

# A Pig Model for the Histomorphometric Evaluation of Hard Tissue Around Dental Implants

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This study aimed to evaluate the frontal bone of Swiss Domestic pigs as an animal model for the histologic-histomorphometric examination of bony tissue around dental implants. We inserted SLA surface implants 4.1 mm in diameter and 10 mm in length into the frontal bones of 9 Swiss-Domestic pigs. Histologic and histomorphometric studies were conducted on the undecalcified sections. Histologic examinations showed that the specimens contain a sufficient amount of bone to provide homogenous bone coverage for standard diameter dental implant placement. The mean bone to implant contact was  $61.9\% \pm 8.7\%$ . Other histomorphometric parameters revealed the regular trabecular architecture at this site. Pigs' frontal bone appears to be a suitable animal model in short-term dental implant studies because it provides a sufficient amount of bone and favorable bone microarchitecture.

**Key Words:** dental implants, osseointegration, animal model, histomorphometry, frontal bone

## INTRODUCTION

**A**nimal studies constitute the main component of histologic-histomorphometric research in implant dentistry. Since the introduction of dental implants to the market, many animal studies have been published and are still being conducted for the improvement of today's dental implant technology.

Determination of the conformity of a new biomaterial or newly designed implant depends on the data retrieved from the animal studies before using the implant is used in clinical practice. Animal studies regarding dental implants are mostly performed on monkeys (macaques and baboons), rabbits, sheep, goats, pigs/mini pigs, and dogs.<sup>1-4</sup>

When the hard tissue around dental implants is to be evaluated, both intraoral and extraoral implantations can be done. Implants should be placed into the safest place, so there will be no or few implant failures and a maximum number of specimens for the histologic or histomorphometric examinations. In addition, the bone architecture of the selected animal model should closely resemble human jaw bones, so a comparable healing response can be obtained. Maxillofacial bones of monkey and dog species are being used for dental implant studies to some degree, and they are considered as good representative for human maxillofacial bones.<sup>5,6</sup> However, it is difficult to use monkeys and dogs because of the need to obtain ethical approval and the increased cost. Therefore, researchers often opt to study small animals such as rabbits. Because the amount of bone at the maxillofacial region of small animals is inadequate for dental implant studies, the anatomic site of choice is usually an extremity bone. However, long bones such as tibia or femur have the disadvantage of having endochondral bone origi-

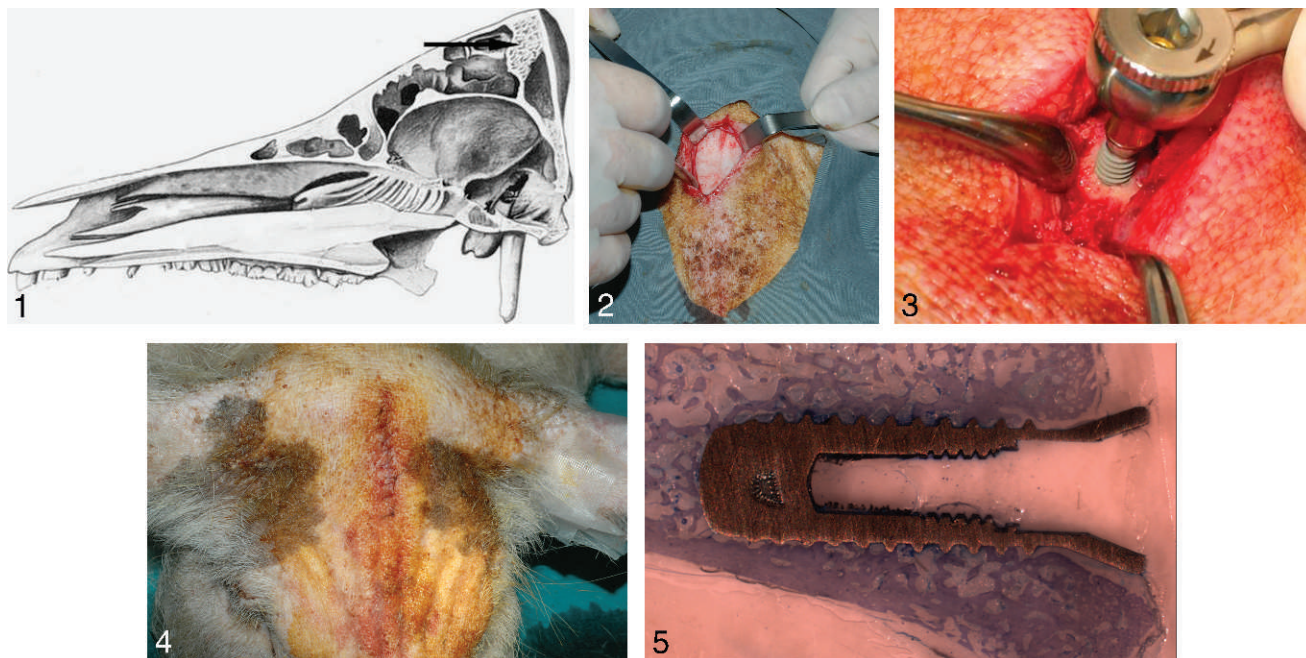
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**FIGURES 1–5.** **FIGURE 1.** Drawing of the sagittal section of a Swiss Domestic pig’s head. **FIGURE 2.** Intraoperative photograph showing the incision and bone exposure. **FIGURE 3.** Intraoperative photograph showing the implant to be installed. **FIGURE 4.** Immediate postoperative photograph showing the wound closure and surgical area. **FIGURE 5.** Photograph of the undecalcified section of a specimen taken without magnification.

nation, unlike maxillofacial bones. From the histologic-histomorphometric point of view, endochondral-originated bone may not adequately represent intramembranous-originated bone. Slotte et al.<sup>7</sup> showed that endochondral-originated long bones behave slightly differently than membranous-originated bones under surgical trauma. Mandibular and maxillary bones of pigs and mini pigs are among the commonly used models for dental implant studies.<sup>8,9</sup> The main disadvantage of this approach is the necessity of tooth extraction before implant installment and the increased complication rate due to contamination of intraoral contents.<sup>10</sup>

This study was designed to evaluate the suitability of an alternative animal model, the frontal bone of Swiss Domestic pigs, for the histologic-histomorphometric examination of bony tissue around dental implants.

**MATERIALS AND METHODS**

Nine Swiss Domestic pigs (age, 6–9 months) were used in this study. Nine SwissPlus (4.1 mm diameter, 10 mm length, Zimmer Dental, Carlsbad, Calif) dental implants were inserted into the superior/posterior edge of the frontal bones of a pig’s cranium. The sagittal section of a Swiss Domestic

pig’s head is shown in Figure 1. The frontal bone is a long flat bone that extends from the nasal bones to the posterior end of the head. It covers the pig’s whole forehead, including the frontal sinuses. It corresponds to the frontal and parietal bones of humans. The superior/posterior edge of the frontal bone incorporates a bulky bony mass that has sufficient bone volume for dental implant placement in young animals. The region used for implant placement is shown with an arrow in Figure 1.

All animals were killed after 8 weeks. Undecalcified hard tissue sections were prepared, and histomorphometric parameters were examined. This animal study was approved by the Ethical Review Committee of Çukurova University Medical Scientific Research Center.

**Surgical procedure**

All animals were premedicated with intramuscular injections of 20 mg/kg ketamine (Ketalar, Pfizer, Turkey) and 2 mg/kg xylazine (Rompun, Bayer, Turkey). After premedication, animals were taken to the operating room, and general anesthesia was obtained by intravenous injection of 40 mg/kg<sup>-1</sup>/h<sup>-1</sup> thiopental sodium (Pental Sodium, İ.E.Ulagay, Turkey). The pig was placed in a prone position, and the posterior edge of the frontal bone was palpated

and then prepared and draped under aseptic conditions. Local anesthesia and hemostasis were provided by supraperiosteal injection of 4% articaine with 1:200 000 epinephrine (Ultracain-DS, Hoechst Marion Roussel, Turkey). The bone was exposed via a linear skin incision, the periosteum was incised, and the periosteal flaps were raised (Figure 2). One SwissPlus dental implant was placed lateral to the climax of the frontal bone. The implant sites were prepared using a standard gentle surgical technique with sharp drills, as recommended by the manufacturer. All drilling procedures were carried out under profuse sterile saline irrigation. (Figure 3). After implantation, closure screws were used to leave the implants submerged, and the wounds were closed in layers with resorbable sutures (Vicryl, Brussels, Belgium) (Figure 4).

#### *Postoperative care*

The animals received analgesic (tramadol, 1 mg/kg) (Contramal, Abdiibrahim, Turkey) and antibiotics (cefazolin, 25 mg/kg) (Cefamezine, Eczacıbaşı, Turkey) intramuscularly before surgery and twice per day during the 3 postoperative days. The animals were given a normal diet.

#### *Histomorphometric examination*

All subjects were killed after 8 weeks with an intravenous injection of 100 mg/kg sodium pentobarbitone (Pental, Bilim, Turkey). The sections of the calvarial bones with the implant inside were harvested from all subjects, and the soft tissues were stripped off. The harvested bones were then carefully sectioned so each specimen consisted of an implant with the surrounding 5 mm of bone. The specimens were fixed in 10% buffered formalin, dehydrated in increasing concentrations of ethanol, from 70% to 99% in 15 days, and embedded in methylmethacrylate (Technovit 7200, Haerus Kulzer GmbH, Wehrheim, Germany). Using an electric diamond saw and grinding system (Exakt, Norderstedt, Germany), 40- $\mu$ m thick transversal sections were prepared and stained with toluidine blue. Digital images of the histologic sections were obtained by a digital camera (Olympus DP 70, Tokyo, Japan) attached to a microscope (Olympus BX50, Tokyo, Japan) at a magnification rate of  $\times 40$ . The obtained images were transferred to a computer so that histomorphometric measurements

were ready to be performed by software (WinTAS version 0.1, University of Leeds, Leeds, UK).

Histomorphometric analyses were performed on the prepared specimens. For the calculation, a bone-measurement area was determined 1 mm away from the implant surface, including the outer contours in which osseointegration occurs. The calculated area of bone was divided by the imaginary fulfilled area of bone surrounding the implant, and the data were expressed as a percentage value. The numeric data was the bone to implant contact (BIC) for each individual implant. Other histomorphometric parameters, including bone volume (BV), bone surface (BS), trabecular thickness (TbTh), and trabecular separation (TbSp), were also calculated in selected bony areas for each specimen. The BV and BIC parameters indicate the ratio of present bone to total tissue volume at the analyzed section and the percentage of implant length having direct contact with bone, respectively. Other parameters, including BS, TbTh, and TbSp parameters identify the microarchitecture of bone. These measurements help differentiate between different types of bone structures, such as the dense cortical bone in long bones and the sparse trabecular bone of maxillary alveolar bone.<sup>11</sup> The parameters related to bone microarchitecture were included to determine the possible similarity of the animal models' bone structure to human maxillofacial bones. The definitions and equations for each histomorphometric parameter are summarized in Table 1.

## **RESULTS**

The mean weight of the animals was  $26.8 \pm 3.1$  (range, 20–26 kg) and the mean height at their shoulders was  $59.4 \pm 4.7$  cm (range, 58–64 cm). All the animals tolerated the operations and medications well and had no significant complications. Thus, no animals were excluded from the study. Nine specimens were obtained eventually. Histologic examination at lower magnification showed that all implants were completely covered with healthy trabecular bone and had no apparent inflammatory tissue. None of the implants encountered the frontal sinus. The histologic slides of the specimens at low and higher magnifications are shown in Figures 5 and 6. The mean BIC was  $61.9\% \pm 8.7\%$ . The mean values of the other bone

TABLE 1		
Bone histomorphometric parameters		
Parameter	Unit of Measure	Description/Equation
Bone to implant contact (BIC)	%	Percentage of implant length at which there is direct BIC without intervening tissues
Bone volume/total volume (BV/TV)	%	Ratio of mineralized and unmineralized BV to total TV estimated from the analyzed section
Bone surface (BS)	mm/mm <sup>2</sup>	Ratio of mineralized and unmineralized BS to total tissue surface area estimated from the analyzed section
Trabecular thickness (TbTh)	μm	Trabecular width × 10/1.2*
Trabecular separation (TbSp)	μm	Trabecular thickness × 10x ([100/BV/TV] – 1)

\*A correction factor for section obliquity.

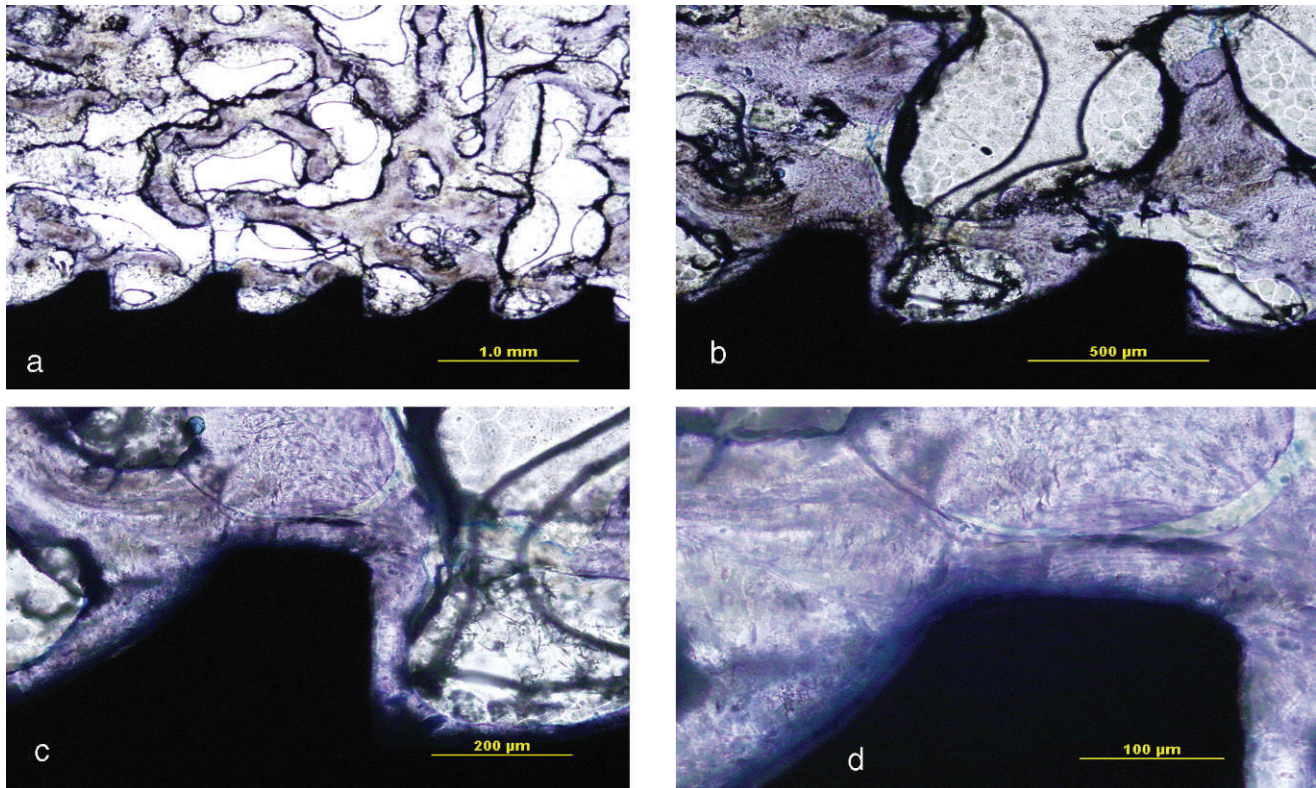
histomorphometric parameters are shown in Table 2. The weekly maintenance fee for each animal, excluding medical treatments, was the equivalent of US \$14. The fee was charged according to the effective interuniversity agreement.

**DISCUSSION**

Determination of bone-implant interface by means of histomorphometric analyses in animal models is the key element of dental implant studies. We

evaluated BIC and bone tissue surrounding dental implant in a novel animal model. Although pigs are relatively difficult to handle during invasive or noninvasive procedures because of their noisy and aggressive behavior,<sup>12,13</sup> they are considered to be closely representative of human bone and therefore suitable species of choice.<sup>14,15</sup> However; their fast growth rate may be a significant handicap for bone studies in pigs. Therefore, the pig model might not be suitable for long-term studies.

Another handicap specific to this anatomic site is



**FIGURE 6.** Photographs of the bone-implant contact (implant body region) taken at magnification rates of (1) ×4, (2) ×10, (3) ×20, and (4) ×40.

TABLE 2

Mean ( $\pm$  standard deviation [SD]) values of histomorphometric parameters\*

N	BIC (%) (Mean $\pm$ SD)	BV (%) (Mean $\pm$ SD)	BS (mm/mm <sup>2</sup> ) (Mean $\pm$ SD)	TbTh ( $\mu$ m) (Mean $\pm$ SD)	TbSp ( $\mu$ m) (Mean $\pm$ SD)
9	61.9 $\pm$ 8.7	49.2 $\pm$ 9.3	37.9 $\pm$ 9.3	276 $\pm$ 59	285 $\pm$ 107

\*BIC indicates bone to implant contact; BV, bone volume; BS, bone surface; TbTh, trabecular thickness; TbSp, trabecular separation.

the decreased bone volume that occurs because of pneumatization of the frontal sinus as the animal ages. Our previous experience showed that after 9 months of age the bone volume at the superior-posterior edge recesses, and the frontal sinus is entered directly after few millimeters of drilling. Figure 7 shows a thin cortical bone without trabecular content at the surgical site in an 18-month-old pig. It is not possible to conduct a dental implant study in such a thin content. We used pre-adult animals (6–9 months old), who had not reached their maximum weight to overcome the handling and bone recession problems.

Rabbits are among the most commonly used animal models because of their convenience in handling. However, it is questionable to what extent rabbit bones may be representative of human maxillofacial bones. Rabbit maxillofacial bones are relatively thin for standard implant placements, and implant installation might be problematic. Freilich et al<sup>16</sup> inserted the implants at the lateral aspect of the mandibular posterior border from a lateral-medial direction by leaving the coronal part of the implant outside. Other options include using specially designed mini implants at the maxillofacial bones or placing implants into other bones, such as tibia or femur.<sup>3,17,18</sup> Both tibia and femur bones have been successfully used for dental implant studies. However, endochondral ossification of long bones may present a significant difference in microarchitecture in comparison with maxillofacial bones. In addition, the large bone marrow space underneath the cortical bones may be a major drawback for implant placement.

Canine models are among the widely used animal models for dental implant studies.<sup>14</sup> After tooth extraction, the mandibular or maxillary bones can be successfully used for dental implant installation.<sup>6,19,20</sup> Canine mandible or maxilla may be more suitable than the pig frontal bone for dental implant studies, as they mimic the same

anatomic structure. However, an important prohibitive factor for the canine model is the difficulty in obtaining ethical approval; in territories where animal studies are monitored by animal rights organizations, ethical committees usually advise against using canine models. Therefore, researchers opt for alternative animal models.

Histologic analyses on undecalcified sections are useful methods for evaluating the implant-bone interface, and morphometric analyses give quantitative data about the rate of osseointegration. A commonly used parameter is BIC, which gives the percentage of bone area that has direct contact with the implant surface. It is calculated with specific computer software and recognized as one of the major histologic parameters of quantity of osseointegration. We found that the mean BIC was 61.9% at the end of the eighth week. The literature has some data regarding BIC rates in human alveolar bone. Histomorphometric analyses on dental implants retrieved from human alveolar bones cannot be performed routinely for research purpose because of ethical reasons; however, numerous case reports and case series have been

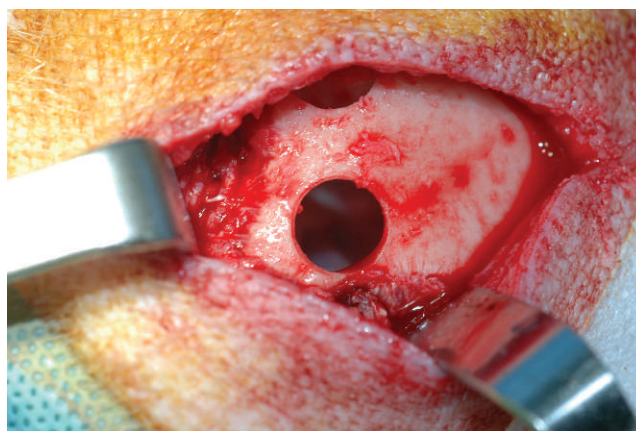


FIGURE 7. Intraoperative photograph showing thin cortical bone without trabecular content at the surgical site in an 18-month-old pig. Note the excessive pneumatization of the frontal sinus resulting in loss of the bony mass.

published.<sup>21–30</sup> These case series used different types of loading protocols and osseointegration periods. In these studies, the duration of period after implant placement varies between 1 and 240 months, and BIC varies from 38.9% to 92.4%.<sup>21–30</sup> A considerable difference between our study and these clinical reports was that the implants were not loaded and left submerged under the periosteum in our study.

We evaluated bone histomorphometric parameters, including BV, BS, TbTh, and TbSp, for the bone area surrounding the implant surfaces. The mean BV value was 49.2% in our animal model. A cadaver study by Nkenke et al<sup>31</sup> evaluated bone volume values in the maxillary and mandibular alveolar bones of 3 cadavers. They determined the mean BV to be  $19.7\% \pm 8.8\%$  for maxilla and  $34.3\% \pm 6.0\%$  for the mandible. These measurements seem lower than our findings. However, the ages of the cadavers at death were 75, 82, and 95 years, and significant bone resorption is expected at these ages, which might account for the difference. A similar cadaver study by Rozé et al<sup>32</sup> evaluated bone histomorphometric parameters in mandibular and maxillary alveolar bones of relatively younger human cadavers (age 53, 67, and 80 years at death). The median BV was 31% for the mandible and 51% for the maxillary alveolar bone.

### CONCLUSIONS

In conclusion, subjective histologic evaluation showed that the superior/posterior edges of the frontal bones of pre-adult Swiss Domestic pigs contain a sufficient amount of bone to provide homogenous bone coverage for standard-diameter dental implant placement. All implants had osseointegrated successfully at the eighth week. Histomorphometric examinations revealed that this area is composed of mainly trabecular bone content, which creates favorable conditions for implant placement. With this study, we determined the mean values of certain histomorphometric parameters for this animal model. It appears that the bone structure of a pig's cranium can represent the human maxillary and mandibular bone for dental implant studies. Histologic examinations of the gross specimens showed that this site provides a sufficient amount of bone, and histomorphometric results confirmed the favorable bone microarchi-

ture. However, the bone at this region recesses because of the pneumatization of the frontal sinus after the animal reaches a certain age. In addition, the fast growth rate of the pigs should be taken into consideration when designing a dental implant study. Therefore, we recommend using this site in young animals (between 6 and 9 months old) for short-term studies.

### ABBREVIATIONS

BIC: bone to implant contact  
BS: bone surface  
BV: bone volume  
TbSp: trabecular separation  
TbTh: trabecular thickness

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