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The "Mechanostat Theory" of Frost and the OPG/RANKL/RANK System

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ABSTRACT

Frost's great interest to elucidate the principles of action underlying skeletal deformities, during, and after growth, urged him to undertake an extensive study of the mammalian skeleton. He suggested that survival of the skeleton (but also of other tissues, such as fibrous tissue, hyaline cartilage, fibrocartilage, cementum, or dentin) requires the functional coordination of modeling and remodeling. Modeling adapts bone to overloads, by enhancing additions of new bone and by changing bone architecture, and remodeling adapts bone to underloads by removing bone next to marrow and conserving normally used bone. There exists a mechanism that monitors bone metabolism (longitudinal growth, bone modeling, and remodeling activities) in relation to mechanical usage, the "mechanostat." Recent literature has presented new information regarding the physiological procedure of osteoclast and osteoblast activation. It has been understood that the OPG/RANKL/RANK proteinic system regulates bone metabolism by exerting biological effects on osteoblasts or osteoclasts. The same proteinic network, also regulates alveolar remodeling during tooth movement, as well as physiological root resorption and root resorption during orthodontic tooth movement. The aim of the present review is the presentation and evaluation of recent information in the field of osteoclast and osteoblast biology, as regards to the "mechanostat theory" of Frost. An attempt will be made to elucidate, whether recent data can support this remarkable theory and reveal the biological mechanisms behind it. J. Cell. Biochem. 116: 2724–2729, 2015.

KEY WORDS: MECHANOSTAT; OPG; RANKL; BONE

Frost's great interest to elucidate the principles of action underlying skeletal deformities, during and after growth, urged him to undertake an extensive study of the mammalian skeleton. He attempted to introduce constructively provocative ideas into the area of hard tissue research. Both chondral and bone modeling constituted his area of inquiry. He perceived that: "bone, cartilage, and fibrous tissues respond differently to mechanics, but can influence each other in complex and purposeful ways" [Frost, 1979, 1990a,b,c,d]. Initially, he described a theory of chondral modeling.

Accordingly, Frost described the hypothesis of "minimum effective strain"(MES), which predicts the time and the site of bone architecture changes, as a result of adaptation to mechanical loads [Frost, 1973a,b, 1977, 1980, 1983]. The MES describes the minimum effective signal of mechanical loads that converts their effect to bone architectural adaptation. Strains below the MES are not considered to produce adaptive bone modeling, whereas those above it change bone architecture, in order to reduce subsequent strains under loads, similar to or below the lower limit of the MES.

In vivo, different threshold ranges seem to control the bone modeling and remodeling reactions to mechanical strain. The 1,500–2,500 microstrain (με) range may constitute the limit of the MES, for the mechanical control of bone modeling. Bone modeling enhances additions of new bone in order to adjust bone mass and keep strains below this limit. The 100–300 με range appears to be the setpoint of the MES under which, remodeling can proceed rapidly. Bone remodeling induces removal of cortical-endosteal or trabecular bone and formation of new bone. However, a net bone loss is allowed during increased bone remodeling. The terms modeling and remodeling drift refer to modeling or remodeling activities, respectively. Normal lamellar bone is fractured when mechanical load attain 25,000 με. Conclusively, there exists a mechanism, the "mechanostat" [Frost, 1987a], that monitors bone mass in relation to mechanical strain. The "mechanostat" was named after home thermostat because it seems to turn "ON" in response to an error and "OFF" in its absence [Frost, 1987b] .

Recent literature has presented new information regarding the physiological procedure of osteoclast and osteoblast activation. It has been understood that the functional coordination of the OPG (osteoprotegerin)/RANKL (receptor activator of NFkB ligand)/RANK (receptor activator of NFkB) proteinic system regulates the metabolism of bone tissue, by exerting biological effects on osteoblasts or osteoclasts [Tyrovola et al., 2008]. The critical biological role of OPG is the inhibition of osteoclast function and the acceleration of osteoclast apoptosis [Lacey et al., 1998; Oshiro

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et al., 2002]. On the contrary, the major biological action of RANKL, together with the protein ligand, M-CSF(macrophage colony stimulating factor) (which binds to its receptor c-fms), is to induce osteoclast activation and in this way promote bone resorption [Matsuzaki et al., 1998; Tsukii et al., 1998; Kartsogiannis et al., 1999]. RANKL is activated when it binds to RANK and this interaction is prevented by OPG because OPG acts as a soluble receptor antagonist, which neutralizes RANKL and, therefore, prevents RANKL–RANK interaction [Udagawa et al.,1999].

The RANKL/RANK/OPG system also regulates alveolar remodeling during tooth movement, as well as, physiological root resorption and root resorption during orthodontic tooth movement. The cellular mechanisms of root resorption appear to be quite similar to those of osteoclastic bone resorption [Oshiro et al., 2001; Kanzaki et al., 2002; Wise et al., 2002; Sasaki, 2003; Casa et al., 2006; Harokopakis-Hajishengallis, 2007] and this similarity explains the change in the concentrations of OPG and RANKL [Low et al., 2005; Casa et al., 2006], in the periodontal ligament (PDL) where root resorption has been observed.

In fact, PDL seems to constitute a perfect model for the study of hard tissue architecture changes due to the application of mechanical loads. During orthodontic tooth movement the application of the orthodontic force creates a compressed and a tensile area adjacent to the tooth. On the compressed side, there appears an increase in RANKL concentration [Shiotani et al., 2001; Oshiro et al., 2002] and remodeling mechanisms are enhanced. In contrast, it has been reported that on the tensile side of an orthodontically moving tooth, OPG expression is induced and bone modeling increases [Kobayashi et al., 2000; Tsuji et al., 2004; Kusumi et al., 2005; Tang et al., 2006]. The relative concentrations of OPG and RANKL on the compressed and the tensioned sides of the tooth regulate bone modeling, remodeling, and root resorption during orthodontic tooth movement.

Conclusively, further data has come into light, in relation to the biological mechanisms behind the activation of osteoclast and osteoblast function, as a response to mechanical load. The aim of the present review is the presentation and evaluation of recent information in the field of osteoclast and osteoblast biology as regards the "mechanostat theory" of Frost. An attempt will be made to elucidate, whether recent data can support this remarkable theory and reveal the exact biological mechanisms behind it.

THE THEORY

Frost [1990] suggested that survival of the skeleton (but also of other tissues, such as fibrous tissue, hyaline cartilage, fibrocartilage, cementum, or dentin) requires the functional coordination of modeling and remodeling. He clearly distinguished modeling and remodeling as two biologically different activities, which differ in their anatomical locations, effects, and responses to mechanical usage, disease, and aging. As a useful rule of thumb he perceived that modeling adapts a bone to gross overloading but remodeling adapts it to gross underloading. Where modeling appears to be enabled by overloading, remodeling increases in response to underloading. There exists a threshold strain range that distinguishes which loading state exists in a given situation [Frost, 1988, 1990, 1994].

BONE MODELING

The limits of the MES for bone modeling appear to be about ~1,500–3,000 με [Frost, 1988]. Strains in or above this range increase additions of new cortical bone. Bone modeling adjusts the architecture of a growing bone to the mechanical strains induced due to typical physical activities, body weight, and neuromotor function. Modeling responds to time-averaged value of peak strains that are equal or exceed the MES range. In that regard, repeated longitudinal bone strains, above about ~2,000 ± 30% με, enhance the increase of bone mass and change bone architecture in such a way so as strains under the same loads are reduced toward the ~2,000 με value. Therefore, modeling drifts have an ON–OFF property. A given modeling drift can act for a while (on) then stop (off) and then return to (on) [Frost, 1987a,b,c] . Strains, in or above the 3,500 με range, cause a SOS-like response from the system, expressed as woven bone formation [Frost, 1987a] or massive anarchic resorption. Strains in the range of 25,000 ± 30% με cause fracture of healthy lamellar bone. During growth, bone architecture keeps bone strains below ~1/10 of the momentary fracture strength of ~25,000 με.

BONE REMODELING

The MES range for remodeling appears to be ~100–300 με. Obviously, the strain range for the initiation of bone remodeling procedures is smaller. Under this threshold of strain values increased remodeling and increased net bone loss are expected. Frost [1990a,b,c,d] has supported that there exists a multicellular mediator mechanism, the "basic multicellular unit" (BMU), (activated cells, osteoblasts, osteoclasts), that turns lamellar bone over in small packets. A BMU's osteoclastic activity couples biologically to its subsequent osteoblastic activity [Frost, 1994] . The human skeleton comprise some 2×10^6 BMUs that act at any moment and some 6×10^6 BMUs that are completed annually [Frost, 1989]. Resorption by osteoclasts removes a small packet of bone and formation by osteoblasts refills the resorption bay [Frost, 1990].

This BMU-type of remodeling takes place throughout life in humans, and since BMU functions for ~4 months, BMUs are continually created in certain bone sites and replace other BMUs that have been completed. Normally, a net deficit remains throughout life on cortical-endosteal and trabecular surfaces and may be positive only on periosteal surfaces. Therefore, increased remodeling removes bone next to marrow and makes a bone weaker, whereas decreased remodeling conserves bone and its strength [Frost, 1994]. Acute mechanical disuse increases BMU activation and Frost [1990] suggests that even strains in the 50–100 με range or less increase BMU activation. Therefore, the strain range of 50–300 με defines the MES under which, BMU activation becomes derepressed and, as a result, it increases and above which, remodeling becomes more depressed.

Strains that exceed ~3,000 με, increase BMU-based remodeling as well, due to increased amounts of mechanical bone microdamage, which need repair by remodeling BMUs [Frost, 1990]. A regional acceleratory phenomenon (RAP) can begin too and further increase BMU creations and bone turnover . For example, woven bone can appear in the marrow cavity ahead of a tooth socket, containing a tooth, subjected to excessive orthodontic forces [Frost, 1994]. Here,

modeling and remodeling collaborate. Drifts of woven bone adjust bone architecture, so as to minimize microdamage, by keeping peak strains below the 1,500 με range, while BMUs repair microdamage to prevent accumulation. Mechanical crumbling, stress fractures, and anarchic bone resorption (not coupled to formation) can result too. Bone's fracture strain is about 25,000 με [Frost, 1994].

THE PLATEAU

Consequently, modeling adapts bone architecture to high levels of mechanical strain by adding bone and remodeling adapts bone to underload by removing bone next to marrow and conserving normally used bone. Increasing mechanical loading depresses remodeling and enables modeling drifts. And there exists a kind of plateau between the two MES ranges, within which, changes in mechanical loading have little observable effect on bone architecture and its tissue responses [Frost, 1988]. BMU creations fall to normal, BMUs tend to equalize their resorption and formation, modeling stays OFF, no RAP or little microdamage arise [Frost,1994] and such effects conserve bone and its strength and prevent an osteopenia. The aforementioned conclusions are depicted more accurately as a curve, that relates the biological activities of hard tissue (remodeling and modeling) to the loading history (magnitude, range, frequency, rate, and duration) of hard tissue. Increasing mechanical loading depresses biological activities (remodeling), then there exists a kind of plateau between the two MES ranges and then the biological activities (modeling) can increase again (Fig. 1).

Frost [1988] pointed out that architectural adaptations of hard tissue are related to the largest and most frequent daily repeated strains (typical peak loads or strains) rather than frequent and small, or rare and large ones. Rubin and Lanyon [1985] had supported that bone remodeling might also be sensitive to strain distribution. Age, genetics, drugs, hormones, or disease may change the aforementioned MES ranges and affect accordingly bone architecture.

RELATIONSHIP BETWEEN THE "MECHANOSTAT THEORY" OF FROST AND THE OPG/RANKL/RANK SYSTEM

Further data has come into light, in relation to the biological mechanisms behind the activation of osteoclast and osteoblast function as a response to mechanical load. The OPG/RANKL/RANK proteinic system regulates bone modeling and bone resorption by exerting biological effects on osteoblasts or osteoclasts, during accumulation of strain.

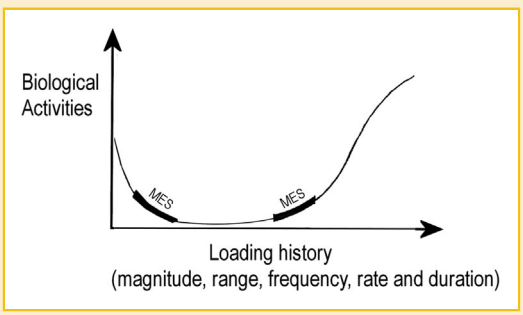

Fig. 1. The "mechanostat theory." Relationship between the biological activities of hard tissue and the loading history (visual configuration of the process).

PDL seems to constitute a perfect model for the study of OPG/RANKL/RANK regulation of hard tissue architecture changes as a result of adaptation to mechanical loads. Melsen [1999, 2001] conducted an investigation, combining strain levels and biological reactions during orthodontic tooth movement. She found that the strain levels in the compression side were below the MES, provoking in this way underload remodeling. On the other hand, in the tension side, the stretching of the PDL fibres generated a strain level corresponding to modelling. Woven bone formation also took place in the direction of movement (compression side), which Melsen attributed to an expression of regional acceleratory phenomena (RAP), developing as a reaction to overloading.

According to recent literature, during orthodontic tooth movement on the compressed side of the tooth, the concentration of RANKL increases [Shiotani et al., 2001; Oshiro et al., 2002]. RANKL promotes osteoclast formation. Kanzaki et al. [2006] demonstrated that the transfer of the RANKL gene to the PDL accelerated tooth movement. In contrast, on the tensile side of an orthodontically moving tooth the concentration of OPG increases. It has been shown that tensile stretching of osteoblasts promotes the production of OPGmRNA [Kobayashi et al., 2000; Tsuji et al., 2004; Kusumi et al., 2005] in a magnitude-dependent manner [Tang et al., 2006]. Such tensile strain also induces a decrease of RANKL concentration and RANKLmRNA expression in cultured osteoblasts. The production of RANKL is not affected by OPG synthesis. There is no difference in RANKL concentration between OPG-deficient and normal mice, after application of orthodontic forces, although, OPG-deficient animals experience severe alveolar bone resorption [Oshiro et al., 2002]. In addition, severe root resorption is correlated to greater increase of RANKL and greater decrease of OPG [Kanzaki et al., 2002; Nishijima et al., 2006; Yamaguchi et al., 2006].

So, the relative concentrations of OPG and RANKL on the tensioned and the compressed sides of the tooth and the OPG/RANKL ratio in the periodontal ligament (PDL) cells regulate bone modeling, remodeling, and root resorption during the application of orthodontic forces. Obviously, the proportion of OPG/RANKL concentrations in the PDL strongly depends on the strain accumulated in the PDL due to the orthodontic force (compression or tension). In the compressed side of the tooth, strain levels below the MES are correlated to increased RANKL expression. On the contrary, on the tensile side a strain level corresponding to modeling is correlated to up-regulation of OPG-synthesis. (Table I)

A nonlinear correlation between the OPG and RANKL concentrations and the tissue reaction during accumulation of strain, for example, during the orthodontic movement, must characterize the PDL, analogous to the curve presented by Frost. Indeed, Tyrovola et al. [2010] studied the levels of OPG and soluble RANKL (s RANKL) in gingival crevicular fluid (GCF) of healthy experimental animals, relative to the degree of orthodontic root resorption in a rat model. S RANKL is the soluble form of RANKL that is not cell-bound on osteoblastic cells. A statistically significant nonlinear correlation between the root resorption ratio and the ratio of OPG/RANKL

TABLE I. Relationship Between the "mechanostat" Theory and OPG/RANKL/RANK System

Increased biological activity [bone remodeling]	Strains in or below ≈100 – 300 με	Compression side of the tooth	Increase of RANKL concentration [↑RANKL]	Lower values of OPG/RANKL [↓OPG/RANKL]
Plateau	Strains between ≈300–1,500 με		Bone formation and resorption equalize	OPG/RANKL values with little observable effect
Increased biological activity (bone modeling)	Strains in or above ≈1,500–3,000 με	Tension side of the tooth	- increase of OPG concentration (↑OPG) - decrease of RANKL release (↓RANKL)	Higher values of OPG/RANKL (↑OPG/RANKL)
Increased biological activity (RAP)	Strains exceed ≈3,000 με	Compression side of the tooth	Acceleration of sterile inflammatory process	- woven bone - increased bone microdamage - massive anarchic resorption
Fracture	Strains 25,000 ± 30% με			

concentrations was detected. This nonlinear correlation is depicted graphically as a curve, in which there appears a plateau (a finite range of OPG/RANKL ratios) for which the dental root was protected against extreme external root resorption (Fig. 2, Table II).

In another study, Nishijima et al. [2006] revealed that the ratio of OPG/RANKL concentrations in the PDL fluctuate in a non linear mode in relation to time, during orthodontic tooth movement, (Fig. 3) or in relation to the application of different magnitudes of compressive force (Fig. 4). They determined the levels of RANKL and OPG in the GCF during orthodontic tooth movement and investigated the effect of compression force on RANKL and OPG production from human PDL (h PDL) cells. These results may refer to both juveniles and adults [Kawasaki et al., 2006].

Densitometry analysis described in the article of Kanzaki et al. [2002] further confirms the aforementioned conclusion. Compressive force, ranging from 0.5 to 3.0 g/cm^2 and applied to PDL cells for 24 h, upregulated RANKL expression in a force dependent manner up to 2.0 g/cm^2, while it downregulated the expression of OPG in a force-dependent manner up to 2.0 g/cm^2.

Yamaguchi et al. [2006] examined the effects of different compressive forces on the production of s RANKL and OPG from PDL cells derived from patients (in good general health), in whom severe root resorption had occurred or from patients assigned to the

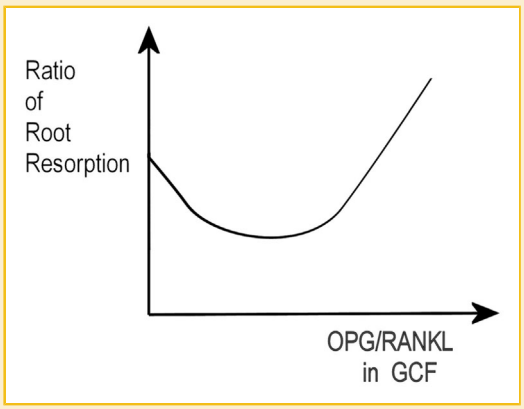

Fig. 2. Relationship between the biological activity of root resorption and the ratio of OPG/RANKL concentrations (visual configuration of the process).

TABLE II. Relationship Between Root Resorption and OPG/RANKL/RANK System

Increased biological activity [root resorption]	Lower values of OPG/RANKL ratio [↓OPG/RANKL]
Plateau	OPG/RANKL values with little observable effect
Increased biological activity (root resorption)	Higher values of OPG/RANKL (↑OPG/RANKL)

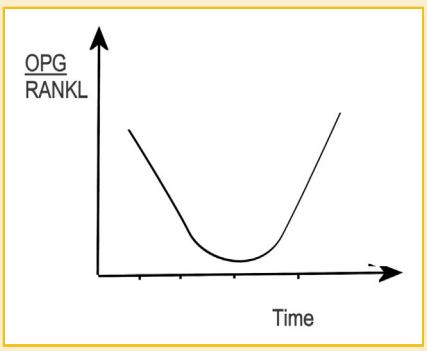

Fig. 3. Relationship between the ratio of OPG/RANKL concentrations and time of force application (visual configuration of the process).

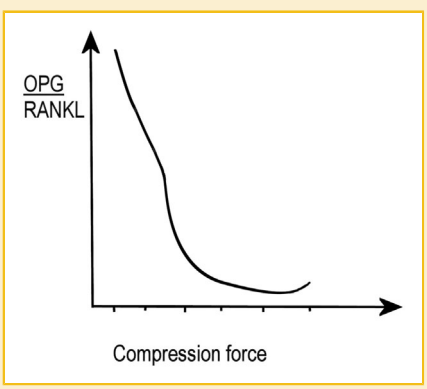

Fig. 4. Relationship between the ratio of OPG/RANKL concentrations and the application of different magnitudes of compression force (visual configuration of the process).

non-resorption group. Interestingly, the increase of s RANKL and the decrease of OPG were greater in the severe root resoprtion group than in the non-resoprtion group, which can be interpreted as a lower OPG/RANKL ratio up to 2.0 g/cm^2 in the severe root resorption group as compared to the non-resorption group.

THE SYNTHESIS

With the aid of mathematical equations the "mechanostat theory" of Frost is revealed, while the OPG/RANKL ratio constitutes the mediating-composing link.

Let the curve that relates root resorption to the ratio of OPG/RANKL (parabolic correlation) (Fig. 2) be represented by the second degree equation $f(x) = \alpha\chi^2 + \beta\chi + \gamma$. Then, the curve that relates the OPG/RANKL ratio to the time of force application (parabolic correlation) (Fig. 3) may be represented by the second degree equation $g(\psi) = \kappa\psi^2 + \lambda\psi + \mu$. The composition of the two equations, that relates root resorption to the time of force application may be $g(f(x)) = \kappa(\alpha\chi^2 + \beta\chi + \gamma)^2 + \lambda(\alpha\chi^2 + \beta\chi + \gamma) + \mu = \kappa(\alpha^2\chi^2 + 2\alpha\chi^2\beta\chi + \ldots) + \lambda(\alpha\chi^2 + \beta\chi + \gamma) + \mu$ for α different from 0 and κ different from 0. Conclusively, the final equation, that represents the relationship between the ratio of root resorption and the time of force application, is a fourth degree non-linear parabolic correlation as well. The same calculations may be applied for the composition of the curve that relates root resorption to the ratio of OPG/RANKL in GCF (Fig. 2) and the curve that relates the OPG/RANKL ratio to the compression force (Fig. 4). The composition of the two equations that relates root resorption to the compression force is a fourth degree non-linear parabolic correlation. Both the time of the force application and the magnitude of compression force represent the loading history induced in the PDL. In addition, root resorption represents the biological activity of osteoclasts.

Undoubtly, the application of these results in humans needs further clinical investigation, since Tyrovola et al. [2010] studied the levels of OPG and RANKL in the PDL of healthy experimental animals, whereas Nishijima et al. [2006] determined the same levels in the human PDL. However, we must take into consideration that the biological mechanisms are common and this is the reason why the rat, as an experimental model, has often been used for the detection and calculation of serum bone markers. More importantly, the aforementioned experimental results (correlations-curves) constitute a qualitative information (apart from quantitative), which provides us with the indication that OPG and RANKL fluctuate in a non-linear mode according to Frost's theory.

The above finding is further enhanced by recent literature, according to which the local effects of the polypeptide leptin on bone tissue are mediated via the RANKL/RANK/OPG system and the mRNA OPG/RANKL ratio. Leptin has been established as a dually acting factor, responsible for the finely tuned bone homeostasis. Leptin mRNA seems to be augmented upon mechanostimulation of the cells by 1,200 με [Gordeladze and Reseland, 2003]. Generally, under normal physiological conditions, leptin , acting locally, and systemically (via the central nervous system) is suggested to be a key mediator of the mechanostat negative-feedback loop, required to normalize bone mass. This regulating system involves a leptin–insulin–osteocalcin feedback loop [Vinoth et al., 2013]. Specifically, serum osteocalcin is reported to be inversely associated with leptin in the aforementioned feedback loop [Lu et al., 2012; Suh et al., 2013]. Therefore, the OPG/RANKL ratio seems to serve as an important link in a functional network, responsible for the regulation of bone modeling, remodeling, and the control of bone homeostasis through the mechanostat principle.

CONCLUSION

As it can be deduced, osteoclastogenesis is controlled by opposing mechanisms in response to mechanical stimuli. There exists an area of OPG/RANKL ratios, in which the hard tissue seems protected against increased remodeling procedures. The ratio of OPG/RANKL concentrations may control the initiation and termination of the bone modeling and remodeling process, as well as root resorption, in a non-linear mode, as was first described by Frost. The ratios of OPG/RANKL concentrations that correspond to the "plateau" of Frost's curve, may have great clinical significance in medicine, and therefore, future investigation is essential for the definition of measurements, that will transform Frost's theory to application in everyday clinical practice.

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