Bone Regeneration by Recombinant Human Bone Morphogenetic Protein-2 Around Immediate Implants: A Pilot Study in Rats

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Purpose: *Difficulties relating to bone regeneration that complicate immediate implant placement include buccal and/or lingual fenestrations, primary anchorage of the implants, and the need for protection from functional loading during the osseointegration period. The objective of this pilot study was to evaluate bone regeneration by recombinant human bone morphogenetic protein-2 (rhBMP-2) around immediate implants placed in maxillary sockets in rats.* ***Materials and Methods:*** *A total of 16 cylindric 0.8×1.8-mm commercially pure, solid titanium implants were placed immediately after gentle extraction of the maxillary first molar teeth of 8 male Wistar rats. The sockets were randomly divided into 3 groups: group 1 (n = 6) received rhBMP-2 with polylactic acid/polyglycolic acid copolymer–coated gelatin sponge carrier; group 2 (n = 5) received only the carrier; and group 3 (n = 5) received no grafting materials following placement. The rats were euthanized at 90 days postsurgery for microscopic analysis.* ***Results:*** *In group 1, the implant body remained submerged completely, including the coronal part, which was fully covered by a significant amount (30% of total height) of regenerated cortical bone, even though the implant could easily be pulled out by a tweezer at the time of placement. Close approximation between the implant surface and regenerated bone could also be detected, indicating good bone-to-implant contact. In contrast, only peri-implant bone regeneration occurred in group 2, and an approximate 0.3-mm coronal part of the implant remained exposed. When no grafting materials were used (group 3), almost one third of the total length of the implant was exfoliated out of the socket when no grafting materials were used.* ***Discussion and Conclusions:*** *Based on previous study and data from 16 sockets of the present study, it could be concluded that rhBMP-2 facilitated the regeneration of bone around immediate implants. In particular, the bone covering the coronal part could have been regenerated shortly after surgery, which helped to maintain the implant body inside the socket during the integration period in rats.* (Int J Oral Maxillofac Implants 2003;18:211–217)

Key words: *bone morphogenetic proteins, dental implantation, dental implants, gelatin sponge, immediate implant placement*

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Placement of implants immediately after tooth extraction is a treatment modality used increasingly commonly in implant-supported oral rehabilitation.[1] To date, there have been several studies documenting this immediate implant placement technique.[1–3] Major difficulties[4,5] relating to bone

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regeneration that complicate immediate implant procedures include buccal and/or lingual fenestrations, primary anchorage of the implants, and the need for protection from functional loading during the osseointegration period. In addition, stability of the implant body during the osseointegration period and osseointegration at the coronal part of the implant may be of concern. There is always a risk of trauma to the implant-bone interface, which can compromise implant success or increase crestal bone loss. To overcome these difficulties, various techniques are being investigated and applied, and special efforts have been devoted to improving the bone-implant interface by regenerating enough bone of sufficient quality around implants. Recent reports have demonstrated bone regeneration around nonsubmerged implants placed immediately in extraction sites and supported by grafting materials or bone augmentation materials.[4–7] Some of these studies involved the use of bioabsorbable materials, which did not significantly enhance peri-implant bone regeneration in immediate implantation.

Recombinant human bone morphogenetic protein-2 (rhBMP-2) is the most actively studied of the recombinant proteins produced by recombinant technology for human bone morphogenetic proteins.[8,9] The most recent report on successful oral application of rhBMP-2 in humans is very encouraging, and it suggests that further studies of various oral applications of rhBMP-2 would be worthwhile.[10] In previous work, the present investigators were able to demonstrate that rhBMP-2 accelerated socket healing so as to preserve the cortical bone volume in rat maxillary root sockets.[11] Bone regeneration around immediate implants supported by rhBMP-2 has not previously been evaluated in animal models. Therefore, the purpose of the present study was to evaluate the bone-regenerative efficacy of rhBMP-2 around immediately placed implants in the maxillary root sockets of rats.

MATERIALS AND METHODS

The protocol for this animal experiment was approved by the Niigata University School of Dentistry's Committee on the Guidelines for Animal Experimentation. Six-week-old male Wistar rats (170 to 190 g, Charles River Laboratory, Yokohama, Japan) were housed under similar conditions (22°C room temperature, 40% humidity, and a 12-hour daylight cycle); fed commercial rat food (MF; Oriental Yeast, Tokyo, Japan); and given access to tap water ad libitum.

In this experiment, a polylactic acid/polyglycolic acid copolymer (PLGA) –coated gelatin sponge (GS) was used as the rhBMP-2 carrier (PLGA/GS).[11] The molar ratio of the PLGA polymers was 1:1, and the weight ratio of PLGA to GS was 4:1, with porosity of approximately 90%.[12]

A total of 16 maxillary first molar teeth were gently extracted from 8 Wistar rats under anesthesia, and the rats were divided into 3 groups. Group 1 (n = 6) received rhBMP-2 and the carrier in their sockets, group 2 (n = 5) received only the carrier, and group 3 (n = 5) received no grafting materials with the implants. The socket walls were delicately trimmed with a spiral-type, low-speed (500 rpm) engine bur for less than 5 seconds under sterile saline cooling, then debrided and cleaned with sterile saline so that each implant could reach the base of the socket and fit tightly. A commercially available, commercially pure titanium implant bur was used to prepare the sites for machined, solid-cylinder implants (diameter = 0.8 mm, length = 1.8 mm, rounded apices). These were placed in each of the 16 sockets, keeping the coronal part approximately 0.1 mm out of the socket. The implants showed no side-to-side movement on probing, but could be pulled out easily by a tweezer. Placement of the implant was followed by placement of the grafting materials (groups 1 and 2 only) and suturing of the gingival mucosa. The implant remained diagonally in the socket because of the diagonal anatomy of the anterior root and its socket of otherwise vertical rat maxillary first molar teeth.

The rats were provided with soft food and monitored every day for the first 2 weeks after the operation. Monitoring was continued at regular intervals over a 90-day period. Rats were sacrificed by perfusion fixation under general anesthesia as described in a previous report,[11] and block biopsies were harvested. The status of each implant was verified using scanning electron microscopy (SEM), contact microradiography (CMR), and confocal laser microscopy (CLM). For examination using the SEM, biopsy specimens were chemically treated to remove soft tissue and then dehydrated and gold-coated before examination. Conventional methods were used to embed the block biopsies in methylmethacrylate resin after fixation in 70% ethanol and Villanueva staining. Using a cutting-grinding technique, 250-µm-thick sagittal sections were obtained; these were then examined by CMR and CLM.

Regenerated bone height around the implants and the position of the implants were evaluated by a similar procedure, as described previously.[11] In breif, 3 vertical lines were drawn on an imaginary

Figs 1a to 1c *SEM images of implant sites. Im = implant; M2 = maxillary second molar tooth (original magnification ×20).*

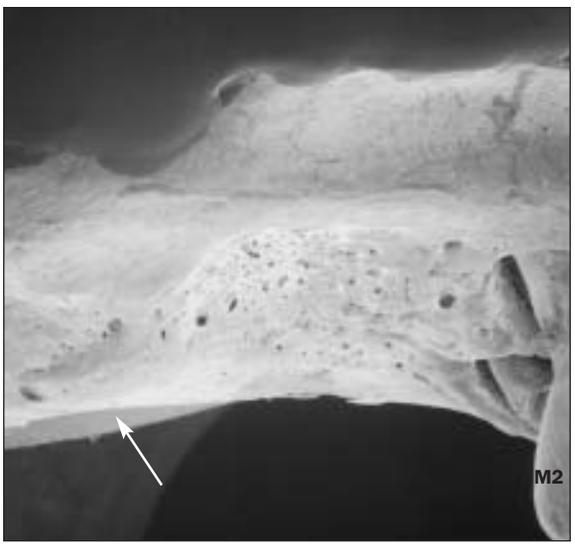


Fig 1a *(Above)* Implant with rhBMP-2 (group 1). Arrow indicates the periosteal surface of the rhBMP-2–induced bone, which kept the implant submerged.

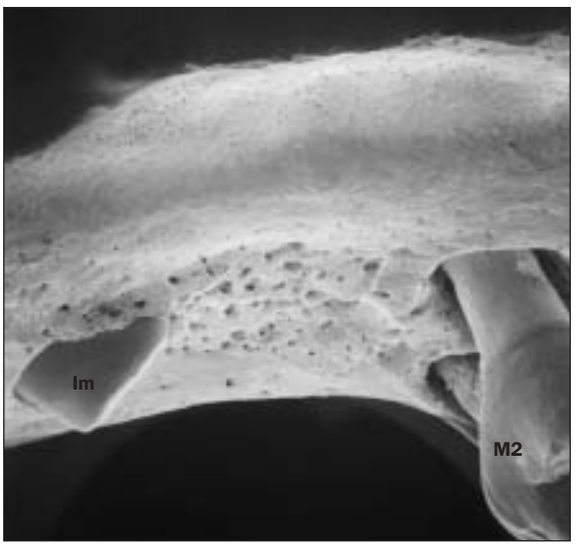


Fig 1b *(Above right)* Implant with the carrier only (group 2); the coronal part is exposed and bone regeneration is different between the 2 sides.

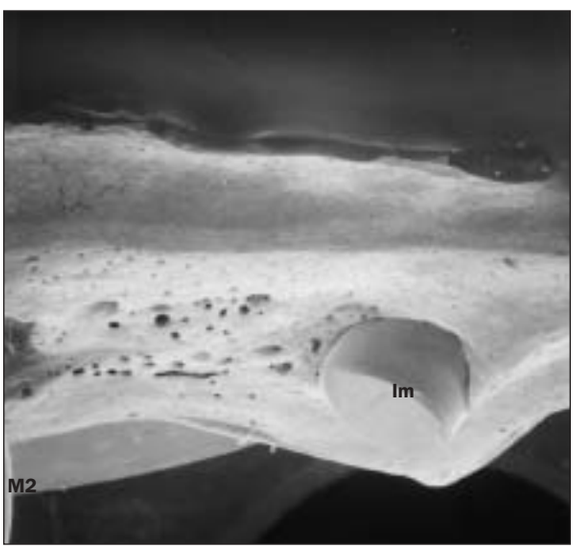


Fig 1c *(Right)* Implant with no treatment (group 3); the entire coronal part is exposed (maxilla opposite of Figs 1a and 1b).

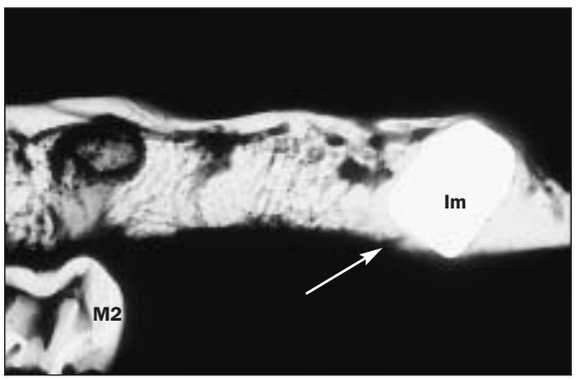
horizontal straight line along the upper border of the maxillary bone (base of the maxillary sinus) on the photomicrographs taken at the same magnification. One vertical line touched the most mesial point of the implants, while the other 2 lines passed through or ended at the coronal edges of the implants. Apical distance between the horizontal line and the apical end of the implants in all groups and the heights of the newly formed coronal bone that covered the implants in group 1 were measured on these vertical lines. Data on respective items for group 1 were compared with those of group 2 and group 3 separately by paired Student t tests, and P values $< .05$ were considered significant.

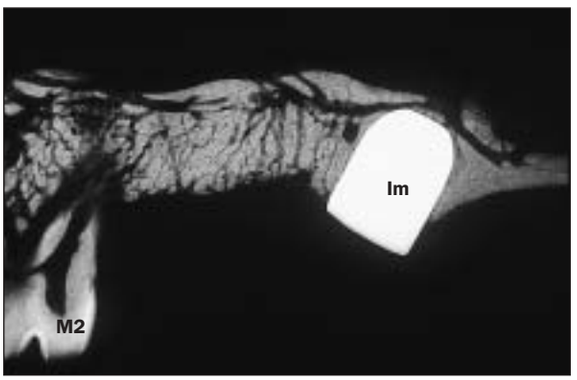
RESULTS

On clinical examination, gingival healing over the implants was uneventful; only 1 implant from group 3 was lost during the period of integration. No infection or soft tissue dehiscence was observed during the 90 days of the postsurgical period.

SEM of the implant sites displayed the fine-textured structural conditions of bone around the coronal part of the implants (Figs 1a to 1c). In group 1, it was very difficult to locate the implants, because new bone almost entirely covered the coronal part of the implants (Fig 1a). The surface of the new bone was smooth, with small osteocyte lacunae, featuring a surface structure similar to that of the adjoining alveolar bone (Fig 1a). In group 2,

Figs 2a to 2c *CMR images of sections sagittally cut from the mesiodistal direction. Im = implant; M2 = maxillary second molar tooth (original magnification ×20).*





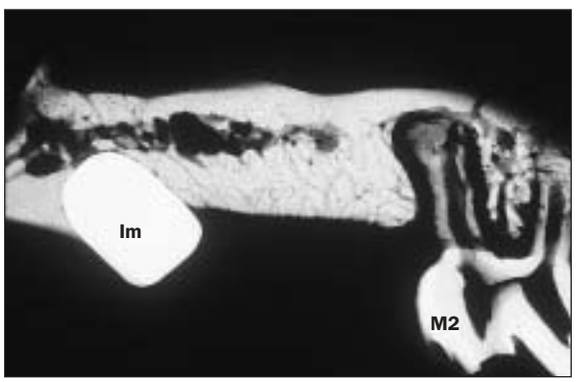


Fig 2a *(Above left)* Group 1 implant, which remained submerged at the same level as the adjoining alveolar bone; the arrowhead points to the bone that covers the implant.

Fig 2b *(Above)* Group 2 implant, which was exfoliated beyond the height of the alveolar bone.

Fig 2c *(Left)* Group 3 implant. Nearly half of each implant was exfoliated out of the alveolar bone (maxilla opposite of Figs 2a and 2b).

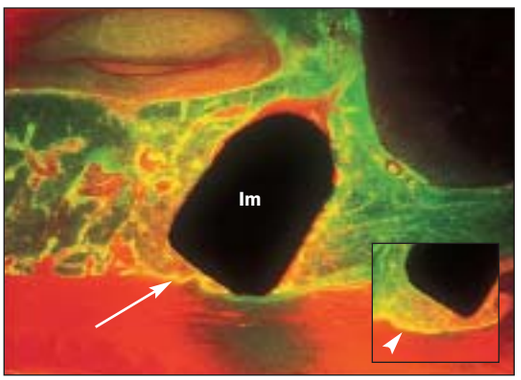
approximately 0.5 mm (average 0.3 mm) of the implant remained exposed on the mesial wall, and the alveolar crest was not firmly attached to the implant (Fig 1b). In group 3, 0.3 to 0.5 mm of the entire coronal part of the implant remained exposed, and the alveolar crest formed only a craterlike profile with the implant (Fig 1c).

CMR showed bone around the implants in group 1, with a wide range of bone-to-implant contact; here the implants apparently remained stable in the original sockets, and, as a result, the implants were the same height as the adjoining alveolar bone. The bone covering the top also was evident (Fig 2a). In group 2 implants, excluding the coronal part, bone adaptation around the implants could be detected, and the implants were well-extruded from the alveolar bone (Fig 2b). In group 3, however, nearly half of each implant body remained exposed, protruding out of the alveolar bone (Fig 2c).

Observation via CLM confirmed that Villanueva-stained mature bone covered each implant in group 1 (Fig 3a), and that bone-to-implant contact was intimate around the coronal part of the implant. Thick cortical bone having a smooth periosteal surface was also evident in another section 250 μm distal to the previous one (Fig 3a, *inset*). In group 2, bone-to-implant contact was fairly good around the apical two thirds of each implant. However, the bone crest showed craterlike defects at the neck region of the implants, and thick bone was formed at the base of the sockets (Fig 3b). Similar features were characterized at the coronal part in another section 250 μm distal to the previous one (Fig 3b, *inset*). In group 3, bone-to-implant contact was variable around the apical half of the implants, no bone regeneration occurred around the coronal half, and the base of the sockets was thicker than in the other 2 groups (Fig 3c). Craterlike defects between the bone crest and implants were present (Fig 3c, *inset*).

Implant positions were not equivalent in the different groups at 90 days after placement (Figs 2 and 3). In group 1, the average apical distance was only 0.17 to 0.64 mm, while in groups 2 and 3, respectively, it was 0.44 to 0.9 mm and 0.91 to 1.28 mm (Fig 4 and Table 1), and the differences were significant (Table 1). These data suggest that the implants were partially exfoliated because of bone formation at the base of the socket, as the usual socket healing procedure in groups 2 and 3 produced exfoliation of 0.31 mm and 0.4 mm, respectively. A significant

Figs 3a to 3c *CLM images of sections cut sagittally from the mesiodistal direction. Im = implant (original magnification ×20).*



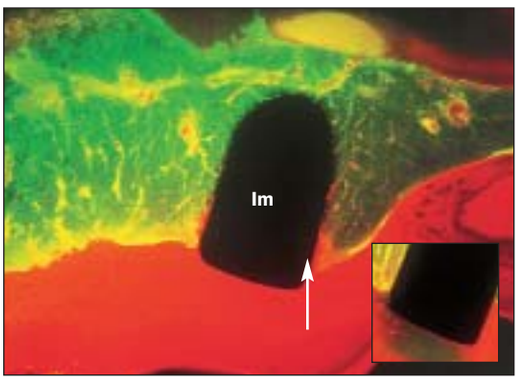


Fig 3a *(Above)* Group 1 implant shows bone regeneration with a wide range of bone-to-implant contact around the coronal part of the implant. The inset indicates thick bone with a smooth periosteal surface *(arrowhead)* submerging the implant.

Fig 3b *(Above right)* Group 2 implant shows the exposed coronal part of the implant and the bone crest that formed craterlike defects *(arrows).* Bone-to-implant contact is fairly good at the apical part of the implant, and thick bone was formed at the base of the socket. The inset shows similar features.

Fig 3c *(Right)* Group 3 implant. Nearly half of the implant is exfoliated beyond the alveolar bone (maxilla opposite of Figs 3a and 3b). The bone-to-implant contact is different from the mesial to the distal sides. The inset shows the craterlike defects *(arrow).*

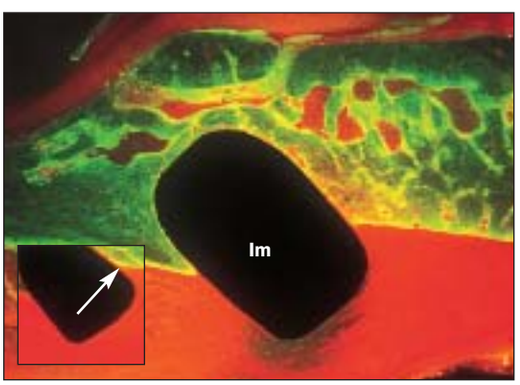


Fig 4 Diagrams represent immediate implants inside the socket and regenerated bone after 90 days. Three vertical lines (MN, OP, and QR) were drawn on an imaginary horizontal straight line (MOQ) along the upper border of the maxillary bone (base of the maxillary sinus). MN is the line touching the most mesial (x) point of the implant, while OP and QR passed through or ended at the coronal edges of the implant. Mx and Oy represent the apical distance between MOQ and the apical end of the implants in all groups, while y'P and zR represent the heights of the newly formed coronal bone that covered the implant in group 1.

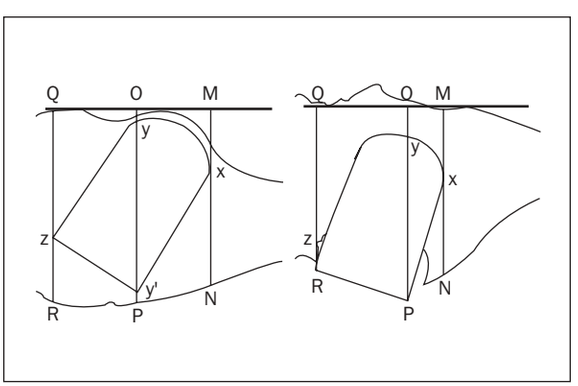


Table 1 Regenerated Bone Height (in mm) and Implant Position in All Groups

	Measurement (mm)							
Group	**Mx**	**xN**	**MN**	**Oy**	**y'P**	**Qz**	**zR**	**QR**
Group 1	0.64	1.18	1.82	0.17	0.18	1.26	0.6	1.86
Group 2	0.9*	0.88	1.78	0.44*	0*	1.82	(–)0.3*	1.51
Group 3	1.28**	0.52	1.8	0.91*	0*	1.84	(–)0.4**	1.44

Data represent the average of 3 animals from each group.
*$P < .05$ and ** $P \leq .001$ were considered significant, while data on the respective items for rhBMP-2 groups were compared with those of the other 2 groups.

amount of coronal bone was observed in group 1 only, and at 0.18 to 0.6 mm was approximately 30% of the total height (MN = 1.82 mm) (Table 1). On average, the alveolar bone crest level was 0.31 mm and 0.4 mm below the edge of the implants in groups 2 and 3, respectively. In group 1, the implant remained inside the socket probably because of the rhBMP-2–induced bone produced around the coronal part of the implant shortly after operation. A shorter apical distance (0.17 to 0.64 mm) indicating bone formation at the base of the socket possibly did not occur because of the implant being helped by quicker-forming coronal bone.

DISCUSSION

In the present experiment, as observed in previous study, rhBMP-2–induced bone formation at the coronal end of each socket kept the implant submerged in the socket, and this new bone remained unresorbed until the time of sacrifice at 90 days. In a previous report, it was documented that rhBMP-2 accelerated rat maxillary root socket healing so as to preserve alveolar bone volume (without implant).[11] In that study, rhBMP-2 induced a large amount of bone formation at the coronal end of the socket during 14 to 28 days after the operation, and the bone remodeled to a plane alveolar ridge by 84 days. In the present study, rhBMP-2–induced bone at the same location remained for a longer period and retained about 30% of the total bone height until 90 days. This result is in agreement with a recent experimental report, which indicated that significantly more bone formation occurred at rhBMP-2–treated sites within the perforations of dental implants compared to sites treated with the vehicle alone.[13] In the present study, the smooth periosteal surface and the small osteocyte lacunae resembling the adjoining alveolar bone indicated that the bone was mature and cortical. The implants that could easily be pulled out and did not have primary anchorage at the time of surgery were retained within the socket covered by newly regenerated bone in all samples in group 1. Apical distance between the implant apex and MOQ was shorter compared to other groups, about 0.4 mm on average (Table 1). Also, the thickness of apical bone in this group appeared to be the same as that observed in a fresh extraction socket in a previous experiment.[11] Thus, it might be suggested that rhBMP-2–induced bone helped the implant remain inside the socket during the integration period by restricting at least vertical movement. Perhaps a similar occurrence around submerged immediate implants without primary anchorage had not been demonstrated before.

Several reports have demonstrated the use of grafting materials or bone augmentation materials to support submerged immediate implants. However, significant enhancement of peri-implant bone regeneration around immediate implants was not shown in those experiments.[4,5] The rhBMP-2 might also have enhanced the bone regeneration so as to increase the range of bone-to-implant contact around the coronal part of the implant. In the no-treatment group (group 3), the implants were pushed out of the sockets, possibly because no bone was formed at the coronal region. In addition, bone formation from the base of the sockets resulted in a risk of exfoliation of the implants. However, in group 2, a larger part of each implant remained inside the socket than was the case in group 3. The bone-to-implant contact appeared to be better as observed in CMR and CLM studies, perhaps because the mass of the carrier worked as a cover at the opening of a socket just after the operation.

In a previous study it was also found that the carrier (PLGA/GS) was resorbed within 84 days when applied in rat maxillary root sockets.[11] The present rat model documented findings suggesting that further studies in larger animals or studies of an oral application of rhBMP-2 via PLGA/GS along with immediate implants would be worthwhile.

CONCLUSIONS

Based on a previous study and data from 16 sockets of the present study, it can be concluded that rhBMP-2 accelerated bone formation around the immediate implants. A significant amount of bone was induced by rhBMP-2 at the coronal part of the immediate implant, and this bone helped to maintain the implant body inside the socket during the integration period in rats. This technique of rhBMP-2 application around immediate implants also appeared to be useful in maintaining alveolar bone height and may thus aid in the successful immediate placement of oral implants.

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