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

Kevin M. Spitler, Katalin M. Gothard *

College of Medicine, Department of Physiology, The University of Arizona, 1501 North Campbell Avenue, Room 4104, Tucson, AZ 85724, USA

Received 25 May 2007; received in revised form 19 November 2007; accepted 20 November 2007

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.

© 2007 Elsevier B.V. All rights reserved.

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 del- rin 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.
The chamber was 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 new silicone elastomer plug was applied. The chamber was checked in glutaraldehyde (MetriCide 28, 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-positive bacterial growth.
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 for neurophysiological recordings, this tissue did not require debridement or anti-mitotic agents (Fig. 2).

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

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
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 cranioptomies 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.

Acknowledgments

We thank P. Zimmerman and K. Brooks for assistance with data collection. This work was supported, in part, by National Institute of Mental Health Grants K01MH-01902A and MH-070836 to K. M. Gothard and National Institute of Mental Health Grant MH072059-01A2 to K. M. Spitler.

References