as the main driver of intracellular degradation of a wide variety of cellular proteins implicated in several physiological and pathological cellular functions [156]. Interestingly, the proteasome pathway controls via transcriptional and posttranslational mechanisms the concentration and turnover of several ECM macromolecules (including proteoglycans/glycosaminoglycans, MMPs/TIMPs, and collagens) [157]. Importantly, the proteasome provides a link between the regulation of extracellular proteolytic events to intracellular proteolysis by modulating MMP/TIMP expression and activity. In particular, it has been shown that proteasome blockade by proteasome inhibitors resulted in a marked modification of gene expression and activity of MMPs (upregulation of MMP-1, -3 and downregulation of MMP-2, -9) and TIMPs (downregulation of TIMP-1). Moreover, proteasome inhibition regulated also the synthesis and activity of other ECM constituents, such as TGF- β (downregulation), decorin (upregulation), and collagen type-I and type-IV (downregulation) [157]. Therefore, since matrix remodeling and degradation can be tightly regulated by proteasome activities, its modulation may be considered as a novel strategy to control the properties of periprosthetic ECMs as has been recently suggested for tumor microenvironment (Figure 2).

4. Potential Therapeutic Perspectives

Much progress has recently been made in understanding the molecular and cellular mechanisms whereby prosthetic wear debris can ultimately cause aseptic loosening and osteolysis. However, the complex nature of the interactions between wear particles and periprosthetic cells as well as the multiple intracellular signaling pathways activated by such interactions results in the reality that development of therapeutic approaches to the treatment of periprosthetic osteolysis is long overdue. In the following lines, we will try to address strong rationale for potential clinical applications of the described molecular mechanisms for periprosthetic loosening and osteolysis treatment.

The finding that proteasome inhibitors (i.e., bortezomib, MG-132) altered the macrophage secretory profile of inflammatory cytokines, chemokines, and inflammatory enzymes [146, 147], which play a key-role in the inflammatory response of periprosthetic tissue to wear debris, reveals a critical role for proteasome in the development of periprosthetic loosening and osteolysis. In a recent review, we have highlighted the novel approach of targeting the proteasome as a mechanism to control the synthesis and bioactivity of ECM effectors in tumors, since the proteasome appears to be an elegant molecular regulator of specific matrix macromolecules [157]. From the data described in the present review, proteasome signaling pathway emerges as a promising target to selectively regulate the synthesis and activity of inflammatory factors

(such as TNF- α , IL-1 β , IL-6, IL-8), their membrane receptors, and matrix degrading effectors (such as specific MMPs) in the periprosthetic microenvironment. To this aim, a promising agent is bortezomib, which exhibits multiple functions by interfering also with other intracellular signaling pathways such as the RANK-RANKL system thereby regulating new bone formation by both inducing osteoblastic function and inhibiting osteoclastogenesis. However, the elevated production of reactive oxygen species (ROS) by activated macrophages and osteoclasts in the presence of wear particles [158] should be considered in this context, since proteasome inhibitors have been also shown to induce ROS [159], which further attenuate the proteasomal system activation [160]. This proteasomal inhibition would potentially result in the accumulation of phosphorylated c-Jun and activation of AP-1 that ultimately induce MMP-1 and MMP-3 expression levels [160]. Therefore, proteasome inhibitors may have a synergistic effect with wear particles on ROS production and strongly induce the expression of specific MMPs within the periprosthetic microenvironment although their inhibitory effect on inflammatory cytokines and their receptors as well as other MMPs has been documented. Moreover, MG-132 has been found to significantly downregulate TIMP-1 expression in organ interface tissue cultures and primary IFT fibroblast cultures (Aletras and coworkers, unpublished data), which is in line with the findings of Fineschi and coworkers in dermal fibroblasts [161], revealing the complex and questionable role of proteasome in regulating distinct molecular effectors that would potentially be beneficial for periprosthetic osteolysis treatment. Therefore, the efficacy of proteasome inhibitors (such as bortezomib) to prevent periprosthetic loosening and osteolysis caused by implant-derived particles is an emerging concept and needs to be further investigated.

Taking under consideration these data, it should be investigated whether an alternative strategy associated with proteasome activation would be more beneficial in the treatment of periprosthetic loosening. Several activators of the proteasome, such as isoflavonoids, should be tested in order to reverse the effects on the expression levels of specific MMPs and TIMPs described previously, as a result of the reduced proteasome activity in IFT. Proteasome activation might be further induced by combined treatment with activators of nuclear factor erythroid 2-related factor 2 (Nrf2), such as sulforaphane [162]. Notably, Nrf2 upregulates the transcription of multiple antioxidant enzymes providing an effective means of reducing elevated ROS levels in IFT.

The observation that genistein and herbimycin A strongly attenuated the expression of MMP-1 and TIMP-1 by macrophages implied that tyrosine kinases play also an essential role in the signaling pathways regulating the remodeling of macrophage ECM in the periprosthetic microenvironment [124]. Therefore, PTKs (e.g., Src kinases) may serve as an additional target for selective inhibition of periprosthetic osteolysis. Importantly, proteasome is implicated in this process since it has been reported that herbimycin A targets the degradation of tyrosine kinases by the 20S proteasome [163]. Moreover, apart from its inhibitory action on PTKs, genistein was found to downregulate the expression of VEGF, a major

angiogenic factor in periprosthetic microenvironment. The interactive network of the VEGF/Flt-1 and RANKL/RANK pathways may play important roles in the initiation, progression, and resolution of aseptic loosening. In a study by Ren and coworkers, it was shown that VEGF may be actively involved in the regulation of RANK/RANKL gene expression, and that it exerted a regulatory effect on the development of particle-induced inflammatory osteoclastogenesis through its unique Flt-1, rather than Flk-1, receptor located on monocyte/macrophage cell lineages [164]. In particular, they found that treatment with R2/Fc (a VEGF neutralizing antibody) but not SU5416 (an Flk-1 receptor inhibitor) resulted in the inhibition of polyethylene particle-enhanced VEGF/Flt-1 signaling and inflammatory osteolysis by trapping VEGF in the periprosthetic milieu (Figure 2). Taken together, these findings provide the biological rationale for a combined VEGF/Flt-1- and RANKL/RANK-targeted treatment strategy, especially in the early stages of wear debris-induced inflammatory response. The fact that the RANK/RANKL/OPG system is of crucial importance for the development of periprosthetic osteolysis together with the finding that Dox inhibits RANKL-induced osteoclastogenesis by its inhibitory effect on MMP-9 enzyme activity [110] provides a reasonable rationale for a pharmaceutical advantage of tetracycline antibiotics against periprosthetic osteolysis. It should be noted that this class of antibiotics, including Dox, has been effectively utilized for the treatment of bone resorptive diseases because of their activity to suppress osteoclastogenesis induced by RANKL.

Given that excessive osteoclast activity represents the cellular endpoint of osteolysis, it is not surprising that the bisphosphonate class of osteoclast inhibitors have come in for much discussion as possible therapeutic agents for this disease. Again, however, despite promising results in animal models, there is no clinical evidence supporting the effectiveness of these drugs in the treatment of osteolysis patients. Alendronate inhibits wear debris-induced osteolysis in the rat loaded tibial implant model of osteolysis [165] and in a similar canine model [9] and is also effective in preventing osteolysis in the murine calvarial model [6]. A single dose of zoledronic acid administered directly after surgery also suppressed particle-induced osteolysis in mouse calvaria [166]. Bisphosphonates inhibit osteoclast formation by blocking the mevalonate pathway of isoprenoid biosynthesis. Their potential effect in periprosthetic osteolysis should be also considered with regard to their ability to inhibit the enzymatic activity of various MMPs. Certain bisphosphonates showed beneficial effects as a result of altering the expression pattern of MMPs/TIMPs by inhibiting and increasing the gene and protein expression of several MMPs and TIMPs, respectively, in breast cancer cells. In particular, it has been shown that zoledronic acid suppressed the expression of metalloproteinases MMP-2, -9, the membrane type MT1- and MT2-MMP, whereas it increased the expression of their endogenous tissue inhibitors [167].

Though not extensively studied, other mechanisms that should be further investigated with regard to their contribution to the remodeling of periprosthetic ECM include SNPs

of certain MMP/TIMP genes as well as the involvement of TLRs in periprosthetic inflammation. An SNP of MMP1 gene was highly associated with total hip replacement aseptic failure [142]. It should be noted that MMPs do not possess only degrading functions but they also play protective and anti-inflammatory roles. Therefore, the association that exists with a particular polymorphic form of MMP-1 does not necessarily show that particular form is associated with increased MMP-1 activity; in fact, the opposite may be true. The possibility that SNP markers may serve as predictors of implant survival and aid in pharmacogenomic prevention of total joint replacement failure should be further investigated. A more comprehensive analysis of MMP and TIMP SNPs is thus required, and given the coverage by existing genome-wide association study (GWAS) platforms, a candidate gene approach is justified. Regarding TLRs, strong evidence indicated that macrophages, which are the most important cellular targets of wear debris, are the main TLR-expressing cells in periprosthetic microenvironment. The increased secretion of MMPs by combined TLR activation may be an important factor that should also be considered during treatment of periprosthetic loosening and osteolysis.

Extended information is available regarding the action of several nonsteroidal anti-inflammatory drugs (NSAIDs) upon significant for the loosening process effector molecules, which though originates from *in vitro* studies with articular chondrocytes and synovial or dental pulp fibroblasts [168–173]. However, little information is available in the literature about their possible role in retarding the periprosthetic loosening and bone resorption process. To this aim, we tested the effect of four widely used NSAIDs (i.e., aceclofenac, piroxicam, tenoxicam, and indomethacin) on cytokine, MMP, TIMP, and prostanoid production by IFT from patients with aseptic loosening of total arthroplasty [174]. The results showed that all the tested drugs exerted uniformly an inhibitory effect on IL-6 and TNF- α , both known to directly cause osteoclastic bone resorption, independently of PGE2 [175–177]. Moreover, all of them modified specific MMPs (MMP-1, MMP-2, MMP-3, and MMP-9) expression and activity, although these drugs did not have a statistically clear effect on MMPs, which might reflect individual responses in terms of susceptibility to osteolysis. However, NSAIDs had a profound stimulatory effect on TIMP-1 production. Interestingly, paracetamol, which was used as a neutral drug, significantly decreased the synthesis of TNF- α and gelatinases (MMP-2 and MMP-9). Considering these observations, NSAIDs could reduce the ability of periprosthetic membrane to cause bone resorption, which is in line with previous reports that have shown that piroxicam, which exhibited about the same effects as the other tested NSAIDs, significantly decreased the IFT-induced resorptive process [178]. Consequently, *in vivo* long-term clinical trials may shed light on the possibility of a beneficial effect of specific NSAIDs on the loosening process.

5. Concluding Remarks

The elucidation and understanding of the cellular and molecular mechanisms that control the composition, turnover, and

activity of matrix macromolecules within the periprosthetic microenvironment exposed to wear debris is highly important for the development of novel therapeutic approaches to the treatment of periprosthetic loosening and osteolysis. One ultimate target would be to disrupt the vicious cycle between the inflammatory response to wear debris particles induced by the secreted proinflammatory and osteoclastogenic cytokines and the periprosthetic osteolytic cascade governed by the uncontrolled action of MMPs. Considering the multicomplex biological mechanisms underlying the particle-induced periprosthetic loosening and osteolysis described in the present review, it may be crucial to develop and use combinations of conventional therapeutic agents as well as new approaches targeting specific extracellular, cell surface, and intracellular molecular effectors and apply them in clinical practice.

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