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# Experimental Study of Bone Growth Around a Dental Implant After Surgibone Grafting

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Histologic and histomorphometric results of bone growth around titanium alloy screw-type implants after Surgibone grafting in New Zealand white rabbits are presented. At 21 days, new bone was formed along the surface of the implant. At 84 days, newly formed bone replaced almost all of the trabecular bone of the graft and reached the shoulder level of the implant. There was a higher percentage of host bone area at 84 days than at any of the earlier experimental periods ( $P < .01$ ). The average mineral apposition rates ranged from 1.82 to 2.35  $\mu\text{m}/\text{day}$  in original bone and 2.55 to 2.80  $\mu\text{m}/\text{day}$  in newly formed bone. The results suggest that Surgibone grafting in combination with dental implants can be used to increase the height of the recipient bone and therefore aid in the fixation of the implant in this animal model.

(INT J ORAL MAXILLOFAC IMPLANTS 1999;14:889-897)

**Key words:** dental implant, fluorochromes, grafts, histomorphometry

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**B**one is the basic foundation for all dental implant systems. It provides stability for implant devices and the prostheses that they support. For the extremely resorbed edentulous arch, few methods can improve the quality of the bone, but it is possible to increase the quantity of bone with reconstructive surgery, including various grafting procedures. Two main methods of increasing

and optimizing denture-bearing areas have been used, particularly before the era of dental implants: vestibuloplasty and ridge augmentation procedures. Although some patients have benefitted from these traditional surgical procedures, clinical results have often been disappointing, especially in very atrophic jaws. Therefore, interest has recently been increasingly focused on dental implants or bone grafts in combination with dental implants. Fresh autogenous bone grafts in conjunction with dental implants have been applied to patients with insufficient width and height of the residual alveolar or basal bone.<sup>1-5</sup> However, this is an expensive procedure that requires hospitalization, as well as the potential risk for donor site morbidity. Xenografts have been used as a method to avoid the second operative site. These have been largely deproteinized bovine bone (Keil Bone, Braun Milsungen, Milsungen, Germany; Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland; and Surgibone, Unilab Surgibone, Mississauga, Ontario, Canada).

The present study focused on evaluating bone growth around the dental implant under light and fluorescent microscopy after Surgibone grafting. The possibility of clinical application of this bone substitute in combination with dental implants for advanced jaw resorption was addressed.

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## Materials and Methods

**Experimental Grouping.** New Zealand male rabbits (Riemens Fur Ranches, St. Agatha, Ontario, Canada), weighing an average of 3 to 3.5 kg, were used for the experiment. Ten rabbits were divided into 5 groups of 2 rabbits each, depending on their time of sacrifice at 21, 28, 35, 42, and 84 days after grafting. They were fed 17% rabbit ration pellets (Shur-Gain, B. W. Feed, New Hamburg, Ontario, Canada) and water ad libitum.

**Operative Procedures.** After rabbits were given an intramuscular injection of 0.75 mL ketamine hydrochloride (Park-Davis, Scarborough, Ontario, Canada), general anesthesia was maintained by inhalation of 2% isoflurane (Abbott Laboratories, Montreal, Quebec, Canada) using a mask. The medial skin of both legs was shaved. Thereafter, the operative fields were prepared with 10% providone-iodine (Purdue Frederick, Pickering, Ontario, Canada) and isolated with sterile drapes. Local anesthesia was supplied by 2% lidocaine hydrochloride with a 1:100,000 epinephrine vasoconstrictor. A skin incision about 3 cm in length was made on the anteromedial area of the right tibial metaphysis. The dissection continued in layers to the bone. The cortical bone of the medial aspect was roughened by a dental bur under continuous saline irrigation. A compound plate of Unilab Surgibone (Unilab Surgibone, Mississauga, Ontario, Canada) measuring 50 × 20 × 5 mm was divided with a sharp fissure bur into 5 plates (blocks), each measuring 9 × 20 × 5 mm. Preparation of the implant site was accomplished using a series of drills and a tap under continuous irrigation. The hole passed through the graft into the tibial bone. The spongy surface of the Surgibone block was faced to the roughened recipient area, the cortical aspect of the block was placed superiorly and fixed by a titanium alloy screw-type implant (University of Toronto, Ontario, Canada). The fascia was closed with 3-0 chromic gut suture, and the skin was approximated by interrupted 3-0 black silk sutures; the same procedure was performed on the left side. Wounds were sprayed with gentomycin and left without covering.

**Polyfluorochrome Labeling Schedule.** The bone was labeled using 3 different fluorochromes. Tetracycline hydrochloride (Sigma-Aldrich, Oakville, Ontario, Canada) was administered via an intravenous injection at a dose of 20 mg per kg body weight at days 1, 21, 42, and 63 of the experiment. Xylenol orange (Sigma Chemical) was given subcutaneously at a dose of 90 mg per kg body weight at 7, 28, 49, and 70 days. Alizarin com-

plexone (Sigma Chemical) was given subcutaneously at a dose of 30 mg per kg body weight at 14, 35, 56, and 77 days.<sup>6</sup>

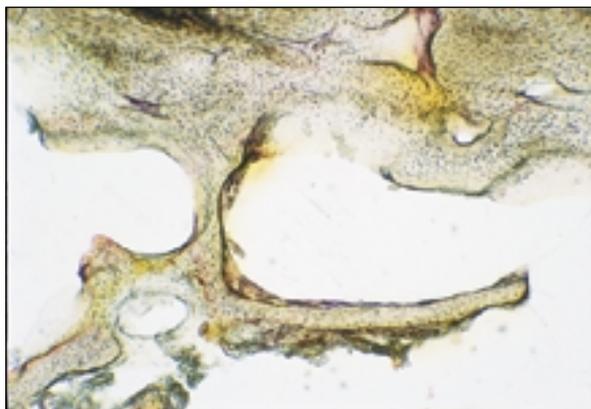
Unilab Surgibone is derived from specially selected adult cattle. After processing, the chemical composition is similar to hydroxyapatite but contains 20 to 29% protein. The microscopic structure of the bone is preserved with large trabecular spaces (Fig 1). This xenograft can readily be distinguished from host bone since it does not take up the fluorochrome label (Fig 2).

**Preparation of Microsections.** *Fixation and Dehydration.* The rabbits were euthanized by an intravenous overdose of pentobarbital sodium at 21, 28, 35, 42, and 84 days after the operation, respectively. Segments of the tibia with Surgibone blocks were removed en bloc with a power saw. Fixation and dehydration were accomplished by using ascending grades of ethanol.

*Infiltration and Embedding.* After dehydration, the specimens were infiltrated and embedded in methylmethacrylate resin.<sup>6</sup> The methylmethacrylate monomer was inhibited with 10 ppm hydroquinone 1000 mL (Rohn and Haas, Philadelphia, PA) and perkadox-16 1.0 G (Nourv Chemical, Burt, NY). When polymerization was completed, the specimen was removed from the vial, and the block was ground to a convenient size using 180-grit silicon carbide paper on the grinding machine (PLANOPOL-2, Struers Co, Copenhagen, Denmark) under water lubrication.

*Sectioning and Grinding.* Sectioning was accomplished by using a low-speed saw (Buehler, Lake Bluff, IL) with a diamond wheel (Norton Co, Worcester, MA). The block was first sectioned into symmetrical halves along the implant's major axis. The surface of each of the halves was polished on 800-grit silicon carbide paper under water lubrication to remove any cutting marks, followed by 4,000-grit to obtain a highly polished block surface. A standard microscope glass slide was glued to the polished block surface using a clear epoxy resin adhesive. After 24 hours, the block with the glass slide attached was returned to the sectioning machine and was sectioned parallel to the glass slide, so that a section about 0.1 mm in thickness attached to the glass slide was obtained. The section was ground and polished to a thickness of approximately 30 µm. One unstained 30 µm section was evaluated using fluorescent microscopy. Another section stained with Stevenel's blue and Van Gieson picro-fuchsin<sup>7</sup> was evaluated using light microscopy.

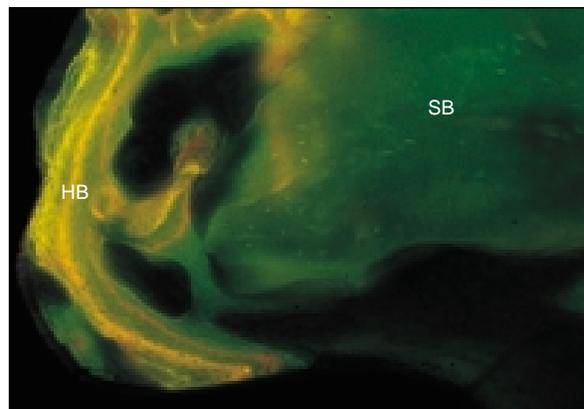
**Histologic and Histomorphometric Observations.** Both the stained sections and unstained sections were examined using a Leitz dialux 22 micro-



**Fig 1** In vitro sample of Surgibone (magnification  $\times 37$ ).

scope (Leitz, Wetzlar, Germany). The static and dynamic histomorphometry parameters were accessed using the morphometry and densitometry program of the Bioquant Meg IV morphometric system (R & M Biometrics, Nashville, TN). Mineral apposition rates were measured using a 100-watt mercury AC (HBO-100) source stabilized by a voltage power supply (IREM model E3-XH5P/L). A  $10\times$  NPL fluotar oil immersion objective (NA 0.45) was used, as well as an I $\frac{1}{2}$  filter block (excitation 450 to 560 nm, emission 515 nm). With this filter block all fluorochromes were seen simultaneously. The filter block is not optimal for viewing the xylenol orange and alizarin-complexone; however, for the purpose of measuring the appositional rate it was satisfactory. The images were accessed on a Dage-MTI series 70 video TV camera (Dage-MTI Inc, Michigan City, IN) equipped with a Newvicon grade 1 tube (resolution: 315 lines/cm). The distances between the different fluorochrome labels, within 0.5 mm from the tips of the dental implant thread, were measured, and the mineral appositional rates were calculated for original bone (OB) and newly formed bone (NB).

The tissue-implant interface of the stained sections was examined under light microscopy. The host bone, Surgibone, and soft or marrow tissue areas were measured for 12 threads, 6 consecutive threads (1 through 6) at each side of each implant. For each thread a total area of 660 mm<sup>2</sup> was measured (objective used  $2.5\times$ , NA 0.08). The percentages of host bone, Surgibone, and soft or marrow tissue were calculated for each thread level and for the total implant. The *t* test was used for statistical analysis of the different groups.

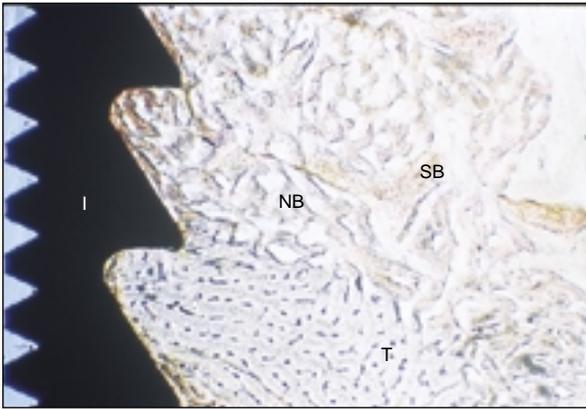


**Fig 2** Surgibone (SB) shows no fluorochrome labeling, while the host bone (HB), which is vascularized, is clearly labeled (magnification  $\times 74$ ).

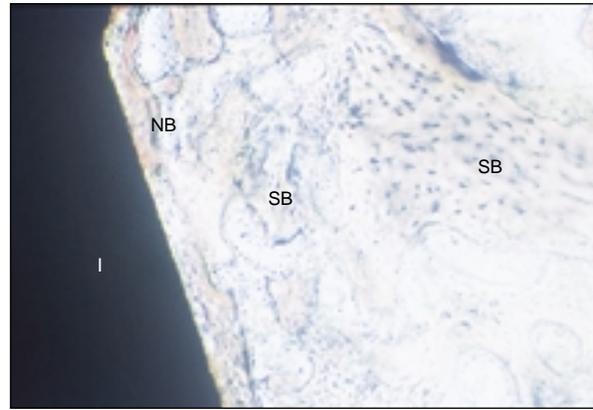
## Results

At 21 days after the initial surgery, the superior portion of the dental implant was in contact with the Surgibone. The middle portion was surrounded by newly formed bone, segments of Surgibone trabeculae, and fibrous tissue. The inferior portion was in direct contact with tibial cortical bone and resided in the marrow cavity. The majority of new bone originated from the host bed, which was growing within the spongy spaces of the Surgibone and along the dental implant (Figs 3a and 3b). There were numerous osteoblasts and some osteoclasts adjacent to the Surgibone trabeculae (Fig 3c). The bone around the inferior part of the implant was mostly mature lamellar bone of the tibia (Fig 3d). A high turnover rate was observed in both the host bone and the Surgibone.

At 28 and 35 days, new bone partially replaced or enveloped the trabecular bone of the Surgibone. In some sections, newly formed bone was observed in direct contact with the implant (Figs 4a and 4b), but at 35 days, there seemed to be absorption of newly formed bone and more abundant fibrous tissue between new bone and the cortical bone of the xenograft. Also at 35 days, the graft was being replaced with fibrocellular stroma, the new bone formation began in this fibrocellular tissue. At 42 days, there was a tendency to resorb more Surgibone trabeculae and to form more new bone around the implant (Fig 5a). Bone-to-implant contact and interface remodeling were often seen at the original tibial level (Figs 5b and 5c).



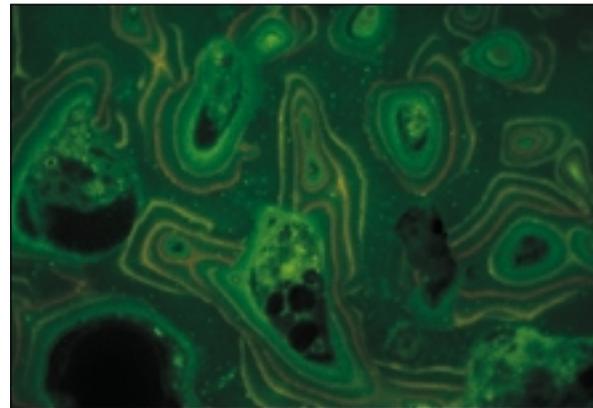
**Fig 3a** Substantial new bone (NB) ingrowth from the surface of the tibia (T) into the trabecular spaces of the Surgibone (SB). The implant threads are shown (I) (magnification  $\times 25$ ).



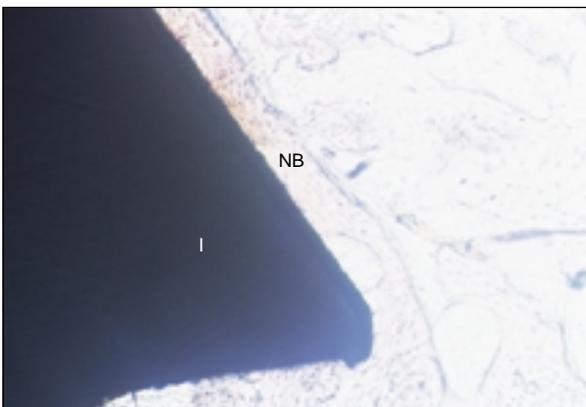
**Fig 3b** Higher magnification of Fig 3a. New bone (NB) is growing along the dental implant (I), and the Surgibone (SB) trabeculae are partially absorbed (magnification  $\times 100$ ).



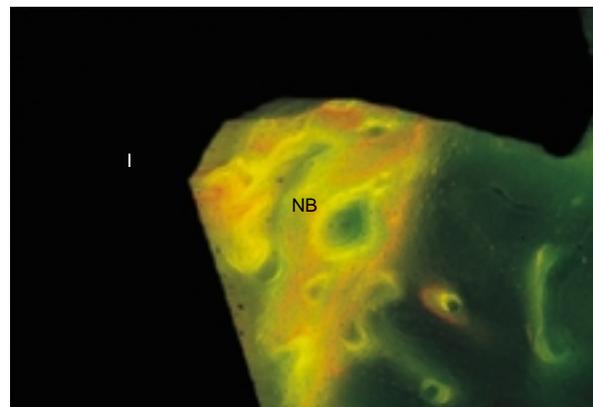
**Fig 3c** Higher magnification of Fig 3b showing newly formed trabeculae (T) lined continuously by osteoblasts (OB) with plump cytoplasm and focally by osteoclasts (OC) (magnification  $\times 200$ ).



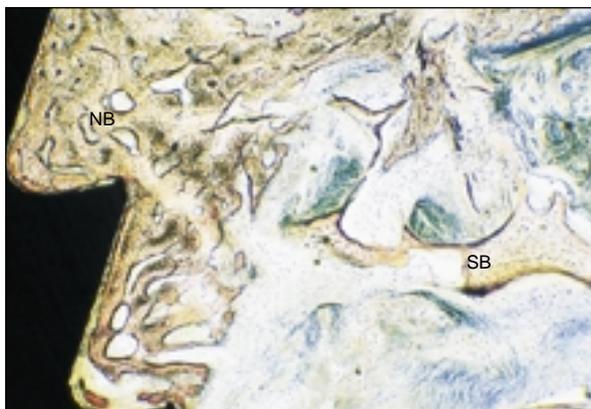
**Fig 3d** Fluorochrome-labeled mature lamellar bone of the tibia (magnification  $\times 74$ ).



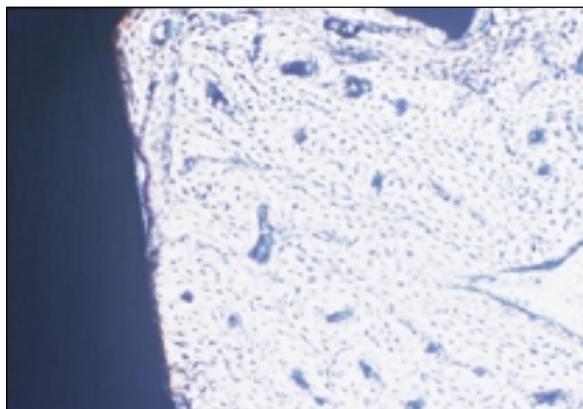
**Fig 4a** Newly formed bone (NB) is seen in direct contact with the implant (I) at the fourth thread level (magnification  $\times 100$ ).



**Fig 4b** Fluorochrome-labeled newly formed bone (NB) is seen in direct contact with the implant (I) (magnification  $\times 74$ ).



**Fig 5a** At the 42-day time period, resorption of Surgibone (SB) is quite evident, along with new bone (NB) formation around the implant (magnification  $\times 37$ ).



**Fig 5b** Light photomicrograph showing direct bone-implant interface with small soft tissue interface between the bone and the implant. As well, the formation of small marrow cavities in the tibia can be observed (magnification  $\times 100$ ).

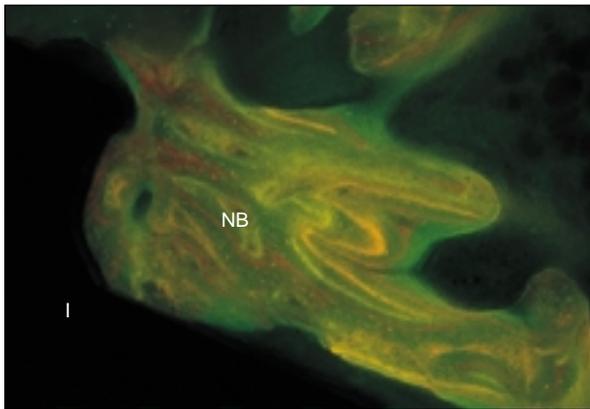
At 84 days, the dental implant had been mostly surrounded by newly formed bone, and part of the new bone had been transformed into relatively mature lamellar bone. However, the implant-bone interface was still subject to continuous bone formation and remodeling. The marrow spaces were filled with new bone, and occasionally small marrow cavities in the new bone were in direct contact with the implant (Fig 6a). Replacement of the cortical part of the Surgibone graft by newly formed bone was also taking place (Fig 6b). In addition, new bone was observed in the gap between the shoulder of the dental implant and the graft (Fig 6c). Bone around the inferior portion of the dental implant had thinned or had been partially absorbed. As a result, bone-to-implant contact in the inferior part of the implant migrated upwards on one thread at the end of the experiment, when compared with that of earlier time periods of the experiment. However, replacement of the Surgibone by newly formed bone resulted in an increase in host bone area. Also of note was the absence of fibrotic marrow and the general normal appearance of the marrow. The percentage of bone area for the whole implant was higher at 84 days than at earlier experimental periods (Fig 7) ( $P < .01$ ). Experimental differences were also found between 21 days and 35 days, 35 days and 42 days ( $P < .01$ ), and between 28 days and 35 days ( $P < .05$ ). When the percentages of bone area for each thread level (1 through 6) were compared, significantly more bone was observed in the 4 upper thread levels at 84 days than that at earlier experimental time periods (Table 1). At 35 days, there was less bone (except for the sixth thread) than at other



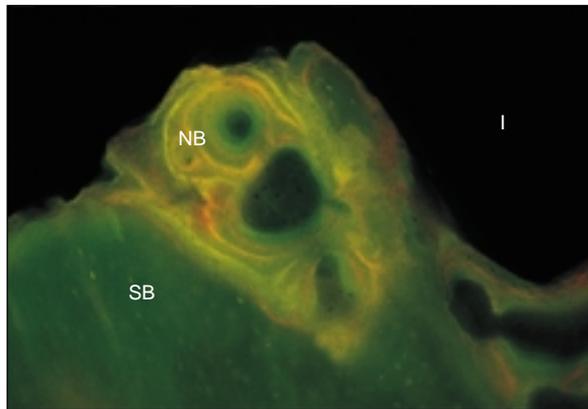
**Fig 5c** Gross specimen at 42 days. Surgibone is secured to the surface of the tibial metaphysis by the implant. New bone growth in the alloplastic graft increases the height of bone from the host along the implant surface.

experimental time periods. The total observed bone area decreased from 21 days to 35 days and then increased from 35 days to 84 days. The graft area followed the opposite pattern, indicating replacement of the Surgibone by the host bone.

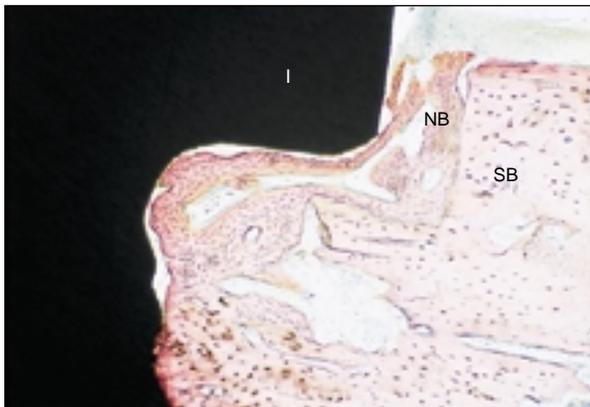
From Tables 1 to 3, it was noted that at the first 4 experimental periods, the Surgibone appeared mostly around the 2 upper threads; the soft tissue and remaining Surgibone were predominantly in the third and fourth thread levels; while the tibial bone was in the fifth and sixth thread levels. In contrast, at 84 days, the Surgibone in contact with the upper portion of the implant was partially replaced. The graft and soft tissue in the middle portion of the implant was almost completely



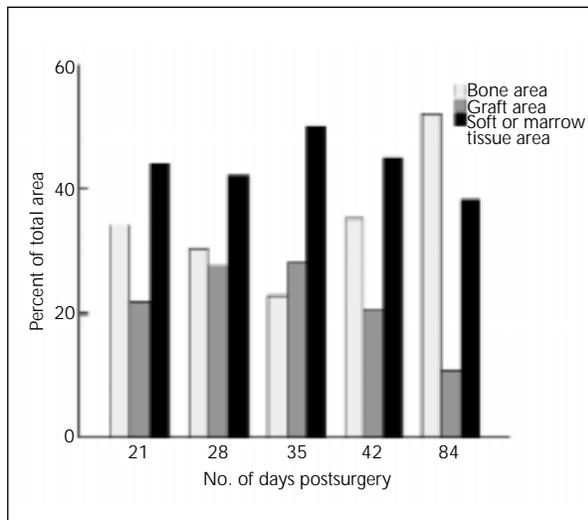
**Fig 6a** Marrow spaces filled with new bone (NB), with a small marrow space in direct contact with the implant (I) (fluorescent photo at the 84-day time period) (magnification  $\times 74$ ).



**Fig 6b** Fluorescent photomicrograph showing replacement of the cortical part of the graft (SB) with new host bone (NB) (magnification  $\times 74$ ).



**Fig 6c** New bone (NB) formation adjacent to the shoulder of the implant (I) at the 84-day time period (magnification  $\times 58$ ).



**Fig 7** Changes in total area measured covered by bone, graft, and soft or marrow tissue over time.

replaced by new bone. Only small amounts of bone were present at the sixth thread level because of the enlargement of the marrow space cavity.

Results of the mineral apposition rates for days 21, 28, and 35 around the implant are presented in Table 4. The apposition rate around the OB was lower than that in the NB on the surface of the endosteum. This difference was seen between OB and NB in the third labeling periods (rate 3) ( $P < .05$ ).

### Discussion

Since the cutting-grinding technique was first described by Donath and Breuner,<sup>8</sup> it has routinely

been used in research associated with dental implants. With this method, endosseous implants can be examined in situ along with any bone substitutes and vital bone. Histologic techniques that required decalcification made it impossible to determine whether the grafting material had been lost as a result of physiologic resorption or simply through the decalcification process.<sup>9</sup> In the present study, new bone formation, replacement of Surgi-bone by the host bone, and the relationship of bone and grafted material to the implant can be clearly demonstrated in the undecalcified sections.

When bone is in direct apposition to an implant, the phenomenon is often referred to as “osseointegration” in current implant terminology. Although

**Table 1 Percentage of Bone Area at Each Thread Level (Mean ± SEM)**

Days	Thread 1	Thread 2	Thread 3	Thread 4	Thread 5	Thread 6
21	0.0	NED	22.7 ± 5.2	44.3 ± 10.0	87.1 ± 5.5	48.7 ± 7.8
28	0.0	1.1 ± 0.6	10.2 ± 4.8	29.9 ± 5.4	74.2 ± 13.0	NED
35	0.2 ± 0.1	0.0	0.0	10.5 ± 5.3	42.8 ± 8.6	83.0 ± 7.2
42	0.0	NED	13.0 ± 6.9	52.1 ± 8.4	87.4 ± 6.9	58.0 ± 7.8
84	18.0 ± 5.4	67.5 ± 5.5	67.1 ± 5.1	85.9 ± 1.3	55.4 ± 4.0	1.9 ± 0.4

NED = not enough data for significance.

**Table 2 Percentage of Surgibone Area at Each Thread Level (Mean ± SEM)**

Days	Thread 1	Thread 2	Thread 3	Thread 4	Thread 5	Thread 6
21	66.8 ± 8.4	32.4 ± 0.9	18.5 ± 4.3	13.3 ± 4.1	0.0	0.0
28	56.5 ± 11.7	60.5 ± 9.2	29.9 ± 5.2	15.2 ± 4.2	4.1 ± 3.4	0.0
35	65.6 ± 3.1	69.7 ± 1.8	21.2 ± 3.8	5.7 ± 1.4	3.6 ± 2.1	1.0 ± 0.6
42	70.5 ± 4.4	41.6 ± 4.9	8.0 ± 3.3	2.2 ± 1.3	0.0	0.0
84	53.3 ± 16.5	7.5 ± 2.2	NED	NED	0.0	0.0

NED = not enough data for significance.

**Table 3 Percentage of Soft or Marrow Tissue Area at Each Thread Level (Mean ± SEM)**

Days	Thread 1	Thread 2	Thread 3	Thread 4	Thread 5	Thread 6
21	33.2 ± 8.4	63.7 ± 2.7	58.8 ± 2.6	42.4 ± 10.8	12.9 ± 5.5	51.3 ± 7.7
28	43.4 ± 11.7	38.4 ± 8.8	59.9 ± 3.2	55.0 ± 2.2	21.7 ± 9.5	32.7 ± 8.8
35	34.3 ± 3.1	30.3 ± 1.8	78.8 ± 3.8	83.8 ± 5.0	53.6 ± 6.9	16.0 ± 7.4
42	29.5 ± 4.4	38.1 ± 4.9	79.0 ± 7.0	45.7 ± 7.3	12.6 ± 6.9	42.0 ± 7.8
84	28.8 ± 11.5	25.0 ± 4.6	32.6 ± 5.3	13.2 ± 2.1	44.6 ± 4.0	98.1 ± 0.4

NED = not enough data for significance.

**Table 4 Apposition Rates in  $\mu\text{m}/\text{Day}$  (Mean ± SD)**

Type of bone	Rate 1	Rate 2	Rate 3	Rate 4
Original bone	2.3 ± 0.4	1.8 ± 0.2	1.9 ± 0.4	2.4 ± 0.3
Newly formed bone	NED	NED	2.8 ± 0.6	2.6 ± 0.1

NED = not enough data for significance.

Control (n = 3): Near marrow cavity 2.07 (± 0.21) to 2.18 (± 0.44)  $\mu\text{m}/\text{day}$ ; remote from marrow cavity 1.73 (± 0.17) to 1.95 (± 0.19)  $\mu\text{m}/\text{day}$ .

Label 1: tetracycline; Label 2: Xylenol Orange; Label 3: Alizarin Complexone; Label 4: tetracycline; Label 5: Xylenol Orange.

Rate 1: between labels 1 and 2; Rate 2: between labels 2 and 3; Rate 3: between labels 3 and 4; Rate 4: between labels 4 and 5.

the term *osseointegration* was coined by Swedish research teams and first used in 1997,<sup>10</sup> it was defined a few years later.<sup>11</sup> Osseointegration was regarded as “a direct contact between living bone and implant.” Osseointegration is by no means limited to 2-stage titanium implants. A direct bone interface or contact can be observed with ceramic, other metals, and 1-stage implants.<sup>12</sup> The present

and previous studies have shown microscopic evidence of an intimate contact of titanium alloy implants with bone.<sup>13</sup> In addition to a cortical bone interface, trabecular (cancellous) bone also directly contacts implant surfaces. It was found that new bone in the interface of the middle portion of the implant in the early periods of the experiment was immature bone, which appeared as a diffuse label

rather than a distinct label under fluorescence. At 84 days, bone-to-implant contact was greatly increased in the second to fifth threads because of the replacement of the Surgibone by newly formed bone.

The proportion of direct bone-to-implant contact varies with the material used, implant design, surface structure, the state of the implant bed, surgical technique, loading conditions, and time.<sup>14-16</sup> It has been demonstrated that with time, screw-shaped implants have a similar or greater proportion of bone-to-implant contact than do other designs.<sup>15</sup> One reason for this discrepancy in favor of the screw-type design may be related to the type of tension that results in bone after placement of a threaded implant. Another explanation for the better outcome of the screws may be the initially better surgical fit that exists in a properly placed threaded implant.<sup>17</sup> An important requirement for mineralized tissue integration of any implant is primary stability. Piattelli et al<sup>18</sup> reported that if the implant is immobile in bone, direct bone contact is possible even in the presence of functional loading. Of all the implants, the screw ensures the best stability, because its placement requires only a narrow implantation bed and manual tapping, after which tight contact of the implant threads with the bone lamellae is achieved.<sup>19</sup> The percentage of bone area, as well as the bone contact, can be used to evaluate the state of osseointegration.<sup>20</sup> In the present study, the percentage of bone area significantly increased and reached about 67% at the second and third thread levels at the end of the experiment, suggesting an increase in bone-to-implant contact. The implant site can also influence the amount of bone-to-implant contact. In this study, the implants were placed in the tibial metaphyses of rabbits. This site may be more favorable to bone formation and an increase in bone height along the surface of implants than the human oral environment.

Remodeling is defined as turnover or internal restructuring of previously existing bone.<sup>20</sup> It is a coupled tissue level phenomenon. Activation (A) of osseous precursor cells results in a sequence of active resorption (R), quiescence or reversal (Q), and formation (F). The duration of the A-R-Q-F remodeling cycle (referred to as "sigma") is about 6 weeks in rabbits, 12 weeks in dogs, and 17 weeks in humans.<sup>18</sup> Remodeling includes all located changes in individual osteons or trabeculae: turnover, hypertrophy, atrophy, or reorientation. Precise quantification of bone formation and turnover requires in vivo administration of multiple fluorochromes. This is a powerful tool for assessing interface development and bone adaptation to dental implants. Interface remodeling is

essential in the establishment of a viable interface between the implant and the original bone. This early remodeling is probably the result of damage to bone during the operation. It has been found that at 2 to 3 weeks, there is a formation of new bone around the implant; thereafter, devitalized bone is replaced by new bone, as seen by fluorescent microscopy.

### Acknowledgments

This work was funded by the Osseointegration Unit, The Toronto Hospital, Toronto, Ontario, Canada. The animal protocol was approved by the Animal Care Committee of The Toronto Hospital.

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