Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/amjoto

Comparison of packing material in an animal model of middle ear trauma☆,☆☆,★

Enrique Perez, MD[a,*], *Ralph Abi Hachem, MD*[a], *Daniel Carlton, MD*[b], *Isabel Bueno, MD*[a], *Stephen Vernon, MD*[c], *Thomas R. Van De Water, PhD*[a], *Simon I. Angeli, MD*[a]

[a] *University of Miami, Miller School of Medicine, Department of Otolaryngology, Miami, Florida*
[b] *Mount Sinai Hospital Icahn School of Medicine, Department of Otolaryngology Head and Neck Surgery, New York, New York*
[c] *University of Miami, Miller School of Medicine, Department of Pathology, Miami, Florida*

ARTICLE INFO

Article history:
Received 23 February 2016

ABSTRACT

Purpose: To compare the performance of absorbable gelatin sponge (AGS) with polyurethane foam (PUF) as middle ear packing material after mucosal trauma.

Materials and methods: Using a randomized, controlled and blinded study design fifteen guinea pigs underwent middle ear surgery with mucosal trauma performed on both ears. One ear was packed with either PUF or AGS while the contralateral ear remained untreated and used as non-packed paired controls. Auditory brainstem response (ABR) thresholds were measured pre-operatively and repeated at 1, 2, and 6 weeks postoperatively. Histological analysis of middle ear mucosa was done in each group to evaluate the inflammatory reaction and wound healing. Another eighteen animals underwent middle ear wounding and packing in one ear while the contralateral ear was left undisturbed as control. Twelve guinea pigs were euthanized at 2 weeks postoperatively, and six were euthanized at 3 days post-operatively. Mucosal samples were collected for analysis of TGF-β1 levels by enzyme-linked immunosorbent assay.

Results: ABR recordings demonstrate that threshold level changes from baseline were minor in PUF packed and control ears. Threshold levels were higher in the AGS packed ears compared with both control and PUF packed ears for low frequency stimuli. Histological analysis showed persistence of packing material at 6 weeks postoperatively, inflammation, granulation tissue formation, foreign body reaction and neo-osteogenesis in both AGS and PUF groups. TGF-β1 protein levels did not differ between groups.

Conclusion: PUF and AGS packing cause inflammation and neo-osteogenesis in the middle ear following wounding of the mucosa and packing.

☆ This work was presented at the American Academy of Otolaryngology-Head and Neck Surgery Annual Meeting, Washington, DC, September 2012.

☆☆ This work was supported by a grant from the Carolina Ear Institute to Thomas R. Van De Water. The funding source had no role in study design, collection, analysis and interpretation of data, writing of the report, and in the decision to submit the article for publication.

★ There are no conflicts of interest to disclose for this work.

* *Corresponding author at:* Mount Sinai Hospital, Department of Otolaryngology, One Gustave L. Levy Place, Box 1189, New York, NY. Tel.: +1 212 241 7107; fax: +1 212 369 5701.

E-mail address: Enrique.perez@mountsinai.org (E. Perez).

http://dx.doi.org/10.1016/j.amjoto.2016.02.009

1. Introduction

Packing of the middle ear cavity during otologic surgery is primarily done to serve as a scaffold for the tympanic membrane and ossicular grafts, achieve hemostasis, aerate the middle ear cavity, and to enhance wound healing in cases of mucosal disease or removal. A variety of middle ear packing agents exist to meet these aims, including both non-absorbable and absorbable materials [1]. Since introduced as a complement to otologic surgery in the 1950s, absorbable gelatin sponge (AGS) (Pharmacia & Upjohn, Kalamazoo, MI) has become the most commonly used middle ear packing agent in clinical practice [2]. Nevertheless, it has been shown that AGS is prone to initiating an extensive inflammatory process leading to fibrosis, adhesions, and new bone formation within the middle ear [3–5]. This has served as the impetus for studying alternative packing materials and particularly those exhibiting better wound healing properties such as hyaluronic acid (HA) [4–6].

Otopore®, i.e. Co-Polyether-Ester Urethane Foam (PUF) (Striker, Kalamazoo, MI), is a synthetic biodegradable foam currently marketed as a middle ear packing agent. Unlike the degradation of AGS, which relies on phagocytosis by macrophages, PUFs degradation occurs mainly via drainage through the Eustachian tube. Furthermore, cytocompatability studies have demonstrated a lack of cytotoxicity from degradation products of this compound [7]. A histology study by Dogru, et al. compared short-term and long-term appearances of middle ears packed with either AGS or PUF in an atraumatic model of rat middle ear packing [8]. Here it was shown that PUF is prone to mild inflammation and fibrosis in the long-term in contrast to the severe inflammatory process and fibrosis associated with AGS [8].

The transforming growth factor (TGF)-β supergene family of low molecular weight cytokines is involved in the recruitment, activation, and proliferation of tissue and cells during inflammatory processes. TGF-β cytokines are known to play a prominent role in wound healing and are important contributors to hypertrophic scarring when present in excess [9]. Among the various isoforms, TGF-β1 has been implicated in the pathogenesis of otitis media with effusion and cholesteatoma formation, two diseases of the middle ear mucosa [10].

We hypothesize that PUF can serve as a better middle ear packing agent than AGS in the healing of the middle ear mucosa. The primary objective of this study is to investigate the characteristics of PUF packing material as an alternative to AGS in a guinea pig model of middle ear trauma-wound healing. Furthermore, we investigate the molecular environment of middle ear mucosal-wound healing via analysis of TGF-β1 signaling in an attempt to understand how this environment can be impacted by the type of middle ear packing agent used.

2. Materials and methods

2.1. Animals

Fifteen 250–350 g pigmented guinea pigs were obtained from a commercial supplier (Cady Ridge Farm, Natick, MA). All animals were treated according to the experimental protocol approved by the University of Miami Animal Care and Use Committee, and in full compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Animal Welfare Act (7 U.S.C. et seq.). The experimental ear of all animals received an intentional, defined pattern of wounding of their middle ear mucosa and a defined, reproducible level of packing material of their middle ear cavity, while the contralateral ear served as a nontreated surgical wound-healing control. Control ears underwent the same procedure as experimental ears but were left unpacked. Animals were euthanized at 6 weeks post-operatively and their temporal bones harvested and processed for histology.

2.2. Surgery

Guinea pigs were anesthetized with a combined mixture of xylazine hydrochloride (15 mg/kg) and ketamine hydrochloride (35 mg/kg). The corneas of anesthetized animals were protected using an ophthalmic ointment applied at the beginning of each case. The surgical site was shaved and cleansed using 30% Betadine in 70% ethanol. Local anesthesia was then provided using 1% lidocaine subcutaneously. A midsagital scalp incision was made and a dura touching stainless steel screw electrode fixed into place just anterior to lambda location in the calvarium. Baseline auditory brainstem response (ABR) recordings were then taken using this permanent electrode along with subcutaneous needle electrodes. Following this step, a retroauricular incision was made to reach the bulla, which was opened using a diamond burr. After visualizing the cochlea, the mucosa was scraped at the junction of basal turn of the cochlea and adjacent bulla wall using a periosteal elevator. The middle ear mucosal lesion was standardized and kept by the surgeon (SA) similar for all lesions. The middle ear cavity was then packed gently using 1-mm^2 pieces of either PUF or AGS being careful to preserve the integrity of the ossicular chain and tympanic membrane. Incisions were sutured closed and antibiotic ointment was applied to the wound site (Fig. 1).

2.3. Electrophysiological testing

ABR testing was done using the Intelligent Hearing Systems Hardware and Software (IHS, Miami, FL) to measure hearing thresholds to pure tone stimuli at baseline (pre-operatively), 1 week, 2 weeks, and 6 weeks post-operatively. Testing was conducted bilaterally to record responses at 0.5, 1, 4, and 16 kHz stimuli. At the time of hearing testing, animals were anesthetized with a combined mixture of xylazine hydrochloride (15 mg/kg) and ketamine hydrochloride (35 mg/kg). Electrical activity was recorded using the chronic screw electrode in the vertex of the skull, referenced to a needle electrode inserted into the neck deep musculature, and grounded to a second needle electrode inserted subcutaneously near the tympanic bulla (Grass Instrument Division, Astro-Medical Inc., West Warick, RI). Stimuli were introduced into each ear via an external ear canal microphone beginning with an intensity of 90 dB SPL and decreasing in increments of 10-dB until threshold responses were identified. Threshold