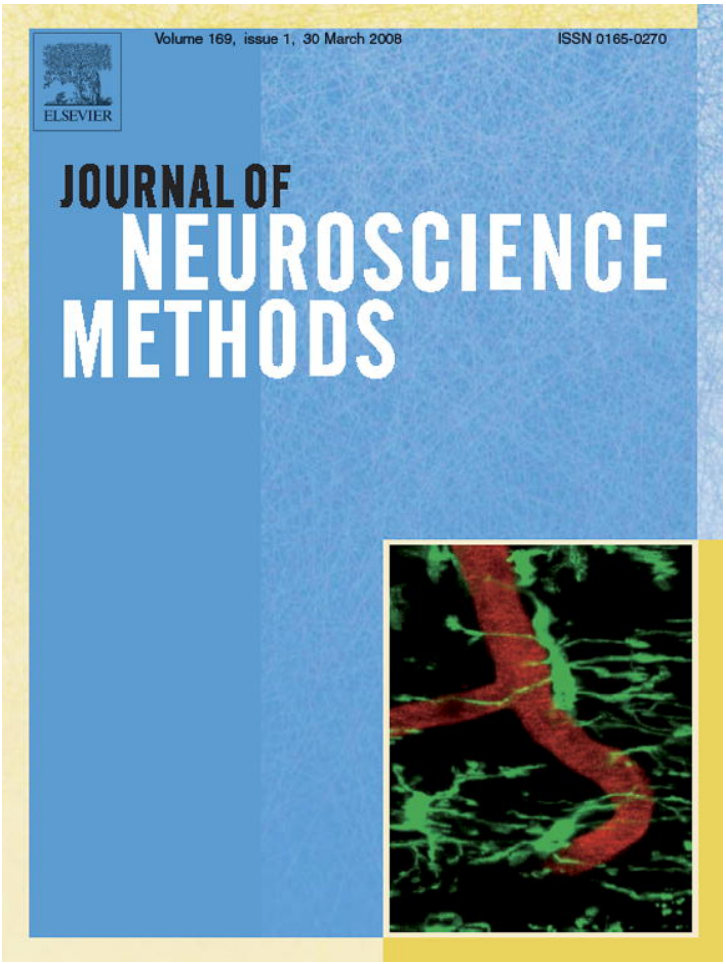

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A removable silicone elastomer seal reduces granulation tissue growth and maintains the sterility of recording chambers for primate neurophysiology

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Abstract

The maintenance of the sterility of craniotomies for serial acute neurophysiological recordings is exacting and time consuming yet is vital to the health of valuable experimental animals. We have developed a method to seal the craniotomy with surgical grade silicone elastomer (Silastic®) in a hermetically sealed chamber. Under these conditions the tissues in the craniotomy and the inside surface of the chamber remain unpopulated by bacteria. The silicone elastomer sealant retarded the growth of granulation tissue on the dura and reduced the procedures required to maintain ideal conditions for neurophysiological recordings.

Keywords: Monkey; Craniotomy; Neurophysiology; Silastic; Silicone elastomer; Granulation tissue; Dura mater

1. Introduction

A standard technique for collecting neurophysiological data in the monkey entails daily transdural penetrations with one or more electrodes through a craniotomy encased in a cylindrical chamber. The chamber limits the exposure of the infection-prone tissue to airborne bacteria. Infections of the dura mater endanger the health of experimental animals, delay experiments, and promote the growth of scar tissue; therefore we developed a method to further reduce the exposure of the dura mater to pathogens.

When the dura is exposed, a small amount (0.1–0.5 ml) of transudate leaks into the craniotomy. Transudate results from capillary permeability and osmotic pressure and contains nutrients such as sugars and amino acids that favor the development of bacteria. Bacteria introduced into this fluid find an ideal milieu for growth and cause a local infection that can spread and cause meningitis. The first sign of this event is the presence of exudate in the recording chamber, a lactescent fluid that contains high concentrations of white blood cells and inflammatory mediators.

We have filled the craniotomy with sterile silicone elastomer that cures without emanating toxic fumes and forms a precise "plug" of the craniotomy. To prevent the inside of the chamber from being populated with bacteria, we mounted o-rings on either the chamber or chamber lid. The combination of a hermetically sealed chamber and the silicone elastomer plug resulted in long term sterility and prevented scaring of the dura mater for up to 14, 24, and 21 months in three different monkeys.

2. Methods

The procedures were performed on three adult male rhesus monkeys (H, T, and Q) used in neurophysiological experiments. The three monkeys were 7, 8, and 6 years of age, respectively, at the date of craniotomy.

For the craniotomy, a thin layer of dental acrylic (Motloid, Chicago) was first applied to the calvarium over the area of interest. A craniotomy of 7–12 mm diameter was then drilled through the cement and the bone. A custom manufactured delrin chamber was centered on the craniotomy and affixed to the bone using anchor screws and dental cement. Alternatively, the chamber was mounted on the skull first and the craniotomy was performed later. A tight-fitting o-ring was either attached to the outside of the chamber or attached to the inside lip of the chamber

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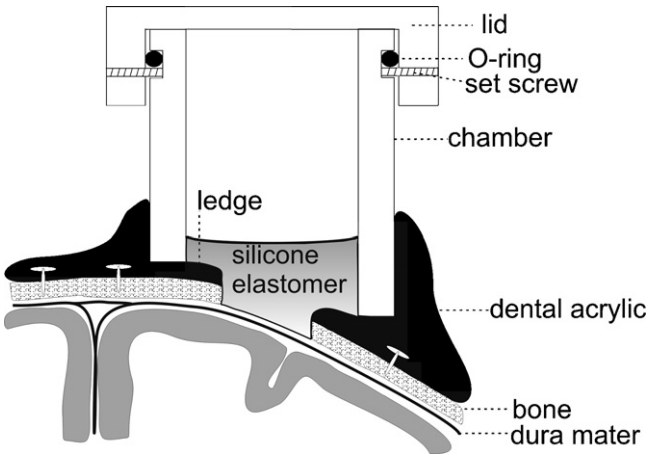

Fig. 1. Schematic diagram of a coronal section through the hermetically sealed chamber. The outer wall of the chamber had a circular recess for the o-ring and set screws that held the lid in place. The chamber was placed on a thin layer of cement, which covered the bone ledges of the craniotomy. Silicone elastomer filled the bottom of the chamber covering the bone ledge.

lid. The dura mater was dried with sterile cotton-tip applicators and sterile silicone elastomer (Kwik-Sil, World Precision Instruments, Sarasota, Florida) was poured into the craniotomy, filling the craniotomy and covering the bone ledges on the bottom of the chamber (Fig. 1).

The Kwik-Sil silicone elastomer received from the manufacturer is packaged in a dual-syringe applicator. A protective cap is removed from the dual-syringe and a sterilized mixer tip is attached. The two liquid components are ejected simultaneously from the syringe and mixed automatically by baffles inside the tip as they enter the chamber. The components flow easily into the chamber, filling the small spaces. The preparation and administration of the silicone elastomer requires 1 min. The curing time is 2–3 min at room temperature. We gas-sterilize the mixer tips to ensure that the silastic that is ejected into the chamber is free of bacteria.

After the silicone elastomer cured, the chamber was hermetically sealed with a lid that fitted over the o-ring. Within 1 week of the surgery, the animal was sedated and the chamber lid was removed to verify the integrity of the silicone elastomer seal. If no fluid was detected in the chamber, the seal was left untouched for several months until the animal was trained for neurophysiological experiments. In cases where fluid was found in the chamber, the old seal was removed, the chamber was washed and dried, and a seal was applied. The chamber was checked in 24 h. If fluid was found again, this process was repeated until the chamber was dry indicating a tight seal. If/when no fluid was found, the chamber was checked on a weekly basis. Typically, 1–2 days were required to obtain and verify a secure seal. The occurrence of a new seal leak was rare (1/25–1/30 seals leaked when the seal was replaced daily). Leaks occurred only when insufficient silicone elastomer was applied or when the application omitted a 2–3 mm region along the walls of the chamber.

When the animal was ready for regular neurophysiological recordings, the following protocol was set in place. The chamber was opened and the silicone elastomer plug was removed with a sterilized tartar scraper tool. The sharpened end of the dental tool was used to break the silicone elastomer bond at the chamber wall, facilitating the removal of the plug. The dura mater under the plug was white and moist and had no vascular scar tissue covering it, permitting penetration with fine microelectrodes. Each electrode (Uwe Thomas, Germany) was housed in a 30 ga cannula. These seven cannulae were pushed through the dura for 3–5 mm and then the electrodes were advanced to the amygdala (Gothard et al., 2007). At the end of the recording session, the electrodes were removed and the chamber was washed with 500 mL sterile saline (0.9% sodium chloride) by continuously pouring and aspirating the saline with a sterilized suction tip. The dura and chamber walls were dried with sterile cotton-tip applicators and sterile silicone elastomer was applied as described previously. When neurophysiological experiments were suspended for longer than 2 days, the integrity of the seal was verified. In cases where the seal was not perfectly formed and fluid was found on top of the silicone plug, the chamber was washed, and the silicone elastomer plug was replaced. Every time the lid was removed for experiments or to verify the seal it was replaced with a sterile lid. The lid was sterilized by immersion in glutaraldehyde (MetriCide28, Metrex, Romulus, MI) for at least 20 h. The lid was removed from the glutaraldehyde with sterilized hemostats and rinsed with sterile water.

The transudate was cultured to detect the presence of bacteria. The sensitivity of any present bacteria to antibiotics was tested (amoxicillin, ampicillin, cephalothin, enrofloxacin, erythromycin, tetracycline, trimethoprim sulfa, penicillin).

3. Results

In the first monkey (H), this method maintained sterile conditions in the chamber, as indicated by a lack of fluid and inflammatory signs, for a period of 14 months. During this period, recordings were performed daily with the exception of a period of 5 months. A single silicone seal lasted for the duration of this recording hiatus. When recordings resumed and the seal was removed, no significant granulation tissue growth was observed. During the life of the craniotomy (14 months), the dura mater did not require debridement or the use of anti-mitotic agents for successful electrode insertion. When sufficient neurophysiological data was collected from monkey H, the chamber was removed.

In the second monkey (T), two unforeseen events occurred that further justify, and qualify, the optimal use of the silicone elastomer sealant. Likely due to a novel combination of anesthetics during surgery, a minor but persistent bleeding occurred in the bone when the craniotomy was drilled. The bleeding was controlled with the application of gel foam (Pfizer, New York). When silicone elastomer was poured over the gel foam it failed to cure. When the bleeding stopped and the dura mater could be dried, a new silicone elastomer plug was applied, which successfully sealed the chamber.

After neurophysiological recordings started, the chamber became contaminated and an exudate formed on the dura mater. The exudate was cultured and determined to contain a gram-

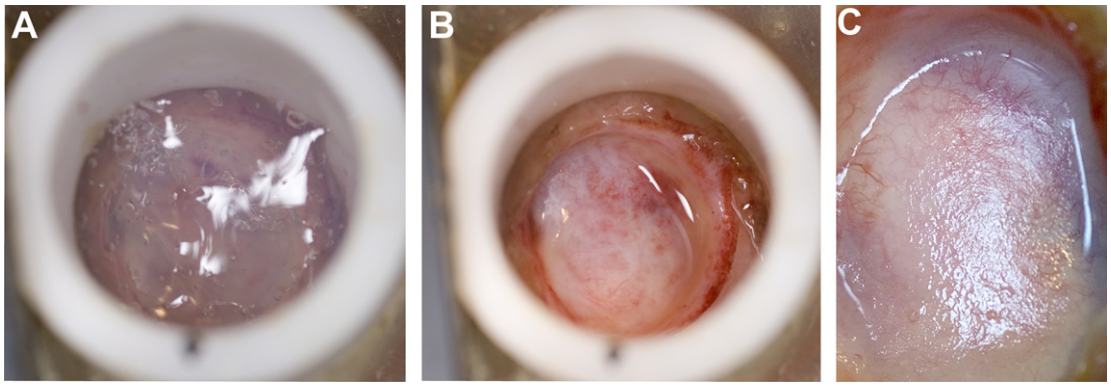

Fig. 2. Recording chamber before and after removal of the silicone elastomer. (A) The bottom of the chamber was filled with silicone elastomer to a level above the ledge of the craniotomy. The silicone elastomer "plug" remained transparent after curing and permitted inspection of the dura mater. (B) Silicone elastomer suppressed granulation tissue growth. After 24 months of silicone elastomer use, the dura mater remained supple and the dural surface did not have vascular granulation tissue. (C) Detailed view of the dura after 24 months of silicone elastomer use.

positive strain of bacteria (*Staphylococcus aureus*). The chamber was washed twice daily with saline but was not sealed with silicone elastomer. Enrofloxacin (Baytril, Bayer, Pittsburgh) was left in the chamber between washes. Additionally, Ceftriaxone (Rocephin, Roche, Palo Alto) was given intramuscularly (50 mg/kg) for 10 days. No exudate was noted after 2 days of treatment and subsequent cultures were negative. The dura remained thin and transparent. The craniotomy was sealed again with silicone elastomer and the chamber remained sterile for the following 24 months. Despite the frequent penetrations of the dura mater for neurophysiological recordings, this tissue did not require debridement or anti-mitotic agents (Fig. 2).

The same procedures were carried out to prepare the third monkey (Q), for neurophysiological recordings. The initial silicone elastomer seal was replaced for the first time 12 months after surgery. At this time the appearance of the dura mater was the same as after a newly made craniotomy. This method has maintained sterile conditions in the chamber for 21 months (Fig. 3). The dura has not required debridement for neurophysiological recording.

The use of silicone elastomer without a hermetically sealed chamber may be insufficient to maintain its sterility of the chamber. It is possible that bacteria enter the chamber via a loosely fitting lid and colonize the chamber. In a fourth monkey (S), the silicone elastomer plug was successful for only 2 months when the dura became populated with bacteria and treatment with antibiotics was required. The lid of the chamber, however, did not have an o-ring and every attempt to sterilize the chamber was unsuccessful and silicone elastomer use was discontinued. A low-grade local infection/inflammation was always present in the chamber, which led to scar tissue growth and required regular debridement.

4. Discussion

Silicone elastomer is an ideal substance to seal the craniotomy because it is biologically inert, does not promote bacterial growth, and can form fit any craniotomy. No macroscopic inflammatory response was observed during silicone elastomer use. Moreover, a silicone elastomer plug increases the likelihood of protecting the dura from exposure to pathogens when used in combination with a chamber fitted with an o-ring. Should infection occur, this technique can be resumed after the chamber is sterilized. Given that the silicone elastomer plug in a hermetically sealed chamber does not require any upkeep, the use of this procedure can reduce the usual workload of chamber maintenance. This technique allows breaks in the experiment for data analysis and behavioral training.

The silicone elastomer formed a seal with the polished inner surface of the delrin chamber. The majority of leaks in the silicone elastomer occurred at the wall of the chamber, and a chamber with either an inner rough surface or circumferential

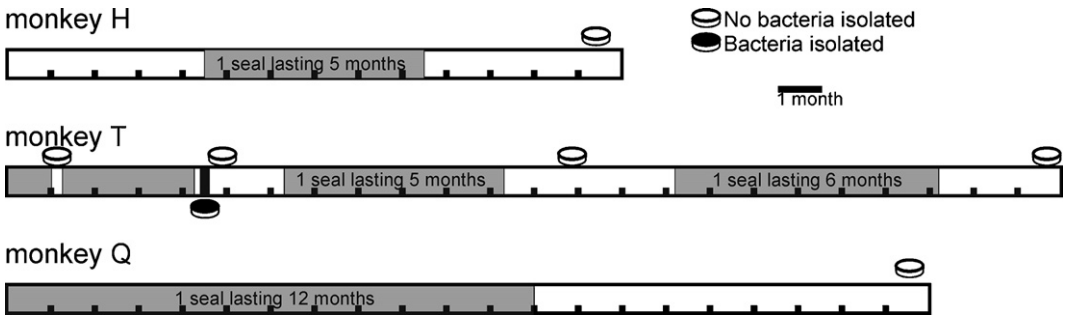

Fig. 3. Timeline of the silicone elastomer use in three monkeys. During behavioral training (grey), a single silicone plug was used to seal the craniotomy. During periods of neurophysiological recording (white), the silicone plug was removed and replaced daily. Periodic cultures of the chamber confirmed the absence of bacteria (petri dish). In one case, bacteria were isolated from the chamber (black mark in monkey T). After antibiotic treatment, subsequent cultures have not isolated bacteria.

grooves is very likely to help in the formation of a strong silicone elastomer seal. The disadvantage of such grooves might be in creating crevices where bacteria might grow and the wash might be less efficient.

An unexpected benefit of the silicone elastomer plug is a suppression of granulation tissue on the dura. Typically, granulation tissue is removed by surgical or chemical means on a regular basis. Recently, anti-mitotic agents have been used to limit granulation tissue growth although debridement is still required (Spinks et al., 2003). Petroleum jelly has been used to prevent granulation tissue growth (Wilson et al., 2005); however this technique seems best suited for neurophysiological recordings using chronic microdrives. The silicone elastomer plug offers the advantage that it can be completely removed in one step whereas the removal of petroleum jelly would be difficult on a daily basis. Teflon is an older solution for reducing granulation tissue in a craniotomy (Saunders and O'Boyle, 1993). These craniotomies were used for brain lesion instead of neurophysiological experiments and the Teflon was sutured to the skull. The stability of the silicone elastomer plug is especially important in the monkey model because of the monkey's high activity level and tendency to hang upside down.

Silicone elastomer in sheet formation has been placed in craniotomies to prevent granulation tissue growth on the dura mater without success. Wilson et al. (2005) reported that silicone elastomer sheets had no effect on granulation tissue growth. After thinning the dura, Gray et al. (2007) secured a silicone sheet to the pia mater. Typically, these sheets allowed 4–6 weeks of neurophysiological recordings before a debridement of granulation tissue was required. The present method is different in that the silicone elastomer is poured over the dura and it solidifies to form a closed, perfect seal. These results suggest that the integrity of the contiguous seal, rather than the properties of silicone elastomer, is responsible for the suppression of granulation tissue.

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