

The use of titanium implants and prosthodontic techniques in the preparation of non-human primates for long-term neuronal recording studies

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Abstract

Investigators that require the chronic implantation of hardware on the non-human primate skull are often challenged with the possible failure of head implants. To improve the success rate of our head implants, titanium anchor screws, referred to as titanium endosseous implants, were implanted in the skulls of six macaques. Techniques adapted from the processing of dentures were utilized to pre-fabricate an acrylic 'skull cap', which provided the mechanical support structure for our study-specific hardware. A two-stage procedure adapted from dentistry (Brånemark et al., 1977) was employed for the placement of titanium endosseous implants in the skull. This two-stage technique incorporates the principles of osseointegration and a healing period. Of the six skull-anchored implants prepared implementing the methods described in this paper, we have not experienced any failures. Additionally, all of the titanium endosseous implants examined post mortem were functionally successful ($n = 30$). Histology results confirmed that there was healthy bone in direct contact with the titanium endosseous implants. The dense cortical bone of the macaque skull is ideal for the implantation of titanium endosseous implants. Titanium endosseous implants have provided secure, functional anchor points for the attachment of hardware to the macaque skull and have resulted in healthy, stable head implants that can remain on the skull for extended periods of time. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Investigators that require the chronic implantation of hardware on the non-human primate skull are often challenged with the possible failure of head implants. The loss of head implant integrity can pose major set-backs to a project, since training animals to perform complex behavioral tasks requires a substantial investment of time. Having experienced such set-backs, our goal was to expand upon techniques presented by Pflugst et al. (1989) and to develop a method for attaching hardware to the skull that would minimize, and ideally eliminate, the failure of head implants.

Brånemark et al. (1969) investigated the long-term stability of titanium implants in the canine jaw and

found bone tissue growing into the surface of the titanium implants; thus, direct bone-to-implant contact was achieved. He introduced the term osseointegration and defined it as a 'direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant' (Brånemark, 1985). Biochemically, osseointegration is true integration, a chemical interaction between the titanium oxide layer of the bulk metal and the bone tissue layer (Steinemann et al., 1986). Titanium dental implants have been used clinically since 1965 and the application of osseointegration techniques into other surgical disciplines has become widespread (Worthington, 1997).

After implanting titanium endosseous implants in the mandibles of 15 *Macaca mulatta*, Young et al. (1979) found that, in most cases, the histology revealed bone in immediate apposition to the titanium implants with

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no discernible fibrous tissue interposed. Titanium endosseous implants were used by Pflugst et al. (1989) to anchor hardware to the non-human primate skull and extremely healthy skull-anchored implants were observed 1–3 years post-implantation. These results suggested an avenue for the success of head implants in our laboratory. The principles of osseointegration, surgical techniques borrowed from dentistry and cranio-maxillofacial surgery, and the adaptation of prosthodontic techniques, have been applied with great success in the preparation of our non-human primates for chronic neuronal recording.

2. Materials and methods

2.1. Overview

Non-human primates were trained to enter primate chairs for daily training or neuronal recording sessions in which they performed behavioral paradigms that produced eye and/or head movements. Six macaques, four *Macaca nemestrina* and two *M. mulatta*, underwent surgery to attach the necessary skull-anchored hardware for long-term neuronal recording, as depicted

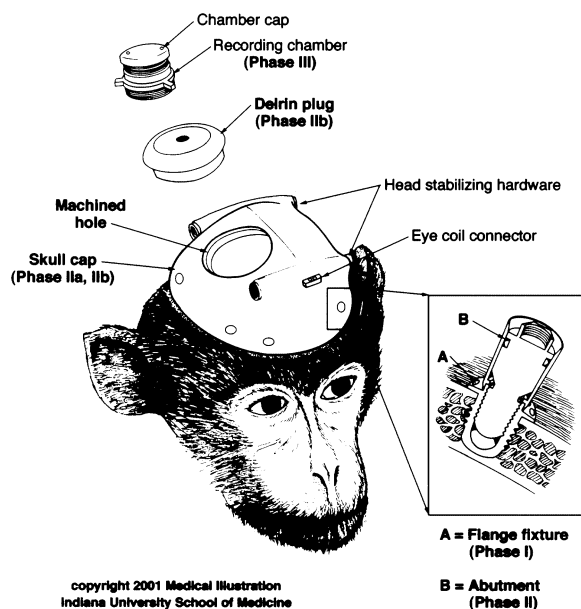


Fig. 1. Diagrammatic representation of a non-human primate (*Macaca mulatta*) supporting skull-anchored hardware. To prepare an animal for long-term studies, three phases were developed: Phase I, Phase II and Phase III. In Phase I, six to eight anchor screws (i.e. flange fixtures) were implanted in the skull and an impression of the skull was made. An acrylic resin skull cap was fabricated (Phase IIa) while the flange fixtures osseointegrated during the healing period. During Phase IIb, cover screws (not shown) were replaced with abutments. The fixture–abutment anchor points and a thin layer of acrylic resin permanently secured the skull cap to the skull (Phase IIb). A delrin plug was placed in the machined hole (Phase IIb) and later replaced with a recording chamber (Phase III).

in Fig. 1. An aseptic surgical technique was employed and standard veterinary pre-, peri- and post-operative protocols were strictly adhered to. The gas anesthetic, isoflurane, was used to induce and maintain the appropriate surgical plane and the analgesic, buprenorphine HCl (0.01 mg/kg), was used during post-operative recovery. All experimental and surgical protocols were approved by the Indiana University School of Medicine Animal Care and Use Committee and complied with United States Public Health Service policy concerning the care and use of laboratory animals.

A three-phase protocol was developed for the preparation of an animal for chronic neuronal recording. Procedures were adapted from dentistry that introduced the principles of osseointegration, new surgical techniques, and a *healing period* of 3–6 months (Brånemark et al., 1977). A healing period was deemed to be important for the successful osseointegration of titanium endosseous implants, as early loading can lead to gradual widening of the fibrous tissue layer and loosening of the implant with consequent implant failure (Worthington, 1994).

In Phase I of the surgical preparation, titanium endosseous implants were implanted in the skull and an impression of the skull was made, detailing skull surface specificities and the endosseous implant locations. Phase IIa incorporated techniques from denture processing to pre-fabricate an acrylic resin ‘skull cap’. The *skull cap* provided the mechanical support structure for study-specific hardware (Fig. 1). In Phase IIb, an eye coil was surgically implanted (Judge et al., 1980) and the skull cap attached to the skull, at which point the preparation was referred to as a *head implant* (i.e. skull-anchored implant). Phase III of the surgical preparation, attachment of a titanium recording chamber, was scheduled after an animal was successfully performing the required behavioral paradigms. The time between Phases II and III was a training period, typically 4–6 months in duration, in which animals learned the complex paradigms required for data acquisition.

3. Phase I: implantation of flange fixtures and fabrication of skull impression

3.1. Hardware and equipment

The skull-anchoring hardware consisted of the flange fixture (i.e. titanium endosseous implant), the cover screw, and the abutment, all of which were made of commercially pure titanium. Osseointegrated flange fixtures provided functional anchor points for the attachment of hardware to the skull (Fig. 1, cross section). The flange fixture had external threads that anchored it into the skull and internal threads that

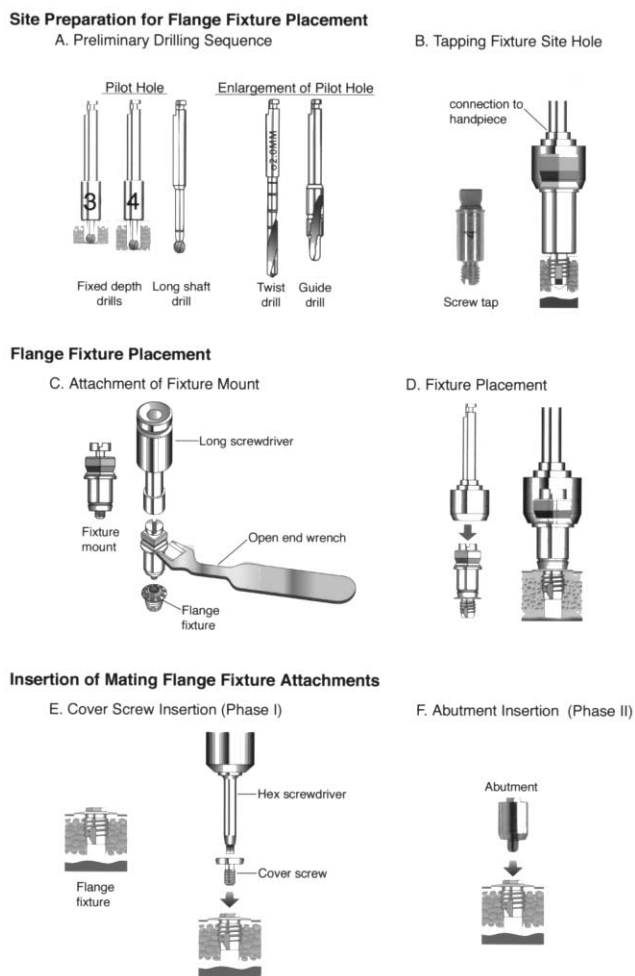


Fig. 2. Steps for Phase I, Implantation of Flange Fixtures. (A) Drill bits needed for preliminary site preparation: 3.0 mm fixed depth drill (Entific, # SEIB 042), 4.0 mm fixed depth drill (Entific, # SEIB 042), long shaft drill (Nobel Biocare, # 25028), twist drill (Nobel Biocare, # 25028), and guide drill (Nobel Biocare, # 25028). (B) The fixture site hole was tapped using the 3.0 mm screw tap (Entific, # SEI 014, 4.0 mm screw tap shown). The connection-to-handpiece (Entific, # DIA 073) facilitated the tapping process. (C) The fixture mount (Entific, # EIB 029), the open-end wrench (Entific, # DIC 010), and the long screwdriver (Entific, # DIB 029) were attached to the flange fixture (Entific, # SEC 001) in preparation for placement in the skull. These instruments were also used to remove the fixture mount from the flange fixture after implantation. (D) Placement of the flange fixture in the prepared bone site. (E) A hex screwdriver (Entific, # DIB 097) was used to insert a cover screw (Entific, # SEC 005) in the implanted flange fixture. (F) In Phase IIb, the cover screws were replaced with abutments of assorted heights (Entific, # SEC 010, SEC 008, SEC 007). Drawings not to scale. Permission granted to use drawings, copyright Entific Medical Systems and Nobel Biocare.

accepted cover screws and later abutments (Fig. 2E, 2F). The flange at the top of the fixture was seated against the skull surface and was machined with holes around its circumference. During the healing period, bone tissue started to osseointegrate into these flange holes and the external threads of the fixture. The cover screw was screwed into the flange fixture to protect the

internal threads from invasive growth of bone and soft tissue during the healing period.

An adjustable drill controller (Aseptico, AEU-267D) that operated at low drill speeds was used for bone site preparation and flange fixture placement. Two drill handpieces (Aseptico, AHP-63 and AHP-60A), each with a different gear ratio, were used in conjunction with the drill controller.

3.2. Implantation of flange fixtures

With the animal stereotaxically positioned, a rostral–caudal incision was made to expose a large portion of the skull. Techniques to minimize tissue violence were employed when retracting the soft tissues covering the skull, since the periosteal layer is important for osteogenesis (Albrektsson, 1985). The basic surgical principles for the placement of flange fixtures (Adell et al., 1985; Tjellstrom, 1990; Tjellstrom et al., 1992) were adapted to meet the specific requirements of our preparation. The sequence for flange fixture placement in the non-human primate skull is summarized in Fig. 2. Six to eight flange fixtures were implanted per animal preparation.

After determining the location of each flange fixture, a preliminary drilling sequence was initiated for each fixture site (Fig. 2A). One of three drill bits was selected, depending on skull thickness, to drill a pilot hole all the way through the skull. The pilot hole was enlarged with the twist drill and the blunt-tipped guide drill, respectively. The preliminary drilling sequence utilized a drill speed setting of 2000–2200 rpm. To complete preparation of the fixture site, the enlarged hole was tapped at a speed of 15–20 rpm with the screw tap and mating connection-to-handpiece shown in Fig. 2B. Placement of the flange fixture into the tapped hole was facilitated by the connection-to-handpiece and the attachment of a fixture mount to the flange fixture (Fig. 2C and 2D). The flange fixture assembly was then threaded into the tapped hole at a speed setting of 15–20 rpm. The connection-to-handpiece was removed and the fixture mount was tightened manually with the open-end wrench to avoid overtightening and stripping of the bone threads. A long, slotted screwdriver was used to remove the fixture mount from the flange fixture (Fig. 2C), subsequently a cover screw was threaded into the flange fixture (Fig. 2E).

Care was taken throughout the drilling and placement sequence not to damage the underlying dura and to limit the amount of irrigation into the subdural space. Throughout the drilling, tapping and fixture placement sequence there was continuous, hand-held irrigation. During the preliminary drilling sequence bone chips were frequently removed. While preparing the flange fixture site and placing the flange fixtures, a perpendicular angle between the skull and the instru-

ments (i.e., drill bits, screw tap, flange fixture assembly) was maintained.

3.3. Stereotaxic reference points

A small indentation was made at five locations on the skull with the aid of a round bur. The stereotaxic coordinates for each point, including the center point of the recording chamber, were logged for future reference. These reference points were transferred to the impression material and utilized in the skull cap fabrication process.

3.4. Fabrication of skull impression

Application cartridges containing impression material (GC America, Inc., # 137406) and a cartridge dispensing gun (GC America Inc., # 152101) were used, along with standard impression techniques (Rudd and Morrow, 1986; Zarb and Jansson, 1985a), to obtain a quality impression of the skull. Soft tissues were retracted to allow the impression material to flow over the skull surface and around each flange fixture. After setting up, the impression was removed and the surgical site irrigated repeatedly, since remnant particles can cause a severe tissue reaction. The midline incision in the skin was closed with a subcuticular suture and standard post-operative procedures were followed.

4. Phase IIa: pre-fabrication of the skull cap

Pre-fabrication of the skull cap commenced at any time during the healing period and incorporated techniques commonly used in the prosthodontic laboratory (Zarb and Jansson, 1985b). The steps involved in skull cap fabrication included: (1) making a working cast of the skull (skull stone); (2) orienting the skull stone in a stereotaxic frame; (3) making a wax model of the skull cap; (4) processing the skull cap; and (5) finishing and machining the skull cap.

4.1. Working cast or 'skull stone'

The skull impression from Phase I was utilized to make a working stone cast, or *skull stone*. Techniques for boxing an impression and pouring a stone cast (Rudd et al., 1986a) were used to make the skull stone. The impression was positioned, detailed side up, in a rubbery compound (Dentsply International Inc., # 605602) which set quickly and stabilized the impression. An all-purpose model stone (Heraeus-Kulzer Inc., # 46277) was poured into the impression and allowed to harden. The hardened stone was separated from the impression and trimmed with a grinder, dental lathe (Baldor Electric Co., # 380WCT), and hand drill in preparation for further processing.

4.2. Orientation of the skull stone

The skull stone was oriented within the stereotaxic frame to replicate the animal's stereotaxic position at the time of Phase I surgery and then immobilized relative to the stereotaxic frame. The four reference points and chamber center point, whose stereotaxic coordinates were logged during Phase I, facilitated orientation of the skull stone. Kopf's head holding accessory (David Kopf Instruments, # 880) was utilized to position and immobilize the transverse cylinders.

4.3. Wax model of the skull cap

A wax model, or wax-up, of the skull cap was crafted (Rudd et al., 1986b) by applying layers of baseplate wax (Dentsply International Inc., # 7709002) to the skull stone surface and around the head stabilizing hardware. At the recording chamber location, a wax plateau was built up to accommodate the machining of a 25–30 mm diameter hole. A metal spatula, a gas burner (Waterpik Technologies, # 002807-000, # 002871-000, and # 002870-000), and an ethanol torch (Buffalo Dental Manufacturing Co., # 82440) aided in the wax-up process.

4.4. Skull cap processing

As in denture processing (Rudd et al., 1986b), the wax from the wax model (i.e. waxed skull stone) was eliminated and replaced with a different material, in this case, acrylic resin. To accomplish this, the waxed skull stone was invested in a three-section flask (Handler Manufacturing Company Inc., # 44G) in a process called flasking. The bottom section of the flask was filled with model stone (Heraeus-Kulzer Inc., # 46277), into which the waxed skull stone was placed. Excess stone was removed and the stone surface smoothed so it was level with the edge of the flask. After the stone hardened, a stone separator (Dentsply International Inc., # 652500) was brushed onto it and the middle section of the flask was added. Another batch of model stone was mixed and poured over the waxed skull stone to fill the remaining half of the flask. The flask cover was put on and the flask was lightly weighted for a minimum of 2 h while the model stone hardened.

To eliminate the wax from the flask, a boil-out procedure was followed. After 10 min in boiling water, the flask sections were separated and the softened wax removed. The transverse cylinders maintained their positions, even though the wax was eliminated, as long as the model stone had flowed into them during the investing procedure.

After the flask cooled, the empty space in the hardened stone was packed with a clear, heat-polymerizing acrylic resin (Dentsply International Inc., Powder,

688506 and Monomer, # 684309). As part of the curing process, the flask halves were put back together and clamped in a press where they remained at room temperature for 5–6 h. Further curing occurred, when the clamped flask was placed in a curing unit. Water bath temperatures were set at 165°F for the first 2.5 h and 210°F for another 0.5 h.

4.5. Finishing and machining the skull cap

The cured skull cap was deflasked and rough areas on the upper surface and along the edges were ground with the dental lathe and assorted drill bits. The skull cap was polished using the dental lathe and a coarse pumice (Whip Mix Corp., # 03433) (Rudd et al., 1986c). Cover screw indentations were visible on the underside of the skull cap and were enlarged to facilitate positioning of the skull cap for the surgical phase that followed.

The center point of the recording chamber was transferred to the skull cap so the hole for the delrin plug could be drilled. The diameter of the machined hole was larger than the diameter of the recording chamber (i) to allow for flexibility in the chamber's final placement and (ii) to provide an adequate workspace at the time of chamber attachment.

The delrin plug functioned as a 'space saver', decreasing the amount of acrylic resin that needed to be drilled away prior to chamber implantation. The delrin plug was machined without threads and a 1/4-20 tapped hole was centered on the top to aid in its removal. The height of the plug was dependent on the recording chamber height and the curvature of the skull.

5. Phase IIb: implantation of eye coil, attachment of abutments and the skull cap

Phase IIb was scheduled after the healing period requirements were met, skull cap fabrication was complete and the animal had adapted to the experimental environment. Following the implantation of an eye coil (Judge et al., 1980), the animal was positioned in the stereotaxic frame and the soft tissues gently blunt dissected from the skull surface to expose the flange fixtures. The cover screws were removed and replaced with abutments (Fig. 2E and 2F), the second component to the flange fixture anchor point. To remove the cover screws and insert the abutments, hex screwdrivers (Entific Medical Systems, # DIB 097 and # DIB 037, respectively) were employed. Bone tissue often grew into the flange fixture holes, making the flange surface uneven. If this was the case, a cover screw mill (Nobel Biocare, # DIB 041-0) was used to remove the excess bone and soft tissue, encouraging the proper seating of the abutment on the flange surface.

The fixed transverse cylinders of the skull cap and the Kopf head holder accessory were an integral part of the skull cap fitting process. Variations in abutment height and the angle of the abutment to the skull surface required further customizing of the skull cap so that it could be lowered to the skull surface without binding. Since the skull was not a flat surface and the skull cap was lowered in the horizontal plane, the pre-drilled fixture holes always required some modification. A Dremel drill and assorted drill bits were utilized for this purpose.

Once adjustments to the skull cap were complete, preparations were made to permanently fix the skull cap to the skull (Fig. 1). The delrin plug was placed in the skull cap chamber hole and secured, at its perimeter, to the skull cap with auto-polymerizing acrylic resin (Dentsply International Inc., Powder, # 651006 and Monomer, # 651003). Soft tissues, including the periosteal layer, were retracted so they did not interfere with seating of the skull cap. The skull surface was flushed with sterile saline to remove blood and tissue debris and gently blotted dry with gauze. These preparations were made prior to mixing a batch of clear, auto-polymerizing acrylic resin, which had a working time of about 5 min. Acrylic resin was poured onto the underside of the skull cap, the skull cap was placed in the head holder accessory, and the left and right head holders were lowered simultaneously until the skull cap seated on the skull. Pressure was applied to the skull cap so the acrylic resin flowed between the skull and skull cap surfaces, replacing air pockets and forming a protective barrier against infectious organisms. Excess acrylic resin was immediately removed from the skull cap edges with a lachrymal dilator (Sparta Surgical Corp., # 16-130). To complete Phase IIb, the eye coil connector was secured to the skull cap with acrylic resin, the rostral and caudal portions of the head incision were sutured, and topical antibiotic ointment was applied around the skull cap.

6. Phase III: attachment of recording chamber

In this short surgical phase, the animal was positioned in the stereotaxic frame so the delrin plug could be removed and replaced with a permanent titanium recording chamber (Fig. 1). The plug was removed, along with the layer of acrylic resin at the bottom of the skull cap hole. Standard techniques were followed to remove a small section of the skull over which the recording chamber was stereotaxically positioned. The application of acrylic resin between the outer wall of the recording chamber and the wall of the skull cap hole permanently secured the chamber to the support structure of the skull cap. The inside of the recording chamber was irrigated with sterile saline before the

protective chamber cap was threaded into it. This surgical phase also provided the opportunity to assess baseline head implant integrity and to perform routine head implant maintenance protocols (Section 7). Completion of Phase III surgery was the final stage in the preparation of a non-human primate for long-term neuronal recording.

7. Maintenance and assessment of recording chambers and head implants

Animals were chaired daily to perform routine head implant and recording chamber maintenance and to assess the condition of each. The condition of the chamber was qualitatively evaluated by monitoring the fluid and the soft tissue at the bottom of the chamber. The recording chamber was cleaned prior to microelectrode penetration and at the conclusion of a neuronal recording session. Standard aseptic protocol consisted of aspirating the chamber fluid, flushing with sterile saline, and soaking with 10% povidone (Medi-Flex, #260286) for 5 min. The povidone was aspirated, the chamber flushed repeatedly with sterile saline and the tissue gently dried with a sterile cotton-tipped applicator. After removal of the microelectrode, the chamber was again flushed repeatedly with sterile saline followed by the prophylactic application of a topical ophthalmic ointment (Bacitracin–Neomycin–Polymyxin) to the tissue surface.

Daily protocols for maintaining healthy head implants included trimming the hair about 1 cm away from the implant, irrigating the perimeter with sterile saline, and applying topical antibiotic ointment. *Head implant integrity* was qualitatively evaluated by (i) determining the physical stability of the head implant and (ii) monitoring the health of the bone and soft tissues underlying and adjacent to the skull cap. Healthy soft tissue did not have a granular, inflamed appearance. The color and vascularization patterns of the bone underlying and neighboring the head implant were used to qualitatively assess the condition of the bone. In order to visualize any possible changes to the bone, the use of clear acrylic resin was incorporated into all of our surgical preparations.

8. Methodology for previous implants

Previously, one surgery was performed to prepare our non-human primates for chronic neuronal recording. Stainless steel anchor screws were implanted in the macaque skull and an acrylic resin skull cap was built up in the surgical suite. Variables such as drill speed, incremental drilling, and irrigation were not controlled

and a screw tap was not used prior to inserting the anchor screw. The anchor screws were functionally loaded 1–2 weeks post-implantation in order to initiate behavioral training sessions and to follow daily chamber and head implant hygiene protocols. Recording chamber and head implant maintenance protocols were the same as those previously described (Section 7).

9. Results

As seen in Table 1, six male macaques, four *M. nemestrina* and two *M. mulatta*, were implanted with titanium flange fixtures for the attachment of a skull cap. One of the six macaques was not used for chronic neuronal recording, thereby negating the need for stereotaxic implantation of a recording chamber. The average age and weight at the time of flange fixture implantation was 5.0 years and 9.1 kg, respectively ($n = 6$). The average age and weight for animals completing the study was 7.3 years and 12.3 kg, respectively ($n = 4$).

In humans, the recommended healing period between the implantation of flange fixtures and the attachment of hardware is a minimum of 3 months in the dense bone of the mandible (Adell et al., 1985). For our non-human primates, the average healing period, the amount of time between Phase I and Phase II surgeries, was 8.9 months. The healing period ranged widely from 3.5 to 23.3 months (Table 1). Notably, the shortest healing period of 3.5 months resulted in a stable, successful preparation suggesting that, as with humans, a healing period of 3 months was adequate.

The average *load duration*, the amount of time between initial loading of the flange fixtures and the completion of the study, was 22.8 months (Table 1, $n = 4$). Throughout this load duration, the anchor points were subjected to the daily functional loads associated with stabilization of the head. Two animals, at the time of this writing, are supporting healthy, stable head implants with recording chambers, the load duration for one is 21 months and the other 3 months. Our results suggest that a head implant surgically prepared with the method described can remain on the skull well beyond 29.4 months (Table 1, Macaque V).

Head implant integrity was compromised for three, male, *M. nemestrina* that were surgically prepared using our previous implantation methods. In these animals, head implant failure resulted after only an average of 2.4 months ($n = 3$). One animal had extensive necrosis of the bone tissue under the skull cap and all three had a spongy, fibrous tissue layer between the skull and the skull cap. This same spongy, fibrous tissue was observed around many of the anchor screws. The average age and weight of these three animals at the time of

Table 1
Data from six non-human primates surgically prepared for long-term neuronal recording

ID	Sex	Species	Fixtures (#)	Phase I		Phase II		Phase III		Euthanasia		Healing period (months)	Load duration (months)
				Weight (kg)	Age (years)	Weight (kg)	Age (years)	Weight (kg)	Age (years)	Weight (kg)	Age (years)		
P	M	<i>M. nemestrina</i>	8	9.0	4.9	9.4	5.2	9.4	5.2	17.0	7.6	3.5	28.7
T	M	<i>M. nemestrina</i>	8	7.5	4.9	8.0	5.4			10.0	6.4	6.5	12.2
U	M	<i>M. nemestrina</i>	8	9.5	4.5	11.0	5.4	11.9	5.9	11.3	7.1	9.8	21.1
V	M	<i>M. mulatta</i>	6	8.2	5.3	9.1	5.8	9.0	6.0	10.8	8.2	6.1	29.4
W	M	<i>M. mulatta</i>	6	7.5	5.4	12.8	7.3	12.5	8.1			23.3	21.0 ^a
Y	M	<i>M. nemestrina</i>	8	12.9	5.2	14.0	5.5	14.0	5.5			3.9	2.7 ^a
Average				9.1	5.0	10.7	5.8	11.4	6.2	12.3	7.3	8.9	22.8

The animals that were implanted with titanium flange fixtures and fit with skull caps were all males, most of them transitioning between adolescence and adulthood. The average healing period = 8.9 months ($n = 6$) and the average load duration = 22.8 months ($n = 4$). Macaque T was not used for behavioral studies.

^a Not euthanized, load duration = difference between time of writing and date of Phase II.

surgery was 5.2 years and 8.1 kg, respectively. Gender, species, age and weight were comparable to those listed for the macaques in Table 1 and offered little explanation as to the cause of these head implant failures.

Head implant integrity was not compromised and head implants were successful for animals surgically prepared with the method described in this paper ($n = 6$). Prior to and at the time of euthanasia, all of the skull caps were securely anchored to the skull and there were no indications of pending head implant failure. The soft tissues around the skull cap were in a healthy state and the bone tissue underlying the skull cap also exhibited a healthy appearance, typically whitish in color with some red vascular areas characteristic of each animal. There was no evidence of a fibrous tissue layer between the skull and the skull cap as observed with the earlier head implant failures. Inside the recording chamber, the color of the fluid was typically clear or a very light pink-white and the tissue covering the dura was smooth and pink.

Macroscopic examination of the flange fixtures was possible during Phase IIb and when the animal was euthanized. At the time of Phase IIb surgery, the flange fixtures typically had bone growing through some or all of the holes in the flange. The photograph in Fig. 3A clearly demonstrates bone growth into the flange after a minimal healing period of 118 days (Macaque Y, R4). In one case, it was noted during Phase I surgery that a flange fixture was loose following placement in the skull. However, examination of this flange fixture during Phase II suggested that osseointegration had occurred, as revealed by the mechanical soundness of the fixture 118 days post implantation (Fig. 3B). Macroscopically, the bone tissue surrounding the flange fixtures from the four euthanized animals was in contact with the external threads of the flange fixture ($n = 30$).

Histologic processing was performed on one flange fixture (Macaque P, R1) to qualitatively determine whether bone was in direct contact with the fixture and how much bone surrounded the fixture. The fixture-abutment selected was functionally loaded for 28.7 months, so the bone surrounding the flange fixture was not in a healing stage but a final, steady-state, or maintenance phase (Roberts, 1988). The bright-field microscopy results in Fig. 4 suggest osseointegration had occurred at the bone-fixture interface.

Fig. 4A shows high quality load-bearing lamellar bone in direct contact with the titanium flange fixture. A higher magnification of the same bone-fixture interface is seen in Fig. 4B. It has been suggested that sustained, intense remodeling activity of lamellar bone near the endosseous implant surface is the mechanism necessary for long-term retention of osseointegrated implants (Garetto et al., 1995). The remodeling rate (percent bone turnover per time) was probably very

high around this flange fixture, especially since remnants of the pilot hole were no longer visible. The pilot hole was replaced with lamellar bone during the healing process, demonstrating the dynamic properties of healthy bone tissue.

For an endosseous implant to have adequate strength at the bone-fixture interface, only 60% of the bone needs to be in direct apposition to the implant surface (Garetto, personal communication). The histology indicated that greater than 60% of the bone was in direct contact with the flange fixture (Fig. 4A). With a majority of the flange fixture surrounded by healthy bone, a protective barrier was formed to help prevent fibrous tissue and infectious organisms from compromising the bone-fixture interface. Fig. 4A also shows that the flange fixture did not protrude into the subdural space, although this may not be the case with younger ani-

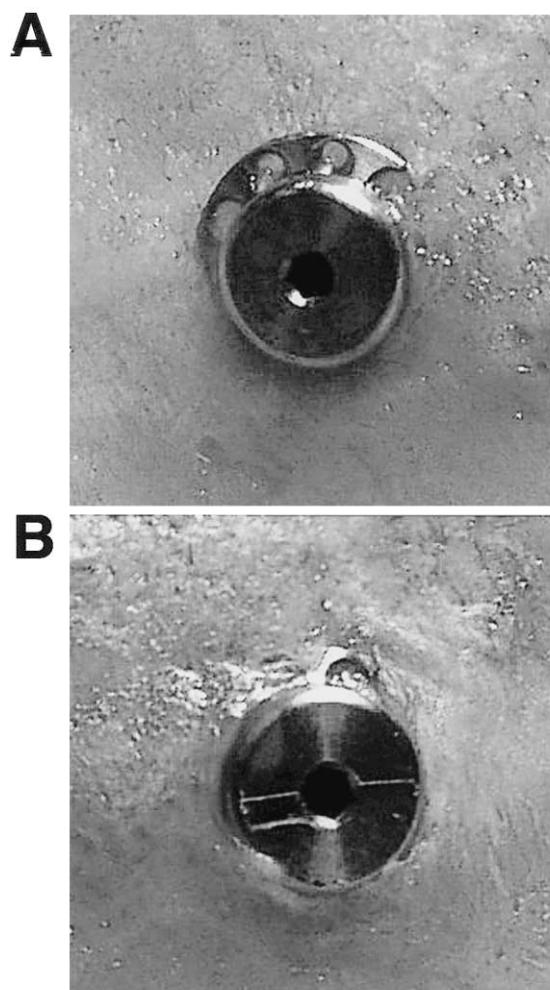


Fig. 3. Macroscopic examination of flange fixtures during Phase IIb surgery. (A) Bone growth into the flange fixture holes after a healing period of 118 days (Macaque Y, R4). (B) A flange fixture from the same animal (Macaque Y, R3), also indicating bone growth into the flange holes. This was of particular interest since this fixture was loose after implantation (Phase I surgery).

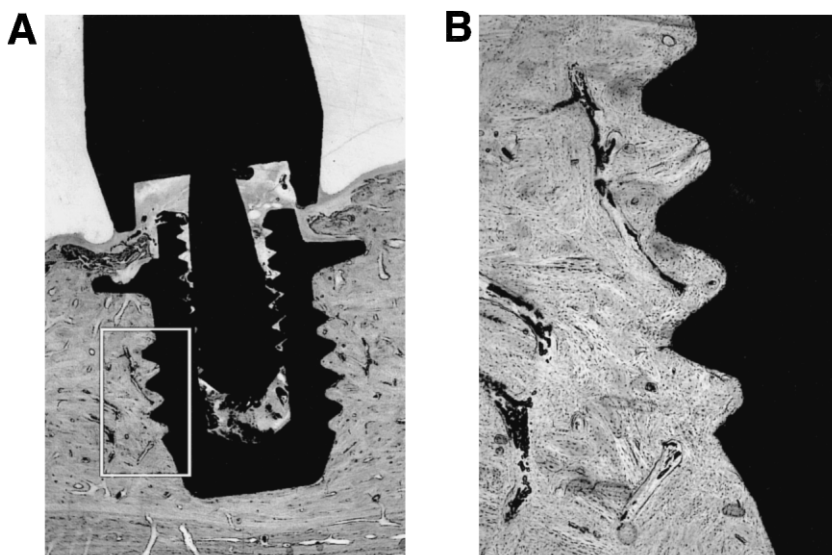


Fig. 4. Cross-section of a titanium flange fixture and abutment in a non-human primate skull (Macaque P, R1). The head implant was stable and healthy at the time of euthanasia and carried a load for 28.7 months. Both photographs show a high quality load-bearing lamellar bone. (A) Bright-field microscopy ($12.5\times$) shows bone in direct contact with the flange fixture; there was no evidence of fibrous tissue at the bone–fixture interface. The amount of bone in contact with the fixture was greater than 60%. Note, the pilot hole drilled during fixture site preparation was replaced with lamellar bone. Also, the thickness of the cortical bone was greater than 3.0 mm so the underlying dura was not damaged. (B) High magnification of a section of the same flange fixture. Area of magnification indicated by box in (A).

mals. Note, that the abutment and flange fixture pictured in Fig. 4 were not paired correctly during the surgical preparation, preventing the abutment from seating on the flange surface properly. Improper seating did not result in a reduction in the strength of this anchor point nor compromise its integrity.

Of the six head implants prepared implementing the methods described in this paper, we have not experienced any head implant failures. Additionally, all of the titanium flange fixtures examined post mortem were functionally successful ($n = 30$). Although histology was not performed on each flange fixture, the stability of each fixture, combined with the apparent normality of the surrounding bone tissue, suggests that osseointegration had most probably occurred. The integrity of our head implants was unaffected by even the relatively heavy weight of our largest macaque (Table 1, Macaque P = 17.0 kg).

10. Discussion/conclusion

The use of titanium flange fixtures in the surgical preparation of a non-human primate for chronic neuronal recording has eliminated the loss of productivity associated with compromised head implants. Titanium flange fixtures have provided secure, functional anchor points for the attachment of hardware to the macaque skull and have resulted in healthy, stable head implants that can remain on the skull for extended periods of time. For an endosseous implant to be successfully

osseointegrated, variables such as implant design, material bio-compatibility, loading conditions, and surgical technique need to be considered. The contribution that each specific variable made to the integrity of our head implants was not systematically studied. However, insights can be gained from the extensive basic and clinical studies performed on endosseous implants.

The design of the selected flange fixtures was ideal for implantation in the dense, cortical bone of the macaque skull as the histology in Fig. 4 revealed. The length of the 3.0 mm flange fixture was well suited for placement in the skulls of our macaques, since skull thickness ranged from approximately 2.5 to 4.0 mm. The bottom of the flange fixture was flat with rounded edges, a design that minimized the potential for damaging the underlying dura. Additionally, the pitch of the threads optimized the number of threads in the skull and, combined with specialized instruments and proper drilling techniques, increased the probability of establishing a functional anchor point.

The biological inertness of titanium most likely contributed to the success of our head implants. Albrektsson et al. (1984) analyzed the bone-implant interface of commercially pure titanium implants and found that the implants were in intimate contact with the bone without any interposed fibrous tissue, proposing the interface was ‘nature-like’. Implants made of titanium were probably not the sole reason for the high success rate of our skull-anchored implants and we do not argue that their use, and the method described in this paper, are the only means of successfully implanting hardware on the macaque skull.

Investigators have found that premature loading may be implicated as a factor affecting the integrity of endosseous implants. Prematurely loaded implants move in bone and this movement can stimulate the formation of soft tissue (Uthoff, 1973; Schatzker et al., 1975). Brunski et al. (1979) found that implants inserted in dog mandibles and subjected to masticatory forces shortly after implantation had a fibrous capsule surrounding them, while implants shielded from these forces post-operatively developed bone in direct apposition.

Premature loading and poor surgical techniques are the most probable reasons for the growth of a fibrous tissue layer at the bone–implant interface (Albrektsson et al., 1981). Osseointegration can only occur if damage to the bone tissue is minimized and living osteogenic tissue is available for bone healing. If low drilling speeds are used, bone temperature is controlled with continuous irrigation of the drilling site, drills are well-sharpened, incremental drilling is employed, and fixtures are not overtightened, the osteogenic tissue will be less likely to incur damage (Adell et al., 1985; Albrektsson, 1990). Eriksson and Albrektsson (1983) found that a bone temperature above 47°C for 1 min led to impaired healing and that there was an increased likelihood that connective tissue would form at the implant–bone interface. Osteogenic tissue was probably not damaged in our implementation of new drilling techniques, as clearly demonstrated by the regeneration of bone tissue around the flange fixture in Fig. 4.

Although pre-fabrication of the skull cap was not thought to be a major determinant of the success of our head implants, there were advantages to fabricating the skull cap in the prosthodontic laboratory. The acrylic resin used in pre-fabricating the skull cap was denser than the type used in surgery and may be less likely to harbor infectious material. Additionally, the pre-fabricated skull cap, when compared with a skull cap built up in the surgical suite, had improved clarity which allowed the condition of the underlying bone to be qualitatively evaluated. Its smooth, polished surface minimized irritation of the adjacent soft tissues and was easily cleaned. Pre-fabrication of the skull cap also reduced the amount of time human and non-human primates were exposed to harmful methylmethacrylate vapors.

Before data acquisition could be initiated in our laboratory, animals had to satisfactorily perform many complex behavioral paradigms. The increased complexity of the paradigms has increased the length of the training period for our non-human primates. To postpone the attachment of a recording chamber and the concomitant drilling of a hole in the skull until absolutely necessary, Phase III was integrated into our surgical preparation. Introducing a third surgery was justified since a third surgery (i) decreased the amount of time for tissue growth over the dura, (ii) decreased the potential for introducing infectious organisms and (iii)

provided for a positive experimental environment. In our opinion, the extended training period prior to neuronal recording facilitated our animal's adaptation to the experimental environment, possibly reducing stress levels and positively affecting performance. Interactions with familiar laboratory personnel can have marked positive effects on physical and behavioral well-being (NRC, 1998). The surgical trauma introduced during Phase III was minimal, the amount of time under anesthesia was short, and the post-operative recovery time was rapid. Investigators who do not employ a lengthy training period could combine Phase III with Phase IIb.

Our use of macaques, weighing 7.5 kg or more, is not believed to preclude the use of titanium flange fixtures in smaller animals. Decreased bone thickness would probably not be a problem, since high success rates were observed with fixtures implanted in the 1–2 mm thick nasal bones of rabbits (Parr et al., 1999). The use of small, immature macaques may impact skull cap fit since changes in skull size and shape may occur during the healing period. However, these changes can be accommodated during Phase IIb by drilling the underside of the skull cap.

The expense of specialized hardware, instruments and equipment was more than offset by the improved reliability of our head implants. To reduce expenses, the number of flange fixtures could probably be decreased from eight to six, as this has already proven successful (Table 1), and some skull-anchoring hardware could be re-used (i.e. cover screws). Establishing collaborations with dental schools or out-sourcing skull cap pre-fabrication to denture processing facilities may also help decrease costs.

The method described in this paper allows for flexibility in design and expands the options available to investigators who require head implant preparations in their field of study. The hardware embedded in our skull cap was study-specific, but many other types of hardware could be accommodated. Titanium implants have been used successfully to anchor percutaneous implants (i.e. prosthetic ears) in humans (Tjellstrom, 1989) and also to percutaneously attach hardware to the macaque skull (Pfungst et al., 1989). Modifications can be made to the method described in this paper as experimental goals dictate.

In conclusion, our results indicate that titanium flange fixtures provide functional anchor points for the attachment of study-specific hardware to the macaque skull. Applying the principles of osseointegration in the surgical preparation of non-human primates for chronic neuronal recording has increased the success rate and long-term survivability of our head implants. Secure, reliable, and healthy head implants will enable investigators to develop novel, complex behavioral paradigms that will enrich our knowledge of brain function.

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Appendix A. Listing of manufacturers

The following is a listing of manufacturers for some of the more specialized instruments and equipment. Most dental supplies (i.e. acrylic resins, flasks, wax, and model stone) were purchased from a dental supplier rather than direct from the manufacturer.

Address	Product #	Product description
Aseptico, Inc., PO Box 1548, Woodinville, WA 98072-1548, USA. Tel.: +1-800-426-5913; www.aseptico.com	# AEU-267D	Drill controller
	# AHP-63	Drill handpiece
	# AHP-60A	Drill handpiece
Baldor Electric Co., PO Box 2400, Fort Smith, AK 72902-2400, USA. Tel.: +1-501-646-4711; www.baldor.com	# 380WCT	Dental lathe (HP = 1/3-0.17, rpm = 3450/1725, 115 V)
Buffalo Dental Manufacturing Co., Inc., 99 Lafayette Drive, Syosset, NY 11791, USA. Tel.: +1-800-828-0203; www.buffalodental.com	# 82440	Ethanol torch
David Kopf Instruments, 7324 Elmo Street, Tujunga, CA 91042- 0636, USA. Tel.: +1-818-352-3274; www.kopfstruments.com	# 880	Semi-chronic head holder
DENTSPLY International Inc., DENTSPLY Caulk, Milford, DE 19963-0359, USA. Tel.: +1-800-532-2855; www.dentsply.com	# 651006	Orthodontic resin powder
	# 651003	Orthodontic resin monomer (clear)
DENTSPLY International Inc., DENTSPLY Trubyte, York, PA 17405-0872, USA. Tel.: +1-800-786-0085; www.dentsply.com	# 7709002	TruWax (Type II Regular, 454 g)
	# 652500	Al-cote separating agent (65 ml)
	# 688506	Lucitone clear dental resin powder (Type I, Class I, 630 g)
	# 684309	Lucitone clear dental resin liquid (430 ml)
Entific Medical Systems, Inc., 3944 N. Hampton Dr., Powell, OH 43065, USA. Tel.: +1-888-825-8484; www.entific.com	See text	
GC America Inc., 3737 West 127th Street, Aksam, IL 60803, USA. Tel.: +1-800-323-7063; www.gcamerica.com	# 137406	Impression material (Type II, medium viscosity, polyvinyl siloxane)
	# 152101	Cartridge dispensing gun
Handler Manufacturing Co., Inc., 612 N. Avenue East, Westfield, NJ 07090, USA. Tel.: +1-908-233-7796	# 44G	Atlas giant flask
Heraeus-Kulzer Inc., Dentist Products Division, 4315 South Lafayette Boulevard, South Bend, IN 46614, USA. Tel.: +1-888- 879-5830; www.heraeus-kulzer.com	# 46277	Labstone (Buff, Type III, 25 lb)
Nobel Biocare USA, Inc., 22895 Eastpark Drive, Bernal Corporate Park, Yorba Linda, CA 92887, USA. Tel.: +1-800-322-5001; www.nobelbiocare.com	See text	
Sparta Surgical Corporation, 2100 Meridian Park Blvd., Concord, CA 94520, USA. Tel.: +1-800-977-2782; www.spartasurgical.com	# 16–130	Lachrymal dilator
Sullivan-Schein Dental (Dental Supplier), 135 Duryea Road, Melville, NY 11747, USA. Tel.: +1-800-851-0400; www.henryschein.com		
Waterpik Technologies Inc., 1730 East Prospect Road, Fort Collins, CO 80553, USA. Tel.: +1-800-525-2020; www.waterpik.com	# 002807-000	Touch-O-Matic burner
	# 002871-000	Loop heat conductor
	# 002870-000	Waxing cup
Whip Mix Corporation, PO Box 17183, Louisville, KY 40217- 0183, USA. Tel.: +1-800-626-5651; www.whipmix.com	# 03433	Pumice (grade CL 60, coarse)

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