

Changes in Bone Metabolism Around Osseointegrated Implants Under Loading

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1. Introduction

The use of osseointegrated dental implants to replace missing teeth is a highly predictable procedure. The scientific literature is replete with reports of high success rates over long periods of time. Since the phenomenon of osseointegration was first introduced by Brånemark, this procedure has gained great popularity.

One of the measurements for success of an osseointegrated implant is that it be load-bearing and transmit these occlusal forces directly to the adjacent bone. Controlling this load is considered a determinant factor in the long term success of the implant. A related consideration is how this load or mechanical stress influences bone metabolism around the osseointegrated implant.

Mechanical stress may lead to an alteration in bone quality and architecture and a distinct reaction within the bone cells at the bone-implant interface. However, there is little published data to support this theory. A few studies have suggested that occlusal overload may contribute to bone loss around an implant and/or loss of integration of a successfully integrated implant. (Rangert et al., 1995; Miyata et al., 2000; Piattelli et al., 2003). Isidor reported implant mobility caused by progressive peri-implant bone loss after the implant were exposed to mechanical occlusal trauma for 18 months (Isidor, 1997, 1998). Others report that peri-implant bone loss and/or loss of osseointegration is associated with biological complications such as peri-implant infection (Lang et al., 2000).

A certain level of mechanical loading is required for normal, healthy bone remodeling (Frost, 1989). Misch observed that the change in bone strength from loading and mineralization after one year alters the stress-strain relationship and reduces the risk of microfracture during following years. Mechanical stress might induce a metabolic turnover of the bone based on the changes in osteocyte responses around the implant, resulting in bone remodeling (Misch, 1999).

This chapter is to investigate the dynamic changes in bone metabolism around osseointegrated implants under mechanical loading.

2. Basic information

2.1 Bone formation

Bone forms by either endochondral or intramembranous ossification. In endochondral ossification or long bone formation, there is an intermediate cartilage phase. All craniofacial bones are formed by intramembranous ossification. Wong and Rabie showed that demineralized intramembranous bone matrix induces bone without an intermediate cartilage stage, mesenchymal stem cells differentiate directly into bone cells (Wong & Rabie, 1999). The mesenchymal stem cells differentiate into osteoblasts and form osteoid in a collagen matrix (Palacci et al., 2001). Mineralization of osteoid occurs and the osteoblasts become trapped in mineralized bone and become osteocytes.

Appositional bone formation occurs when osteoblasts produce bone on existing bone surfaces. Examples of appositional bone formation occur in the periosteal enlargement of bones during growth and remodeling. Histological studies show woven bone formation by appositional growth may only begin to form the second week after implant insertion, at a rate of 30 to 50 microns per day. The bone to implant contact is weakest and at the highest risk of overload at approximately 3 to 5 weeks after implant placement (Strid, 1985).

2.2 Osseointegration process

Osseointegration is defined as direct bone deposition on an implant surfaces at the light microscopic level (Brånemark et al., 1997). It generally follows three stages: (1) incorporation by woven bone formation, (2) adaptation of bone mass to load (lamellar and parallel-fibered deposition) and (3) adaptation of bone structure to load (bone remodeling) (Schenk & Buser, 1998). During the third stage, when functional loading has been initiated, the bony structures adapt to the load by improving the quality of the bone; replacing pre-existing, necrotic and/or initially formed more primitive woven bone with mature, viable lamellar bone. This leads to functional adaptation of the bony structures to the load. The dimensions and orientation of the supporting elements change. *In vitro* studies have illustrated the importance of loading forces on the nature of the interface between an implant and the surrounding tissues (Brunski, 1988). Even if implants were initially integrated, the application of excessive loading can create microfractures and mobility which may promote bone resorption around the implant and may promote repair by the undesirable growth of fibrous tissue (Roberts et al., 1989). An undisturbed healing period along with adequate quality and quantity of bone available at the implant site are essential for proper osseointegration. (Wood, 2004). In addition, primary implant stability is determined by bone quality and quantity, implant design, and surgical technique (Sennerby et al., 1998).

2.3 Implant mechano-biology

Factors that influence mechano-implant studies are mechanical loading and detachment of cells. Previous *in vivo* data have demonstrated that optimal mechanical loading led to more favorable bone quality and quantity than situations with no loading. The testing equipment used for most of these studies were stretching devices borrowed from orthopedic stress-strain research measuring elastic stress relative to joint prostheses. Their application in dental implant research is not optimal as there is mostly shear stress and little elastic stress. More recently, *in vitro* studies of osteoblastic behavior on titanium following loading have been developed. Movement seems to generate negative effects on osseointegration in terms of decreased Alkaline Phosphatase (ALP) activity and osteocalcin, which is consistent with

the results in mechano-cell studies. The suppressed ALP activity may be attributed to PGE2 production (Bannister et al., 2002). With respect to osteoblast maturation following stimuli, biphasic ALP activity and triphasic osteocalcin level in a 3D study differed from 2D cultures characterized by typical gene expression pattern with time (early up-regulation of collagen and ALP) and osteocalcin production (Akhouayri et al., 1999). It was assumed that actin cables and collagen fibers would be aligned to amplify the mechanical forces and to supply bone maximum strength with little amount of material. This research has demonstrated that number of complex elements exist in the human body environment and it is still necessary to improve experimental designs in order to provide reliable *in vivo* implant data.

2.4 Implant under loading

It is clear that successful osseointegration depends on unfavorable loading. Other factors to consider are the timing of initiation, magnitude, and duration of the load or stress. Excessive occlusal loading will lead to disintegration while adequate loading leads to adaptive remodeling of the bone around the implant (Quirynen, 1992). However, there are some differences in data reported among researchers.

Gotfredsen et al. demonstrated that implants subjected to a static lateral expansion load showed increased bone density and mineralized bone-to-implant contact compared with control implants (Gotfredsen et al., 2001a, 2001b, 2001c, 2002). Melsen and Lang reported that there was significantly higher bone apposition around loaded implants than unloaded implants but the dimensions of the applied load did not affect the turnover characteristics of the peri-implant alveolar bone (Melsen & Lang, 2001). Vandamme et al. also indicated significantly more osteoid in contact with the implant was found for the loaded conditions compared with no loading. Well-controlled micromovement favorably influenced bone formation at the interface of an implant (Vandamme et al., 2007). In an animal model, Berglundh et al. described osteoclastic activity as early as four days following implant placement and new bone was noted at one week post placement (Berglundh et al., 2003). These results suggest that bone metabolic activity is changed by mechanical stress and that it depends on the loading conditions.

These reports indicate that functional loading does promote osseointegration and that overloading or favorable loading may contribute to implant failure. Occlusal overload could result in progressive marginal bone loss or loss of osseointegration. Long ago, Adell recognized that early implant failure may be associated with overload (Adell et al., 1981). In a more recent study, Miyata et al. reported the outcome of occlusal overloading at three different occlusal heights (100 μ m, 180 μ m, 250 μ m) on implant prostheses for four weeks. Bone destruction was observed in the 180 μ m and 250 μ m excess occlusal height groups (Miyata et al., 2000).

Bruxism, a non-physiological parafunctional habit is more significant than the forces associated with normal mastication. Excessive micromovement creates stress or occlusal overload and leads to soft tissue encapsulation and prevents osseointegration, thus causing implant failure (Brunski et al., 1979). The occlusal scheme may jeopardize the success rates of immediately loaded implants; they found that 75% of failures in immediately loaded implants occurred in patients with bruxism (Balshi & Wolfinger, 1997). To avoid fibrous encapsulation and subsequent implant failure, implants must withstand functional load with less than 150 microns (Schincaglia et al., 2007). It is the excess of micromotion caused by excessive loading during the healing phase that interferes with bone repair. A threshold

of tolerated micromotion exists, that is somewhere between 50 μm and 150 μm in human case. Therefore, keeping the amount of micromotion beneath the threshold of deleterious micromotion might enable the loading protocols to be shortened (Szmukler-Moncler et al., 2010).

2.5 Immediate and early loading

Immediate and early loading of dental implants are concepts introduced to shorten treatment time and further improve a patient's quality of life (Barone, 2003). Linked to this is the fact that appropriate mechanical stimulation is a positive factor for bone formation. In fact, several studies have demonstrated that early functional loading is helpful in preventing marginal bone resorption and enhancement of osteointegration at the implant surface. In a human clinical case report, Ganeles et al. demonstrated that Straumann® implants placed in the posterior mandible and maxilla are safe and predictable when used with immediate and early loading procedures (Ganeles et al., 2008; Esposito et al., 2009). In poor quality bone, survival rates were comparable with those from conventional or delayed loading. The mean bone level change was not deemed to be clinically significant and compared well with the typical bone resorption observed in conventional implant loading (Kinsel & Liss, 2007; Fischer et al., 2008). Several animal studies also reported favorable results when using immediate and early loading principles.

In a study of radiographic evaluation in dogs, Corso et al. showed that immediate masticatory loading of single-standing dental implants did not jeopardize tissue integration, provided the implants had excellent primary stability (Corso et al., 1999). Cha et al. asserted that immediate loading of mini-implants in the dog model is possible for orthodontic applications with a high bone-implant contact and 100% survival rate (Cha et al., 2009). Some immediate loading studies involving the biting stress of mastication showed the potential to increase bone density and prevent crestal bone loss. With respect to histological bone implant contacts, no significant difference between immediately loaded implants and those of delayed loaded or those of unloaded was observed. It was postulated that mechanical stimulation quite possibly enhanced bone formation (Romanos et al., 2002, 2003). Kawahara et al. studied the effect of immediate loading at the implant interface in dogs and concluded that micromotion of less than 30 μm did not impede bone ingrowth (Kawahara et al., 2003). For an immediate loading model with varying degrees of implant displacement, micromotion had a positive effect on bone formation around a roughened implant surface and a negative effect on a turned implant surface (Vandamme et al., 2007). De Smet reported that early loading enhanced bone reaction around implant and contributed to stability of the implant (De Smet et al., 2005, 2006). At the same time, it has been reported that excessive loading leads to crater-like bone defects around implants, indicating bone resorption (Duyck et al., 2001).

The benefit of immediate loading was further borne out by clinical and histological studies indicating that immediately loaded implants had a higher bone-to-implant contact (BIC) value than non-immediately loaded implants. In one case report, the BIC was 64.2% greater with immediately loaded Osseotite® implants (Testori et al., 2002). In another study, histological comparison of non-submerged unloaded and early-loaded implants in a monkey model found a tight contact with new bone to implant surfaces in both study groups (Piattelli et al., 1997, 1998). But the authors found that the bone of the loaded implants had a more compact appearance than non-loaded controls and the mean BIC of immediate-loaded implants was 67.2% in the maxilla and 80.7% mandible.

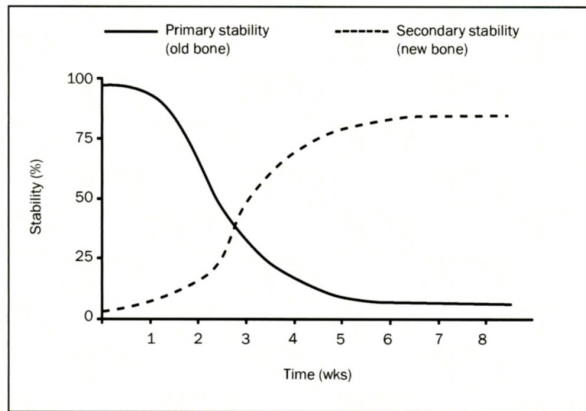


Fig. 1. Changeover from primary stability created at the time of implant placement to secondary stability created by deposition of new bone (osseointegration) in humans. (Raghavendra et al., 2005)

They felt that the most critical factor in successful osseointegration of an implant is the stability in the bone at the time of placement since a static condition was thought to be a prerequisite during the early healing period. This initial mechanical stability is later replaced by biologic stability as the bone remodels and osseointegration occurs (Raghavendra et al., 2005). This reportedly occurs during a 2 to 3 week transition phase following implant placement. Ostman listed requirements for long-term success with immediate-loaded implants as high primary implant stability, moderately rough implant surface, prolonged implant stabilization by splinting, controlled occlusion, and biocompatible prosthetic material (Ostman et al., 2008).

The actual type of bone healing around unloaded and loaded implants was investigated by Slaets et al. (Slaets et al., 2006, 2007). These studies showed that the manifestation and duration of the biological processes including the osseointegration of the implant were dependent on the type of bone, cortical or trabecular. Furthermore, the immediate loading protocol caused no differences in the sequential events leading to osseointegration in cortical bone (Slaets et al., 2009). In an unpublished study using bone scintigraphy, the current authors reported on both immediate and early loading of implants. The bone metabolic activity increased for the first seven days after load application and then decreased gradually until returning to the baseline level despite continuous load-application with same magnitude. These results suggest that this change may be attributed to adaptive bone remodeling and immediate and early loading might not prolong the period until achievement of osseointegration.

3. Nuclear medicine approach with bone scintigraphy

Regarding bone metabolic activity, such as bone remodeling and adaptation, histological studies can merely depict static and cross-sectional aspects of the bone activity and phenomena in the remodeling process. In contrast, a nuclear medicine approach with radionuclide bone scanning, including scintigraphy, is widely used to evaluate the dynamic and longitudinal processes in biological response and to help in comprehending the condition of osseointegration (Bambini et al., 2004). Areas with an observed accumulation of

radiopharmaceutical isotopes show an increased level of bone metabolic activity, suggesting that this method enables measurement of the changes in bone metabolic activity around implants *in vivo* (Fleisch, 1998; Chisin et al., 1988). *In vivo* scintigraphic imaging using Tc99m-MDP enables the same region to be observed numerous times without sacrificing the host animal. Although the specific binding location of Tc99m-MDP remains unknown, it is associated with areas of bone growth and osteoblast activity (Lysell & Rohlin 1985; Kanishi, 1993; Schwartz et al., 1993; Okamoto, 1997). Furthermore, bone scintigraphy makes it possible to observe the accumulations with more sensitivity and higher reactivity over time than with conventional radiology (Bijvoet et al., 1995).

3.1 Materials and methods

3.1.1 Animals and insertion of Implants

Thirty-two 12-week-postnatal male Wistar rats were used. Nine rats were used to investigate the biological process of the osseointegration after implant insertion; and twenty-three rats addition to those nine rats were used to investigate the bone metabolic activity under loading. The rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally with supplemental ether inhalation. Two titanium implants (Orthoanchor®, Dentsply-Sankin, Japan), 1.2 mm in diameter and 9.25 mm in length, were installed in the tibiae perpendicular to the bone surface; the implant heads were exposed about 5 mm. In each rat, one implant was installed 10 mm from the knee joint. The other was installed 13 mm from the first and in the distal aspect.

3.1.2 Loading with coil spring

Healing and osseointegration had gained at eight weeks after insertion. To clarify the biological responses around the implants under continuous loading, closed coil springs (Sentalloy®, Tomy International, Japan) with 0.5 N were attached to the implant heads of nine rats for seven weeks to apply a continuous mechanical stress (Fig 2a). Closed coil springs with 1.0, 2.0, or 4.0 N were also attached to the implant heads of eight, seven, and eight, respectively. The group of rats with two 2.0-N springs is defined as the 4.0-N loading group (Fig 2b). It comprised a tension coil and a hook attachment. The effective length of the tension coil was 12 mm. The springs thus applied the same magnitude of loading continuously within their effective length (10 to 22 mm).

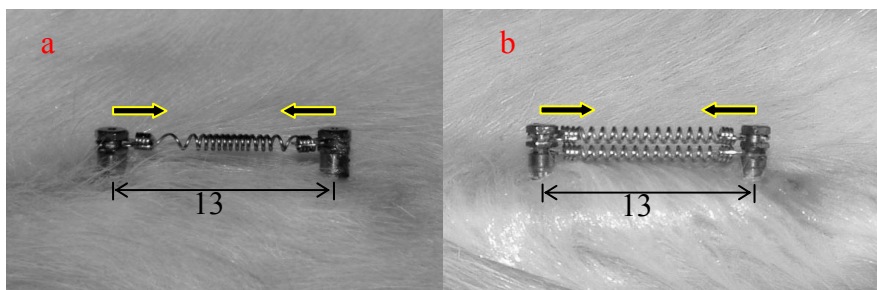


Fig. 2. Attached closed coil springs. Eight weeks after implant insertion, closed coil springs were attached to implant heads. With loading of 0.5, 1.0, or 2.0 N, closed coil spring was set prospectively (a). Two closed coil springs with loading of 2.0 N each were set in parallel for total loading of 4.0 N (b). Arrows show loading directions.

3.1.3 Scintigraphic imaging

Scintigraphic images of the bone were taken using a gamma camera (ZLC7500, Siemens, Japan) with a modified high-resolution pinhole collimator (pinhole diameter: 2 mm). Technetium-99m methylene diphosphonate was used as a radioisotope tracer. Sodium pertechnetate ($\text{Na}^{99\text{m}}\text{Tc-O}_4$) was eluted from a generator (Ultra-Techne Kow[®], Daiichi Radioisotope Laboratories, Japan) and mixed with methylene diphosphonate (Techne[®] MDP injection solution, Daiichi Radioisotope Laboratories, Japan). The Tc99m-MDP was injected into a vein of a tail of each rat (74 MBq/rat). Static-planar acquisition was initiated two hours after the injection and finished at 50,000 counts with a 512×512 matrix size. The rats were fixed on an exclusive table in the dorsal position, with the implant heads turned to vertical. The images were taken from the backside direction of the rat.

To clarify the bone metabolic activity around the implants during osseointegration, images were taken at 1, 4, 7, and 10 days and 2, 3, 4, 7, and 8 weeks post-implantation. To clarify the bone metabolic activity around the implants under continuous loading, images were taken at three days and every week up to seven weeks after loading with the coil springs.

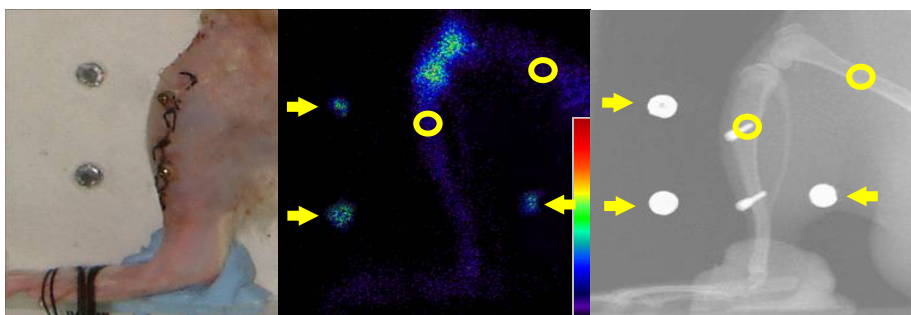


Fig. 3. Image analysis. Photograph on left shows tibia fixation on exclusive table. Device fixes tibia horizontally to equalize distance of implants from pinhole collimator. Scintigram in center and x-ray image on right show planar image. Guide tubes (arrows) were used to overlap two images and to define region of interest (open circles).

3.1.4 X-rayimaging

To identify the region of the reference site and the installed implants, an x-ray image was taken of each rat on the table using an imaging plate (IP; BAS-SR2505, Fuji Film, Japan). Each IP was then scanned with an imaging analyzer (BAS5000, Fuji Film, Japan). The exclusive table had three markers. Before the scintigraphic imaging, Tc99m-MDP was placed in each tube, resulting in an accumulation of Tc99m-MDP in the tube regions on the scintigrams for each rat. These markers indicate the points of overlap with the lead regions in the x-ray images, enabling identification of the implant and reference sites (Fig 3).

3.1.5 Data processing

The scintigrams were translated into TIFF format (16 bits) with a data processing unit (Scintipac 700, Shimazu, Japan) and conversion software (picMAO, Shimazu, Japan). Analysis processing was conducted with image analysis software (Osiris, Geneva University Hospital, Italy). After identification of the implant and reference sites by the overlapping the x-ray images and scintigrams, a round ROI (region of interest, 161 pixels) was defined

around both sites and the accumulations of Tc99m-MDP in both regions were measured. As a metric, the ratio of the metabolic activity around the implants to that around the reference site (uptake ratio) was used. The collected data were analyzed using Friedman, Steel, and Tukey tests with statistical software (SPSS 11.0, SPSS Inc., Chicago, IL, USA.). P-values <0.05 were deemed statistically significant.

3.2 Results

3.2.1 Metabolic changes after insertion of Implants

The uptake ratio increased during the first week after implant insertion and then decreased gradually. It was significantly higher than baseline on days 4, 7, and 10 and during the second and third weeks. However, it was not significantly higher on 4 weeks and 7 weeks, in other words, metabolic activity had returned to the baseline level (Fig 4). No clinical mobility of the implants was observed during the healing period. These results suggest that osseointegration is obtained about four weeks after implant insertion. In addition, the timing of the peak level of and subsequent decrease in bone metabolic activity found in this study correspond very well to those of a previous report on Tc99m-MDP activity around implants using bone scintigraphy (McCracken et al., 2001). Therefore, it should be possible to observe in real time the osseointegration process and the degrees and stages of bone metabolism using this method longitudinally.

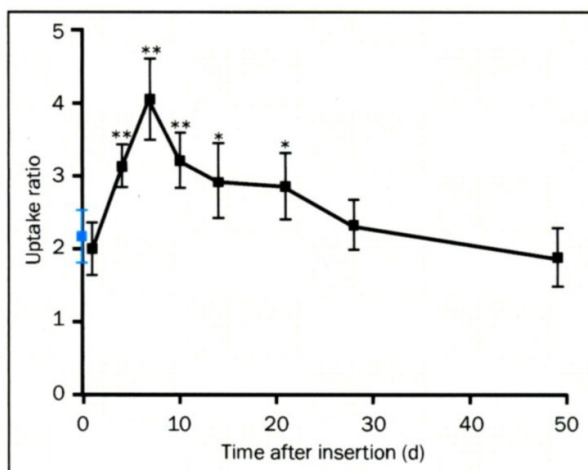


Fig. 4. Change in uptake ratio after insertion of implants using 9 rats. There were significant differences at days 4, 7, and 10 and second and third weeks after insertion. Blue = control. (* $p < 0.05$, ** $p < 0.01$) (Sasaki et al., 2008).

3.2.2 Effect of loading period

The uptake ratio changed with the loading. With 2.0- and 4.0-N loading, both changes of activities over the seven-week experimental period were almost the same in terms of magnitude and timing. The ratio reached a maximum during the first week (more than twice that without loading) and then decreased a little. Metabolic activity had returned to the baseline level. The ratio then returned to baseline level of about two on seven weeks

after loading. The ratio from three days to six weeks after loading was significantly higher than without loading. There was no significant difference seven weeks after loading. The results for the 0.5- and 1.0-N loading groups were similar but differed from those for the 2.0- and 4.0-N loading groups. With the smaller loadings, the uptake ratio gradually increased after loading and returned to the baseline level at seven days. It then decreased, reaching about two on seven weeks after loading. With 1.0-N loading, the uptake ratio did not differ among measurement points.

3.2.3 Effect of loading magnitude

The uptake ratios with the 2.0- and 4.0-N loadings were significantly higher than those with the 0.5- and 1.0-N loadings (Tukey test, $p < 0.05$) (Fig 5). This indicates that the metabolic activities are affected by the magnitude of the mechanical loading on the implant. The uptake ratio showed dynamic changes, and the peak levels were similar in the heavy loading group, i.e., there was no difference between 2.0- and 4.0-N loading. It is conceivable that the bone metabolic activity may have an upper limit as far as the loading does not exceed the physiologic threshold of bone adaptation (Frost, 1994). On the other hand, it is possible that the bone metabolic activity increases remarkably when excessive loading is applied to the implant, causing implant disintegration.

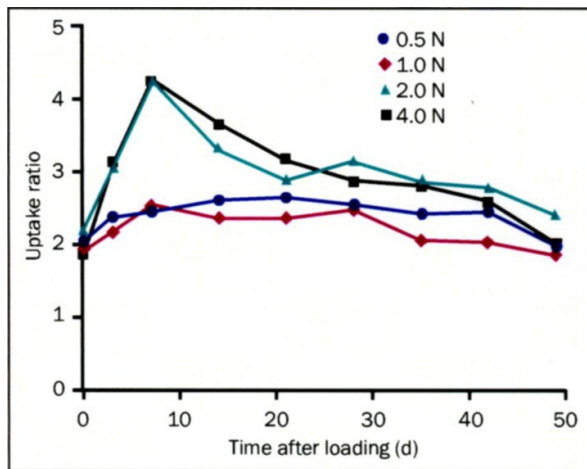


Fig. 5. Change in uptake ratio after loading with coil springs using 32 rats. There were significant differences between 2.0- and 4.0-N loadings and 0.5- and 1.0-N loadings. With 2.0- and 4.0-N loading, the ratio from 3 days to 6 weeks after loading was significantly higher than without loading (Sasaki et al., 2008).

3.3 Discussion

The bone metabolic activity gradually decreased from the peak level despite a static force being applied to the implants and it eventually returned to the pre-loading level. This change can be attributed to an adaptive bone remodeling process similar to those previously reported. Saxon et al. demonstrated that mechanical loading on the rat ulna greatly improved bone formation during the first five weeks of loading, while continual loading

reduced the osteogenic response. Moreover, restoring the same level of loading after a period of no loading increased bone formation again (Saxon et al., 2005). Warden et al. investigated the use of mechanical loading with rat ulna to induce bone adaptation and found that fatigue resistance was advanced than control because the structural properties changed due to loading (Warden et al., 2005). Mechanical loading is thus an important factor in the formation and maintenance of skeletal architecture. Bone morphology adapts to the functional loading patterns by responding to the size and distribution of strains that loading engenders in the bone tissue (Lanyon, 1987, 1992). It is concluded that the bone around the implants adapted to the mechanical stress of long-term loading by structurally changing and that the responsiveness to the loading diminished over time.

Mobility normally occurs during the osseous remodeling process (Ganeles et al., 2002). Remodeling is a variable process with balanced osteoclastic and osteoblastic activity, so that a stable implant is preserved during osseointegration (Schnitman et al., 1997). However, clinically stable implants may exhibit mobility on the micro-level when loaded. Sennerby and Meredith revealed that all implants display varying degrees of stability or resistance to load (Sennerby & Meredith, 2008). That is, functional adaptation and maintenance of the bony structures around implants would be caused by cells (i.e., osteocyte, osteoblast, or osteoclast) in active response to environmental biophysical stimuli, in other word, mechanical stress. Microstrain levels 100 times less than the ultimate strength of bone may be responsible for remodeling rates within the structure, since the bone cell membrane are able to act as a mechanosensory system in bone (Cowin & Moss-Salentyin, 1991). In other words, the cellular behavior of bone cells is largely determined by the mechanical environment of strain or deformation of the bone cell (Jones et al., 1991).

At the interface of implant, osteoblasts and osteocytes play roles of transducers of received strain, leading bone modeling and remodeling phase. Verborgt et al. found that fatigue loading produced a large number of osteocytes in bone surrounding microcracks, and stated a strong association between microdamages, osteocyte apoptosis, and subsequent bone remodeling (Verborgt et al., 2000). Noble et al. showed that mechanical loading of the bone can be used to regulate osteocyte apoptosis, which has a mechanism for the precise targeting of osteoclasts for bone adaptation (Noble et al., 2003). Miyata et al. speculated that long-term occlusal stress on implants within the physiologic tolerance might stimulate blood circulation, which has an intraosseous bone-inducing factor that promotes bone metabolism and, consequently, enhances bone remodeling to obtain the width needed to counter occlusal stress (Miyata et al., 2000). Furthermore, Isidor indicated that depending on the properties of the tissue, a given force may affect different bones or bone tissues differently, but mechanically loaded bones adapt to the load. If the strain in the bone surrounding an oral implant is in the mild overload range, apposition of bone seems to be the biological response. On the other hand, strain in the bone beyond this range will at some point result in fatigue fracture and bone resorption (Isidor, 2006).

4. Conclusion

Changes in bone metabolic activity around dental implants are dependent on mechanical stress relative to timing, direction, quality, and duration of loading conditions. The mechanical stress induces metabolic bone remodeling and adaptation around osseointegrated implants with structurally changing.

5. Acknowledgment

This work was supported by grants-in-Aid for scientific research (grant no. 14370626, 19592226), from the Ministry of Education, Science, and Culture of Japan.

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