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No evidence to indicate topographic dependency on bone formation around cp titanium implants under masticatory loading

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Abstract In vitro studies have proved the topographic dependency upon osteogenesis on titanium plate by investigating the cell-adhesion, -shape, -proliferation, -differentiation, ALP activity and osteocalcin production of osteogenic stem cells, MG36, MC3T3-E1 and wild strains of bone formative cells from animal and human. However, this in vivo study on bone growth around cp titanium dental implants under masticatory loading did not demonstrate significant difference among the different surface roughness in the range of Ra 0.4–1.9 μm, Rz 2.8–11.2 μm, Rmax 3.6–28.1 μm and Sm 2.9–41.0 μm, which was estimated by measuring the bone contacts, bone occupancies and bone bonding strengths at the implant/bone marrow interface.

It is revealed that the topographic dependency on the osteogenetic activity is apt to be covered with wide variation in bone healing potential under the clinical condition with functional biting load.

1. Introduction

In vitro studies have proved the topographic dependency upon cell-adhesion, -shape, -proliferation and -differentiation on titanium implants. The topographic effect upon the cell-differentiation from osteogenic stem cell to osteoblast has been discussed from biochemical standpoint of alkaline phosphatase activity and osteocalcin production (r-carboxyglutamic acid containing bone protein) [1–8]. These in vitro studies have been used as a method to monitor in a controlled and reproducible way when the surface properties were varied and improved [9–12]. However, in vitro data do not correlate constantly with in vivo data, i.e. in vitro study is an effective method to evaluate new materials and designs of an implant, while it frequently shows different findings from the in vivo data of animal experiments and clinical investigations [13]. The more statistical and analytical in vitro assay is the less positive substitution for animal experiments and clinical investigations. Improvements of materials and designs for implant should be evaluated in three criteria; in vitro, in vivo and clinical [14]. This in vivo study was performed under masticatory loading to clarify the topographic dependency on bone formation around the cp titanium implants with different surface roughness to compare with the in vitro data [8].

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2. Materials and methods

2.1. Surface roughness and experimental procedure

Rod-type implants of 2 mm diameter and 12 mm length were made of cp titanium (JIS-H4600, grade 2, Toho Titanium Co., Tokyo, Japan). Three different surface roughness of mirror-like surface, MS (Rz 0.62 $\pm$ 0.08 μm, Rmax 0.95 $\pm$ 0.25 μm), small roughness, SR (Ra 0.4 $\pm$ 0.01 μm, Rz 2.9 $\pm$ 0.16 μm, Rmax 3.6 $\pm$ 0.36 μm, Sm 2.9 $\pm$ 0.3 μm) and large roughness, LR (Ra 2.0 $\pm$ 0.12 μm, Rz 11.2 $\pm$ 0.58 μm, Rmax 29.1 $\pm$ 8.6 μm, Sm 39.2 $\pm$ 9.1 μm) were provided for the 8mm length of endosseous part of the implant root by barrel polishing, hydrofluoric acid etching, corundum blasting and hydrofluoric acid + hydroperoxide post pickling (Fig. 1)

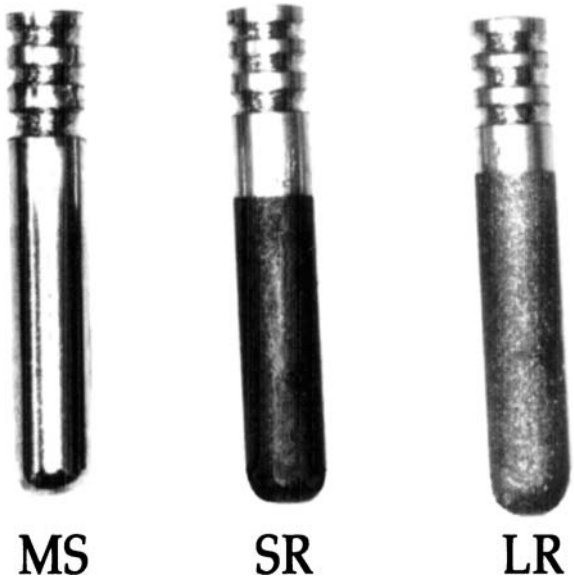

Fig. 1 Rod type implants of cp titanium, 2 mm diameter, 12 mm length with large surface roughness of 8 mm length (LR), small roughness (SR) or mirror-like surface (MS).

[8]. The surface topography was investigated by scanning electron microscopy (S-800, Hitachi, Tokyo, Japan) and mechanical stylus profilometry (Tokyo Seimitsu, Tokyo, Japan) on the titanium plates treated with the same method to the rod-type implants. Each of six implants with three different surface roughness MS, SR and LR, the total of thirty-six implants was installed into the jawbone six months after the extraction of P_3 and P_4 of four beagles from 4 to 6 years old, weighing 10.8 to 12.4 kg and the health condition certified and ethic permission granted by the Ethics Committee on Animal Experimentation, Ehime University School of Medicine. One day after the implantation, the implants were fixed with a temporary hard resin bridge (Plastique, Shofu Co., Kyoto, Japan) by connecting to the proximal teeth of P_2 and M_1. One week post-implantation, the hard resin bridge was removed and replaced with superstructure of a metallic crown bridge made of type IV Au-Pd-Ag alloy (Fig. 2). Surgical procedures for the installation were carried out under general anaesthesia. The mucoperiosteum was elevated from the jawbone and each implant hole had the interval of 3–4 mm to the next. A low speed drilling device at 1000 rpm were used (Microdispenser-8000, Implatex, Tokyo, Japan) under cooling irrigation of phosphate buffer solution (Hanks solution 1000 ml, penicillin 50000 IU and streptomycin 0.05 g, Nihonseiyaku Co., Osaka, Japan). Finally, the implant holes were irrigated with KN solution (NaCl 7.0 g, KCl 0.2 g, NaH_2PO_4 1.15 g, $CaCl_2$ 0.1 g, $CaCl_2$ 0.1 g , d-glucose 2.0 g, penicillin 1000 IU, streptomycin 0.02 g, ascorbic acid 0.05 g, dexamethasone 4 mg, aqua dest. 1000 ml) [15]. The implants were installed with press-fit into the holes [16] and mucoperiosteum flap and exposed jaw-

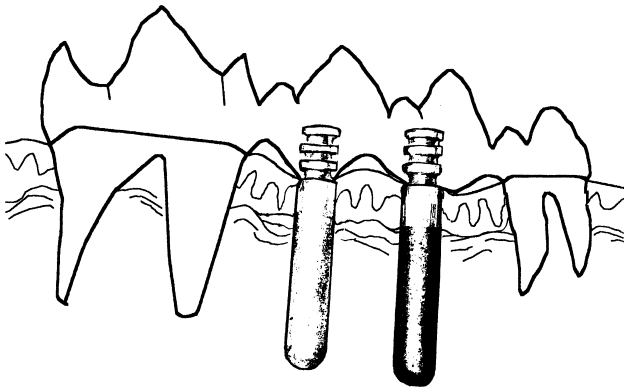

Fig. 2 Two rod-type implants combined to the proximal teeth of M_1 and P_2 with superstructure made of Au-Pd-Ag alloy.

bone were disinfected by flashing with electro-acidic water and neutralized with KN solution before the suture [17].

2.2. Bone bonding strength

After 6 and 24 weeks loading, the dogs were sacrificed under barbital anaesthesia by perfusing 3% glutaraldehyde solution regulated with 0.2 mol/L cacodylate buffer in pH 7.4 into the carotid artery. After removing the bridges, thirty-six tissue blocks including each one implant were provided and refixed with 3% glutaraldehyde. The bone bonding strength of the implant to the bone tissue was measured by push out test with universal testing machine (Shimazu, Kyoto, Japan). For the push out test, the tissue block was locked in a manner so that

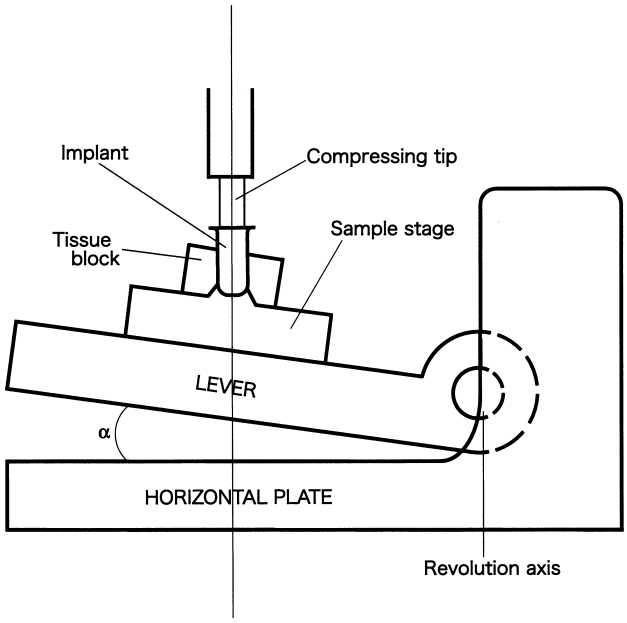

Fig. 3 Diagram of the horizontal platform for the push out test. The angle (α) was adjusted so that the long axis of the implant could be fixed perpendicular to the horizontal plate.

Table 1 Bone contact to the titanium implants with different surface roughness

Surface treatment	Surface treatment (μm)	Post-implantation	
		6 weeks	24 weeks
BP	Mirror-like	22.4 ± 9.96	74.9 ± 10.94
4HF60	Small roughness Rz 2.7 ± 0.18, Sm 2.6 ± 0.3	26.4 ± 8.29	69.8 ± 9.04
SB · 4HF120	SB · 4HF120 Rz 10.6 ± 0.53, Sm 36.0 ± 9.0	33.4 ± 7.44	66.6 ± 11.02

BP: barrel polish, 4HF60 : 4HF60 sec. and 4HF-8H_2O_2 15 sec.,
SB · 4HF120 : corundum basting and 4HF120 sec. + 4HF − 8H_2O_2 15 sec.
6 weeks : young bone contact (linear %), 24 weeks : mature bone contact (linear %),
±: sample standard deviation, n = 5.

the implant was loaded with vertical direction to horizontal plate by adjusting the angular position (α) of sample stage (Fig. 3).

2.3. Histometric investigation on the bone contact area and bone occupancy

The tissue blocks were decalcified with 5% trichloroacetic acid and the histological specimens were made by sectioning with 30 to 40 μm thickness and HE staining. The bone contact length (linear percent) to the implant surface and the bone occupancy (area percent) within 2000 μm, limits surrounding the implant were measured by a histometric method with NIH Image version 1.61 connected to a Mac G4, on phase contrast micrographs (phase contrast LWD 0.25, NIKON, Tokyo, Japan). The measurement was limited within five mm length of the implant root in the bone marrow, one mm away constantly from the endosteal edge of the cortical bone because the bone growth rate on the implant surface closely related to the distance from the endosteum of the cortical bone.

3. Results

3.1. Bone contact and occupancy

At six weeks post-implantation, osteoid young bone was formed around the implant surface. The young bone contact

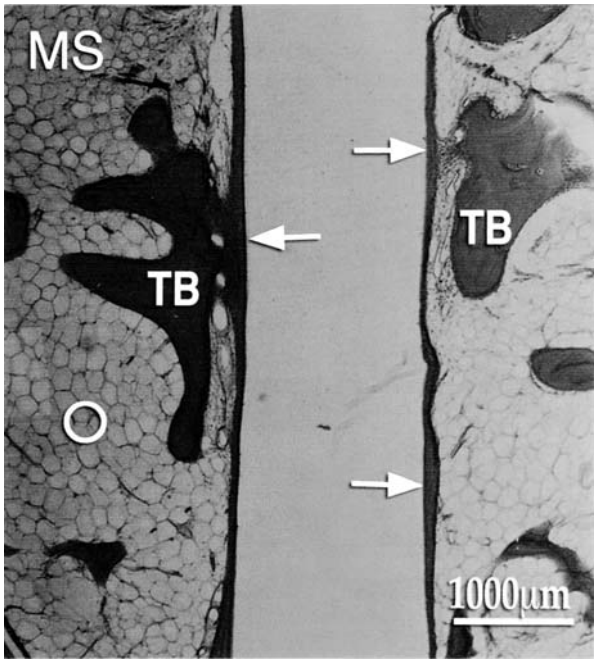

Fig. 4 Osteoid layers (↗) originate at the implant surface with migration cells from endosteum of trabecular bones (TB) in yellow marrow of fatty tissue (○). Implant : MS, 6 weeks post-implantation.

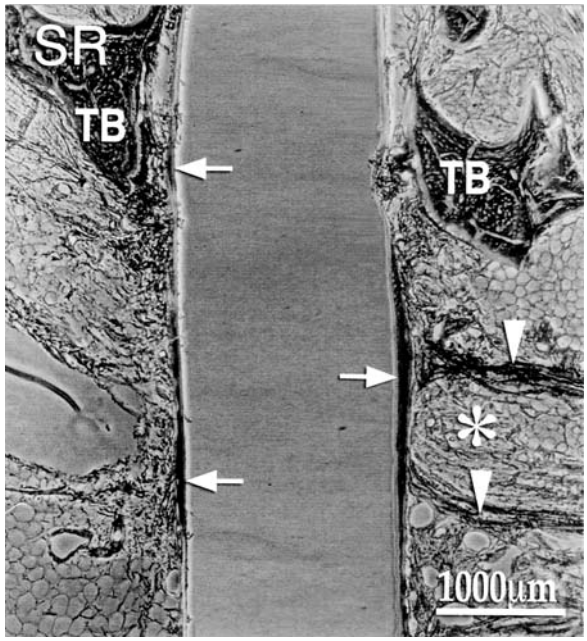

Fig. 5 Osteoid layers (↗) originate at the implant surface with cell migration from endosteum of cortical bone (△) and trabecular (TB). Implant : SR, 6 weeks post-implantation.

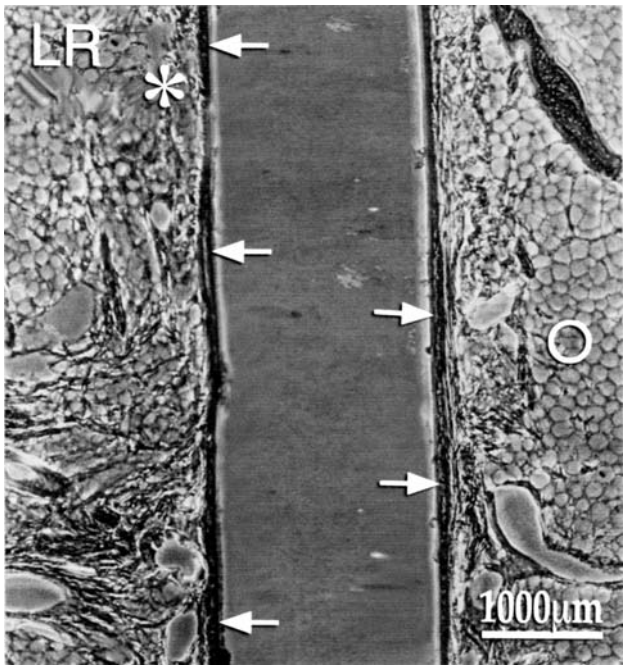

Fig. 6 Osteoid layers (↗) originate at the implant surface in yellow marrow of fatty tissue (○). Implant : LR, 6 weeks post-implantation.

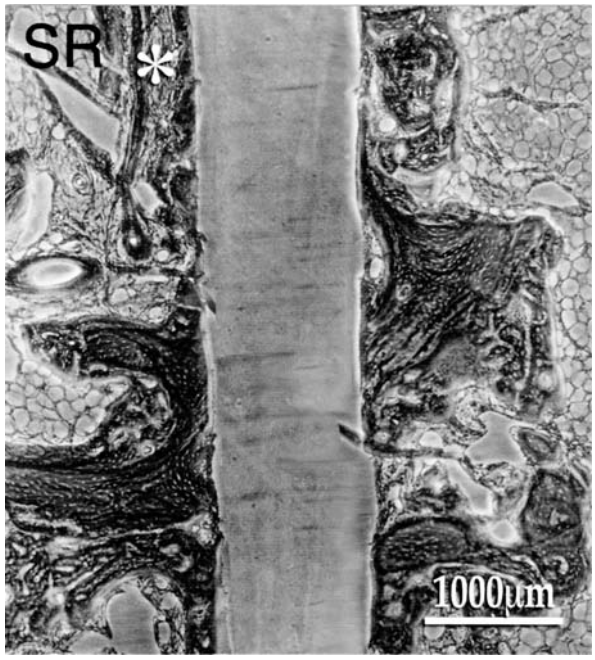

Fig. 8 Implant sheath bone covers the implant surface and turnover phenomenon of bone formation/resorption represents at the implant/bone interface in response to magnitude and direction of biting stress. Implant: SR, 24 weeks post-implantation.

to the implant surfaces was 22.4 ± 9.96% in MS, 26.4 ± 8.29% in SR and 33.4 ± 7.44% in LR, and no contact of mature bone was observed. At 24 weeks post-implantation, the mature bone contacts demonstrated 74.9 ± 10.94% in MS, 69.8 ± 9.04% in SR and 66.6 ± 11.02% in LR. No significant differences were statistically confirmed among the different surface roughness in the young bone contact at 6 weeks post-implantation and mature bone contact at 24 weeks post-implantation (Table 1 and Figs. 4–9).

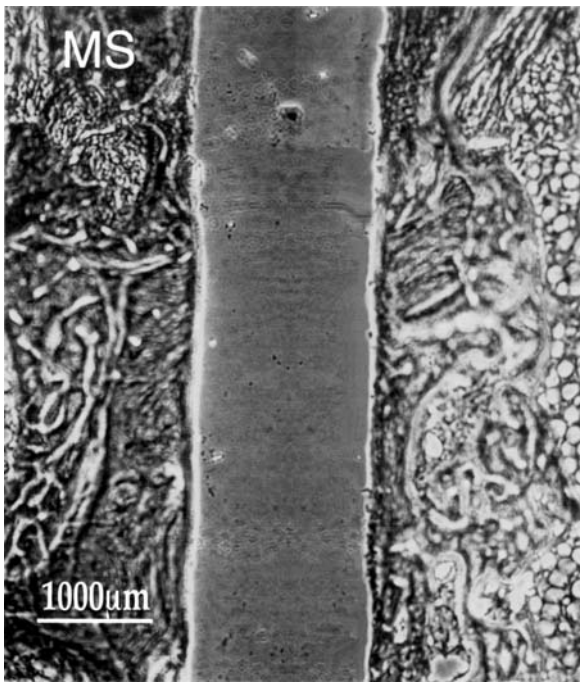

Fig. 7 Implant sheath bone covers major part of the implant surface. Fatty tissue fills outside. Implant : MS, 24 weeks post-implantation.

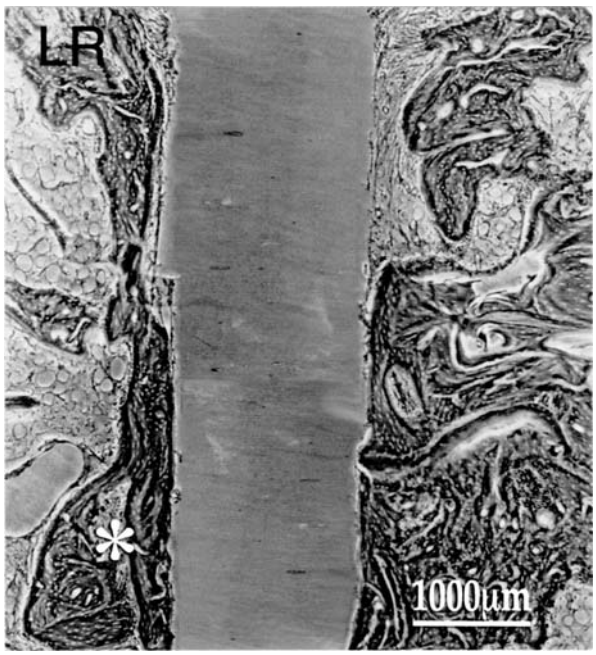

Fig. 9 Cone-shaped bone resorption occurs at the upside of implant, where most of the stress concentlation is generated, but close bone contact is kept at the apical area. Implant : LR, 24 weeks post-implantation.

Table 2 Bone occupancy around the titanium implants with different surface roughness

Implant	Surface roughness	Post-implantation	
		6 weeks	24 weeks
BP	Mirror-like	19.1 ± 5.35	65.2 ± 15.9
4HF60	Small roughness	17.0 ± 7.94	68.1 ± 16.6
SB · 4HF120	SB · 4HF120	15.9 ± 6.09	73.0 ± 12.4

Bone occupancy (area %), ±: sample standard deviation, n = 5.

Bone occupancies up to 2 mm from the implant surface were 19.1 ± 5.35% in MS, 17.0 ± 7.94% in SR and 15.9 ± 6.09% in LR at 6 weeks post-implantation, and 65.2 ± 15.92% in MS, 68.1 ± 16.62% in SR and 73.0 ± 12.4% in LR at 24 weeks post-implantation (Table 2 and Figs. 4–9). The bone occupancies increased with almost the same speed in all implants, regardless of the different surface roughness. No significant difference was confirmed statistically in the bone occupancies of three different surface roughness of MS, SR and LR.

The implants were covered with implant sheath bone at 24 weeks post-implantation. The implant sheath bone covered the majority of the implant surface and resisted functionally to biting load with turnover phenomenon of bone resorption/formation corresponding to magnitude and direction of biting stress (Figs. 7–9).

3.2. Morphological investigation

Bone formation at implant/bone marrow interface: Histological investigation at 6 weeks post-implantation represented yellow bone marrow of fatty tissue including sparse trabecular bone in all specimens because the dogs might be of old age. The implant surface was covered with cell layer migrated from endosteum of cortical bone and trabecular

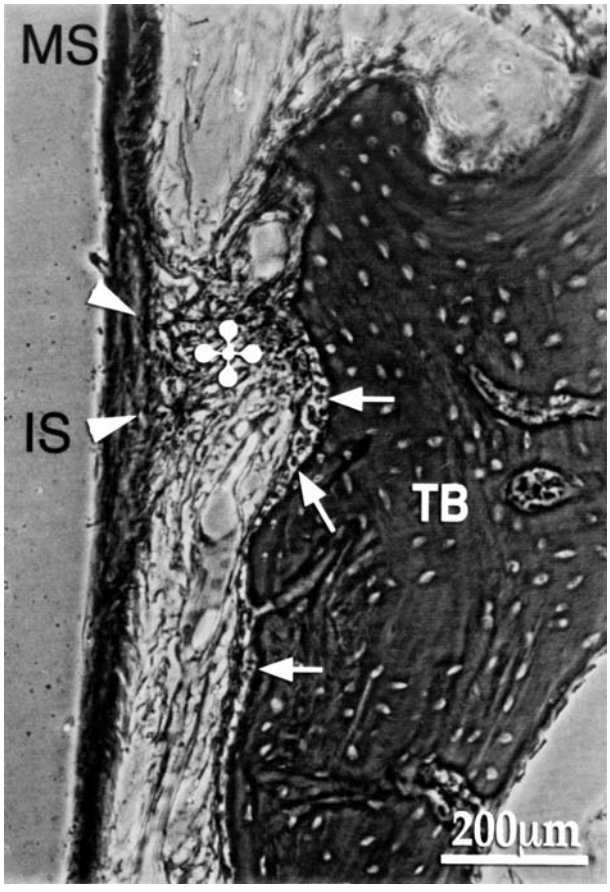

Fig. 10 Large magnification at right upper arrow in Figure 4, osteoid layer originates at the implant surface with cell migration to the implant surface from endosteum of trabecular bone (TB). IS: implant space, ✣: cell migration, TB : trabecular bone, △: osteoid layer, ↗: osteoblasts.

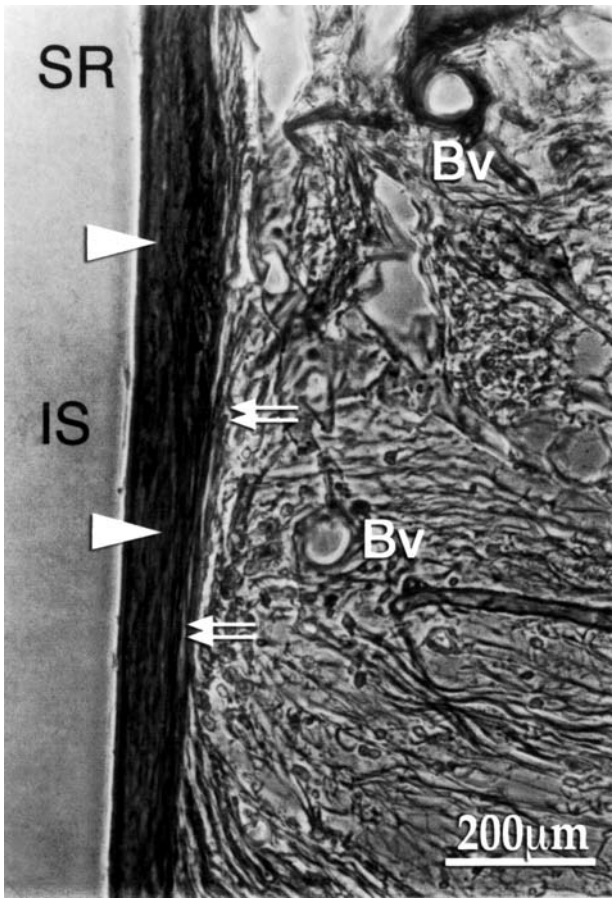

Fig. 11 Large magnification at white aster mark in Figure 5, cells migrate from the endosteum of cortical bone toward the implant surface and contact to osteoid layer (△). Implant : SR, 6 weeks post-implantation, IS: implant space, Double arrows: cell migration covers the osteoid layer, Bv: blood vessel.

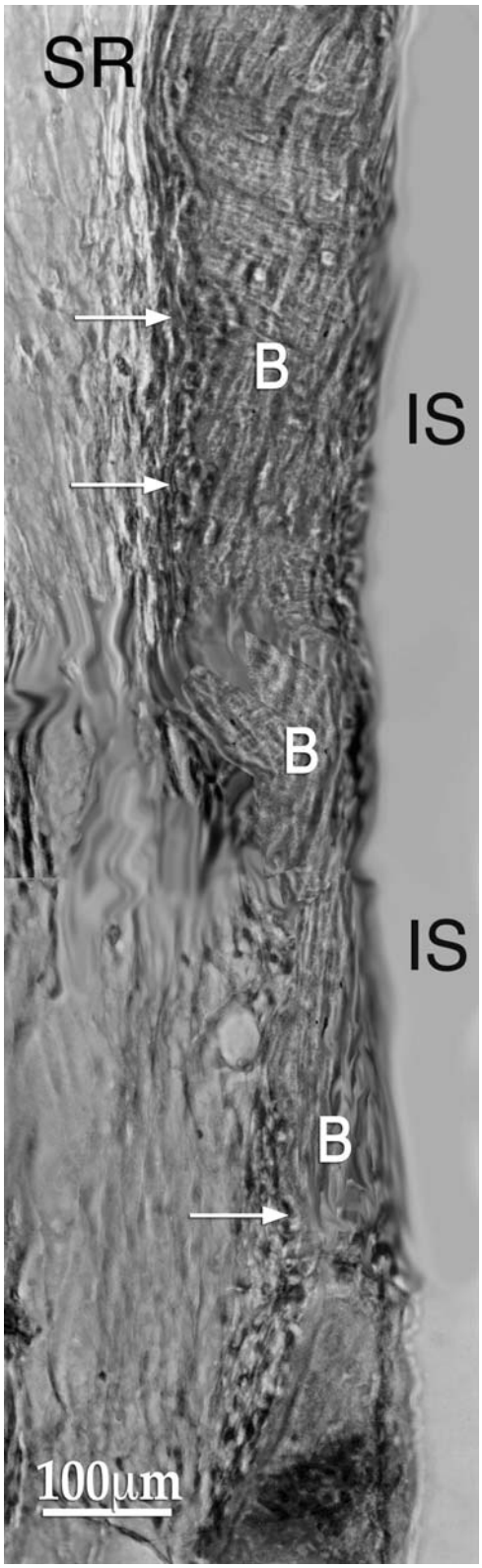

Fig. 12 Large magnification at white aster mark in Figure 8 demonstrates sheath bone formed at the implant/bone marrow interface. Bone formation/resorption in response to the stress distribution in the sheath bone caused by functional loading of mastication. Implant : SR, 24 weeks post-implantation, IS: implant space, B: sheath bone, ↗: osteoblasts.

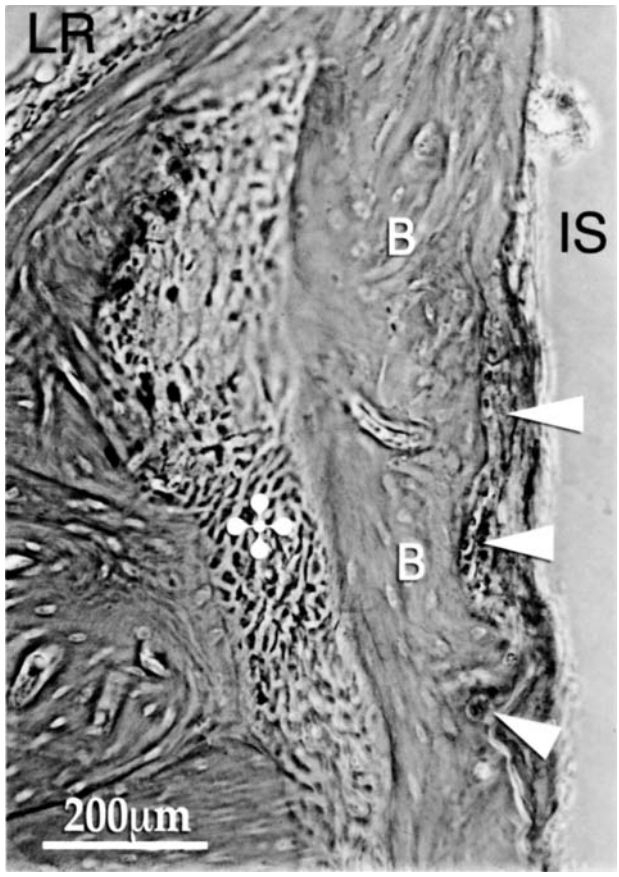

Fig. 13 Large magnification at white aster mark in Figure 9 demonstrates mechanosensitive response of bone formation and resorption at the implant/bone marrow interface, fusion of cell migration (✣) from both side of trabecular bone and implant sheath bone (B) may be a trigger for new bone formation. Implant: LR, 24 weeks post-implantation, △: osteoblast like cells, ↗: osteoclast.

to the implant surface. The cell layer could accelerate bone formation with cooperation of blood cells adhered previously to the implant surface (Figs. 10, 11).

The osteoid layer changed to mature bone with time passage after the implantation under the functional loading (Figs. 12, 13). The mature bone could cover the most of the implant surface as implant sheath bone, repeating the turnover phenomenon of bone formation and resorption corresponding to the mechanical stress distribution in the sheath bone under biting load of functional mastication (Figs. 7–9).

3.3. Bone bonding strength

Bone bonding strengths of all implants were 0–6 N at the six weeks post-implantation. At the 24 weeks post-implantation, the bonding strength increased to 27.5 ± 6.15 N in MS, 73.2 ± 33.73 N in SR and 108.2 ± 48.91 N in LR. The bonding strength of MS was lower than that of SR or LR, however no significant difference between SR and LR was

Table 3 Bone bonding strength to titanium implants 24 weeks post-implantation

Surface roughness	Bonding strength	MPa
BP : mirror-like	27.5 ± 6.15 N	0.45 ± 0.12 MPa
4HF60 : small roughness	73.2 ± 33.73 N	1.45 ± 0.67 MPa
SB · 4HF120 : large roughness	108.2 ± 48.91 N	2.15 ± 0.97 MPa

Push out test, ±: sample standard deviation, rough surface = 50.26 mm^2

represented, because of wide range of the sample standard deviations (Table 3).

4. Discussion

Martin et al. [3] suggested that implant surface roughness may play a role in determining phenotypic expression of cells from in vivo and in vitro data. Anselme et al. [4, 5] proposed an attempt at modelization of cell-surface interaction including the influence of fractal dimensions parameter and reported that cultured human osteoblasts preferred surfaces with relatively high-micro-roughness amplitude and with a low level of repeatability. Perizzolo et al. [6] indicated that surface topography and chemistry could affect osteogenesis and interactions between chemistry and topography could occur. Boyan et al. [7] reported that both Cox-1 and Cox-2 were involved in the response of osteoblasts to surface roughness with respect to the production of PGE_2 (prostaglandin dinoprostone), TGF-β1 (tissue growth factor) and osteocalcin. These in vitro studies have suggested that larger surface roughness demonstrated lower adhesive strength of cell, smaller proliferation, and higher ALP activity and BGP production compared with those of smooth surface. However, Kawahara et al. [8] reported that the accelerating effects of surface roughness upon ALP and BGP dissolved after 21 days cultivation or more in the in vitro assay, and pointed out that biostatistical attention should be paid on the clinical merit of the surface roughness by long term investigation of animal experiments under clinical like condition with functional biting load.

In vivo studies reported that rough surface generally demonstrated an increase in bone formation compared to polished surface [18–23]. The bone contact area and bone bonding strength to implant surface have clarified that rougher surface had faster bone growth, larger bone contact, and higher bone bonding strength [24–31]. Thomas and Cook examined the variables that influenced the apposition of bone at the implant surface, and reported their statement "Of 12 parameters studies, only surface characteristics had a significant effect on the integration of the implant, and rough surfaces resulted in the highest amount of bone to implant contact, whereas smooth surface had more areas of soft tissue contact with the implant surface and less apposition to bone" [25]. It has been common knowledge that rougher surface resulted in larger amount of bone contact and higher bonding strength than smooth surface. This common knowledge, however, may be ambiguous conception for clinical case, concerning the data of this in vivo study under the functional loading that demonstrated no significant difference of the bone contact and bone occupancy between the different surface roughness of MS, SR and LR (Tables 1, 2). Larsson et al. suggested that the biological response to surface roughness were much more complex than had previously been thought [32].

As the variation factors, the following conditions should be considered; species and age of animal, kind of bone (tibia, jaw, calvaria, etc.), location of investigation (cortical bone, bone marrow, cartilage) and time passage post-implantation, especially with or without load bearing. The bone healing potential in this experiment might be comparatively low level and wide variability due to old age of the experimental dogs with fatty bone marrow. The bone contact and bone occupancy were apt to be wide variation due to large fluctuation of cells migration including osteogenic stem cells through fatty marrow from the endosteum of cortical bone to the implant surface. Therefore, no significant difference is confirmed among MS, SR and LR in the bone contact and bone occupancy (Tables 1, 2), even in the bone bonding strength between SR and LR (Table 3). It is unclear what type of surface topography is suitable, because both favorable/unfavorable response to the microtopographic difference may depend on the clinical condition. Although the microroughness might control microdisplacement at implant/bone interface, the implant fixation was afforded by macroanchoring (mm level of implant shape) rather than microanchoring (μm level of surface roughness), as reported by Brunski [34], Brunski et al. [35] and Kawahara [9], which should be positively noticed in clinical evaluation for dental implants.

5. Conclusion

Under the masticatory loading, the bone growth rate and vicissitude of implant sheath bone at the implant/bone marrow interface do not demonstrate significant difference among the different surface roughness of MS (Rz 0.62 ± 0.08 μm, Rmax 0.95 ± 0.25 μm), SR (Ra 0.4 ± 0.01 μm, Rz 2.9 ± 0.16 μm, Rmax 3.6 ± 0.36 μm, Sm 2.9 ± 0.3 μm) and LR (Rs 2.0 ± 0.12 μm, Rz 11.2 ± 0.58 μm, Rmax 29.1 ± 8.6 μm, Sm 39.2 ± 9.1 μm), clarified by investigating the bone-contact area and bone occupancy at 6 weeks and 24 weeks post-implantation. On the bone bonding strength at 24 weeks post-implantation, significant difference between MS and SR or LR is proved statistically, but not significant between SR and LR.

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