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Osteocyte density in the peri-implant bone of implants retrieved after different time periods (4 weeks to 27 years)

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Received 14 February 2013; revised 29 April 2013; accepted 10 June 2013
Published online 00 Month 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.33000

Abstract: Bone tissue is characterized by a constant turnover in response to mechanical stimuli, and osteocytes play an essential role in bone mechanical adaptation. However, little to no information has been published regarding osteocyte density as a function of implantation time *in vivo*. The aim of this retrospective histological study was to evaluate the osteocyte density of the peri-implant bone in implants retrieved because of different reasons in a time period from 4 weeks to 27 years. A total of 18 samples were included in the present study. Specimens were divided into 3 groups depending on the loading history of the implants: loading between 4 weeks and 7 months (group 1); loading between 1 and 5 years (group 2); loading between 14 and 27 years (group 3). All the samples were histologically evaluated and osteocyte density was obtained using the ratio of the number of osteocytes to the bone-area (mm^2). The osteocyte density values significantly increased in the Group 2 (1–5 years) compared with Group 1 (4 weeks–7 months), and significantly decreased in the Group 3 (14–27 years) compared to Group 2. No significant differences were detected between Group 1 and Group 3. The decrease in osteocyte density observed in samples that were *in vivo* for long periods of time under loading is possibly because of the fact that once the bone structure is well aligned and biomechanically competent, a lower number of osteocytes are necessary to keep the tissue homeostasis under loading. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2013.

Key Words: bone remodeling, dental implants, human retrieved implants, osteocyte, osteocyte density

How to cite this article: Piattelli A, Artese L, Penitente E, Iaculli F, Degidi M, Mangano C, Shibli JA, Coelho PG, Perrotti V, Iezzi G. 2013. Osteocyte density in the peri-implant bone of implants retrieved after different time periods (4 weeks to 27 years). J Biomed Mater Res Part B 2013: 00B: 000–000.

INTRODUCTION

Osteocytes are stellate-shaped cells inside skeletal lacuno-canalicular network, characterized by numerous elongated cell processes (50–60) extending into matrix channels or canaliculi (diameter 250–300 nm).[1] These cells comprise more than 95% of mature adult bone cells.[2–4] The osteocyte shape seems to be correlated to their specific location as they have a rounded morphology in trabecular bone, and a more elongated form in cortical bone.[5] Their cytoplasmic processes tend to run in a radial pattern in several directions forming an intercellular network of cells.[6] They have connections with other osteocytes and with cells lining the bone surfaces, osteoblasts, and osteoclasts, through gap-junctions[7]; they are also connected to capillaries, for bone oxygen and nutrition.[8] Osteocytes represent also the final differentiation phase of osteoblasts.[9] This differentiation involves a restructuring of the cytoskeletal and intracellular structures,[10] where osteoblasts loose their membrane apical and basolateral polarization[11] along with a decrease of their protein synthesis and secretion in the mineralization phases of the osteoid matrix.[10]

Bone tissue is characterized by a constant turnover in response to mechanical stimuli, such as loading, and osteocytes have been found to be the cells playing an essential role in bone mechanical adaptation to loading, and the osteocyte network has been regarded as the main mechano-sensor mechanism.[12,13] In one of our previous studies,[14] a higher osteocyte density was found in the bone structure in proximity to immediately loaded implants when compared to their nonloaded (submerged healing) counterparts, suggesting that, during the early stages of healing, mechanical loading resulted in early bone formation dynamics and final morphology. While it can be naturally speculated that the mechanical loading was the responsible stimulus for such higher osteocyte density, the literature on the topic is sparse and little to no information has been published regarding osteocyte density as a function of implantation time *in vivo*.

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TABLE I. Implants Features

Samples	Diameter (mm)	Lenght (mm)	Coating	Implantation	Retrieval	Type of Prostheses
GROUP 1						
1	3.5	7	SB/AE	07/2005	10/2005	Single Crown
2	3.5	8	SB/AE	03/2006	04/2006	Single Crown
3	3.3	10	SB/AE	12/2000	06/2001	Single Crown
4	3.5	8	SB/AE	03/2001	09/2001	Overdenture
5	3.5	8	SB/AE	01/2006	02/2006	Single Crown
6	3.5	10	SB/AE	04/2006	05/2006	Single Crown
7	3.5	8	SB/AE	03/2006	04/2006	Single Crown
GROUP 2						
1	4.5	11	SB/AE	05/2002	06/2007	Single Crown
2	4.5	11	SB/AE	03/2002	05/2007	Single Crown
3	4.5	11	SB/AE	03/2010	07/2011	Single Crown
4	5	11	SB/AE	05/2002	01/2005	Single Crown
5	3.5	14	SB/AE	03/2007	06/2010	Single Crown
6	3.5	14	SB/AE	03/2007	06/2010	Single Crown
GROUP 3						
1	3.5	11	SB/AE	01/1991	10/2009	Single Crown
2	3.5	11	SB/AE	0271989	04/2012	Fixed Bridge
3	3.5	11	SB/AE	06/1991	07/2011	Fixed Bridge
4	3.5	11	SB/AE	03/1985	01/2012	Overdenture
5	3.5	11	SB/AE	06/1995	07/2009	Single Crown

AE, acid-etched; SB, sandblasted.

Thus, the aim of the present retrospective histological study was to evaluate the osteocyte density of the peri-implant bone in implants retrieved because of different reasons in a time period from 4 weeks to 27 years.

MATERIALS AND METHODS

The Archives of the Implant Retrieval Center of the Department of Medical, Oral and Biotechnological Sciences of the University of Chieti-Pescara, Italy, were searched for human implants retrieved for different causes (psychological causes, fractures, misalignment, prosthetic problems, hygienic reasons, part of a study on human retrieved implants) after a loading period varying from a few weeks to more than 20 years. All the patients accepted to participate to the present study and signed an informed consent form. All histologic sections selected pertained to screw-shaped implants that were in clinical function (mechanically loaded). A total of 18 samples were found and included in the present study. Specimens were divided into three groups depending on the loading history of the implants: group 1—loading history of the implants between 4 weeks and 7 months (n. 7) (Dentsply Implants Manufacturing GmbH, Mannheim, Germany); group 2—loading history of the implants between 1 and 5 years (n. 6) (Dentsply Implants Manufacturing GmbH, Mannheim, Germany; Implacil De Bortoli, Sao Paulo, Brasil); group 3—loading history of the implants between 14 and 27 years (n. 5) (Dispo, Milano, Italy) (Table I).

All retrieved implant specimens had been immediately fixed in 10% buffered formalin, and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). The specimens had been dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit® 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens had been sectioned, along their longitudinal axis, with a high-precision diamond disk at about 150 μm, and ground down to about 30 μm with a specially designed grinding machine. The slides had been stained with acid fuchsin and toluidine blue.[15] The slides were observed in normal transmitted light under a Leica DMR light microscope connected with high-resolution video camera (Leica DFC 320) and interfaced with a monitor and a computer. The optical system was associated with a software package with image-capturing capabilities (Leica QWin Plus V 3.5.0, Leica Microsystems, Heerbrugg, Switzerland). The osteocyte density was obtained using the ratio of the number of osteocytes (counted manually for each specimens in the peri-implant bone tissue at a magnification of 200×) to the bone-area (mm^2) with the above-mentioned software package. The measurements were done in the peri-implant bone (mean window 1.168 +/−0.65 mm) along the whole perimeter of the implants.

STATISTICAL ANALYSIS

Data were evaluated by means of Kruskal-Wallis test[16]; differences between groups were assessed by Dunn's Multiple Comparisons Test.[17] Statistical significance was set to $p = 0.05$. All the data are presented as means +/− standard errors (SE).

RESULTS

Relative to implants retrieved after a loading period ranging from 4 weeks to 7 months (Group 1), the osteocyte density values increased sharply in the implants of the group retrieved after a loading period of 1–5 years (Group 2). Then, the osteocyte density values significantly decreased

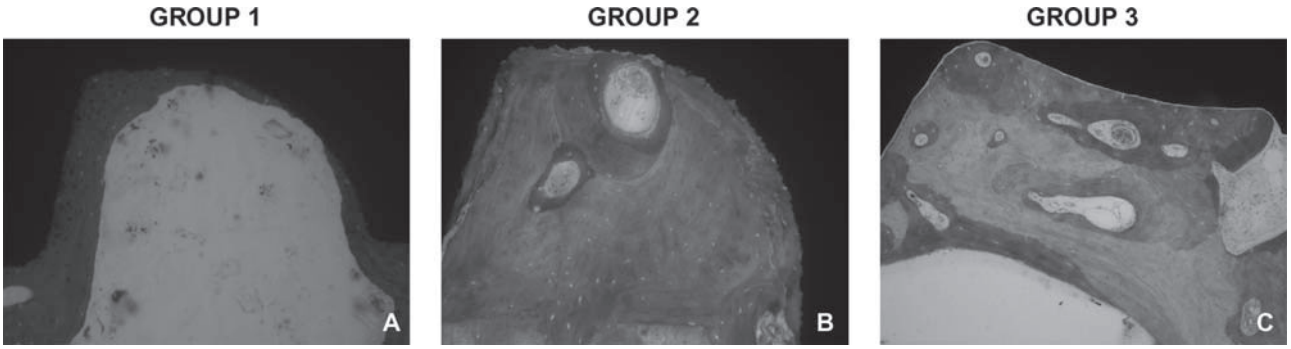

FIGURE 1. Histological images of peri-implant bone tissue in the three study groups. **(A)** Wide osteocyte lacunae, typical of recently mineralized bone tissue, can be observed in Group 1 samples retrieved after a loading period of 4 weeks–7 months. **(B)** Compact bone with remodeling areas can be detected in Group 2 samples retrieved after a loading period of 1–5 years. **(C)** Compact bone with many remodeling areas, characterized by different affinity for the staining, can be seen in Group 3 samples retrieved after a loading period of 14–27 years. In the recently remodeled bone, with a greater affinity for the staining, few osteocyte lacunae are present, whereas in the old bone no osteocyte lacunae are less evident. Toluidine blue and acid fuchsin; original magnification 100×.

around implants retrieved after 14–27 years (Group 3) of loading relative to the group retrieved after a loading period of 1–5 years [Figure 1(A–C)]. Statistically significant differences were observed between Group 2 and Group 1 and 3, whereas no significant differences were detected between Group 1 and Group 3 [Figure 2(A–F] (Tables II).

DISCUSSION

Mechanical load of bone stimulates the interstitial fluid flow through the pericellular space surrounding osteocytes and their processes. This flow activates signaling molecules in the cells that are able to regulate the activity of effector cells, that is, osteoblasts and osteoclasts.[18] In an attempt to understand how osteocytes transduce a mechanical signal into a chemical response, several groups suggested that cytoplasmic processes are more mechanosensitive than cell bodies,[19–22] as cytoplasmic processes present a more robust structure relative to the cell body. Indeed, their cytoskeleton is composed by antiparallel actin filaments cross-linked by alpha-actinin, whereas the cytoskeleton of the cell bodies is constituted by actin bundles cross-linked by fibrin.[23,24] Nonetheless, osteocytes cell bodies have also been shown to be mechanosensitive.[25] Such cytoskeletal structure, the shape of the cell bodies and their orientation are influenced by mechanical forces acting on a specific bone.[26] The orientation of osteocytes according to loading direction, has previously demonstrated bone's response mechanical forces.[18] Osteocytes have also been characterized by a focal adhesion as multiple actin-associated proteins that bind cytoskeletal network to extracellular matrix. As such, those proteins are differently distributed across osteocytes in different bones, further supporting the adaptive capacity of these cells to mechanical loading directions.[26] The interstitial fluid flow mechanically induced, stabilizes beta-catenin in osteocytes because of a focal adhesion kinase, which mediates intracellular signaling.[27]

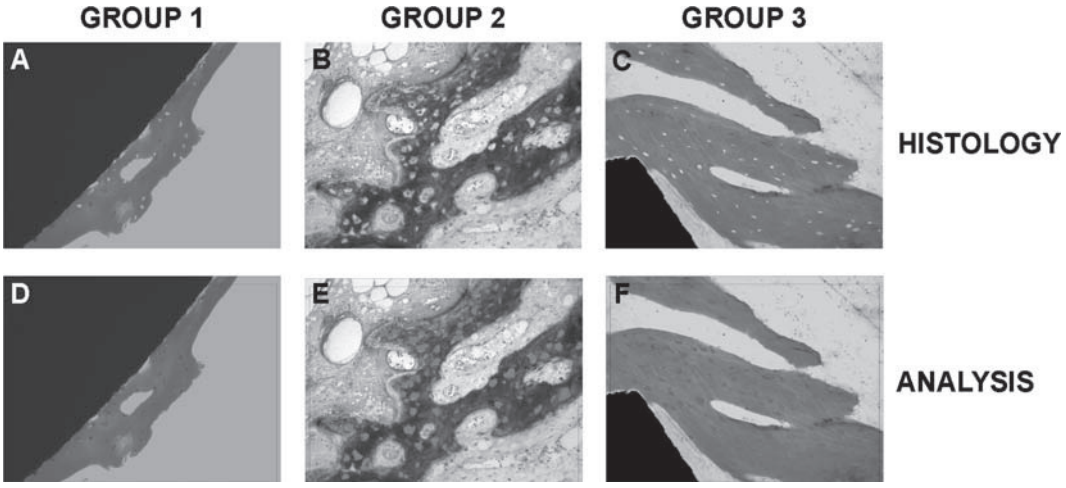

FIGURE 2. **(A)** Histological image showing low osteocyte density in the peri-implant bone tissue of Group 1 samples retrieved after a loading period of 4 weeks–7 months. **(B)** Histological image of high osteocyte density observed in peri-implant bone tissue of Group 2 samples retrieved after a loading period of 1–5 years. **(C)** Histological image of low osteocyte density observed in peri-implant bone tissue of Group 3 samples retrieved after a loading period of 14–27 years. **(D-E-F)** Image showing how the count of the number of osteocytes was undertaken. Osteocytes lacunae were highlighted in red. Toluidine blue and acid fuchsin; original magnification 100×.

TABLE II. Mean Values ± Standard Errors of Osteocyte Density in the Examined Groups

Group 1 (4 weeks–7 months)	Group 2 (1–5 years)	Group 3 (14–27 years)
415.26 ± 129.42 #/mm^2	1620.01 ± 281.81#/mm^2	426.05 ± 128.96#/mm^2
– Dunn's Multiple Comparisons Test		
– GROUP 1 versus GROUP 2	**	$p < 0.01$
– GROUP 1 versus GROUP 3	ns	$p > 0.05$
– GROUP 2 versus GROUP 3	*	$p < 0.05$
– *significance		

The transduction of mechanical stimuli would then initiate by the slide of actin microfilaments in the osteocytes processes, which would open stretch-activated ion channels altering the intracellular ionic concentration. The interconnection of cells in the osteocyte network plays a central role in the transmission and propagation of chemical signals. Even though the mechanical loading induces biological responses and adaptation of bone mass, the real key player for transduction of stimuli into signal activating osteocytes remains unknown.[18] Once mechanical stimulation is applied, signaling molecules produced by osteocytes induce and regulate osteoclast and osteoblast activities, thereby modulating bone modeling/remodeling.

The surface of osteocytes, as pre-osteoblastic cells, express a factor RANKL that bind its receptor RANK on surface of pre-osteoclastics: this interaction plays a key role for differentiation, activation and survival of osteoclasts. The osteoclastogenesis is inhibited by OPG expressed on mature octeocytes.[28] It has been recently demonstrated that apoptotic osteocytes do not produce pro-osteoclastogenic signals unlike nonapoptotic osteocytes nearby (100–300 μm) the microdamage area, which express RANKL and OPG that increase as a function of distance from the affected zone.[29] Neighboring osteocytes release, in addition, VEGF for enrolment of capillaries needed for remodeling. Those findings show the direct role of osteocytes in osteoclasts stimulation for bone remodeling. Osteocytes apoptosis is possibly dependent on different factors and it has been postulated that mechanical stimulation and the induced fluid flow contributes to metabolites and oxygen transport through the lacuno-canalicular network, resulting in a decrease of apoptosis rate. The importance of mechanical loading for osteocyte survival is of key importance[30] and thus lack of loading is a stimulating factor for osteocyte apoptosis.[31]

In addition, it was shown that decline in osteocyte lacunar density in human cortical bone is associated with the accumulation of microcracks and increase in porosity with age.[32] Generation of microdamage induces interruption of fluid flow, loss of nutrition, hypoxia and detachment of osteocyte cells from extracellular matrix[30]; all those factors stimulate osteocyte apoptosis contributing to bone remodeling.

Not many studies of osteocyte density in the oral tissues are currently available in the literature. In a study of Marchetti et al.,[33] the osteocyte lacunar area was evaluated and its relationship with bone remodeling determined at different healing time points (70 and 180 days). No differences were observed between the two groups. Comparing immediate loaded and non loaded dental implants after a healing period of 8 weeks, a higher osteocyte density (0.068 cells / μm^2) was demonstrated in the loaded group, versus the nonloaded group (0.029 cells / μm^2).[14] Kuchler et al.[34] had observed a time-dependent decrease in osteocyte density evaluated in newly formed bone of augmented sinuses, in a time period of 12 weeks.

Mechanical stimuli, that is, loading, is a driver of bone matrix turnover and bone remodeling. Bone remodeling is expected to occur along the direction of the stimulus in order to increase the biomechanical competence of the bone subjected to loading. Recently, a series of studies have evaluated the histomorphology[35,36] and biomechanical properties[37] of bone around plateau root form human retrieved dental implants, which were in function early after surgical placement to over a few decades, demonstrated that both hardness and elastic modulus of bone increased over time. Such property increase was observed after a period of 5 years where no increase was detected until then. The results of the present study appear to be in chronologic agreement with the work by Baldassarri et al.[37] in that bone properties do not really present an appreciable increase until several years *in vivo,* meaning that bone dynamically remodels over several years until more favorable alignment results in higher mechanical properties. The pattern described in the present study is possibly related to the fact that initial remodeling presenting woven bone up to a year will have a given osteocyte density that is primarily related to the initial healing kinetics. Shortly after 1 year up to 5 years, substantial remodeling occurs and with that remodeling a larger number of osteocytes are included in order to provide adequate homeostatic support for a condition that is aligning itself to better biomechanically bear mechanical loading.

CONCLUSIONS

The decrease in osteocyte density observed in samples that were *in vivo* for long periods of time under loading was possibly because of the fact that once the bone structure was well aligned and biomechanically competent a lower number of osteocytes were necessary to keep the tissue homeostasis under loading.

ACKNOWLEDGMENTS

This work was partially supported by the Ministry of Education, University, Research (M.I.U.R.), Rome, Italy.

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