Acta Oto-Laryngologica. 2015; 135: 14–25

ORIGINAL ARTICLE

Effect of absorbable gelatin sponge in the middle ear: in vitro and in vivo animal model

STEFANIA GONCALVES¹,², JUAN ARMANDO CHIOSSONE-KERDEL¹,², ANDREA S. BIANCO¹, JOSE M. ERCOLINO¹ & JORGE HERNANDEZ-ROJAS¹

¹Experimental Neurophysiology Laboratory, Universidad Central de Venezuela (UCV) School of Medicine, Ciudad Universitaria, Caracas and ²Fundación Venezolana de Otorología (FVO), Caracas, Venezuela

Abstract

Conclusion: GelitaSpon gelatin sponge (GS) showed faster reabsorption than gelfoam (GF) in vitro, and GS-packed middle ears resulted in a faster hearing recovery and less inflammation than the ears packed with GF soaked in saline. Soaking GF sponges with boric acid (BA) appeared to offset the inflammatory response of saline-soaked GF, making this inflammatory response comparable to that of GS-packed ears. Objective: To describe the reabsorption and inflammatory effects of absorbable gelatin sponge in the middle ear. Methods: For in vitro evaluation, GF and GS were used as disks immersed in saline solution or 3% BA and placed in gel. Images were captured with a microscope and processed using image processing Toolbox. For in vivo tests, 16 female albino Sprague Dawley rats were divided into four groups: bulla opening; GF + 0.9% saline; GF + BA; and GS + 0.9% saline. An anterior approach to the right bulla was used for surgery. Preoperative and postoperative auditory brainstem response thresholds were measured. Results: In vitro, there was marked degradation of GF by day 14, while GS showed complete degradation by the third day. In vivo, hearing recovery occurred by day 21. GF produced a more severe inflammatory response, which could be reduced by treating the GF with BA.

Keywords: Absorbable gelatine, boric acid, inflammation, middle ear surgery

Introduction

Absorbable gelatin sponges have been used since the 1940s, when they were first introduced for bleeding control during neurosurgery. Gelfoam (GF; Pfizer, Inc., New York, NY, USA) is produced from purified porcine skin, and is composed completely of collagen, which is the most abundant protein in animals and the principal component of bones, skin, cartilage, and connective tissue [1]. GF is not soluble in water, is non-elastic and is supplied as a classical porous sponge that can absorb 45 times its weight in water or blood. Since the mid-1960s GF has been widely used in otologic surgery as it is reabsorbed in about 6 weeks by the action of collagenases and subsequent phagocytosis of its fragments [2]. However, some studies have indicated that GF can produce inflammation and aberrant fibrosis when used for middle ear packing [3]. Other types of collagen sponges that are specifically made for otologic surgery, such as the GS Gelita-Spon® Final™ (Invotec International, Inc., Jacksonville, FL, USA) have been introduced in recent years. The main difference with these products is the reduced amount of cross-linkages between collagen fibers, which produces less overall mass that results in much earlier reabsorption and reduced risk of aberrant fibrosis.

The importance of the permanence and effects of these materials in the middle ear differs depending on the surgical procedure. However, details concerning the reabsorption rate and the inflammatory response generated by these absorbable gelatin sponges have not been described. As such, there is controversy over their use for middle ear surgery, with some studies...
indicating that absorbable gelatin sponges can promote aberrant scarring and fixation during mechanical middle ear reconstruction, and other studies contending that the formation of new fibrosis is beneficial for stable middle ear reconstruction.

Boric acid (BA; orthoboric acid, H₃BO₃) is a widely used weak acid of boron that is available in a crystalline form, is water soluble, and has a low level of toxicity (LD₅₀ = 5.14 g/kg) [4]. This molecule has been used as an antiseptic, anti-inflammatory agent [5], insecticide, flame retardant, and neutron absorbent [6]. In addition, BA has been widely used in otology, especially for prevention of chronic ear infections, but its effects in the middle ear and its possible side effects have been poorly studied.

The aim of this study was to determine the specific properties of GF, GS, and BA in the middle ear in the context of otologic surgery. We aimed to characterize the pattern of degradation of the commonly used absorbable gelatin sponges, GF and GS, and the effects that BA can cause on their pattern using an in vitro model. Furthermore, we present preliminary results of the effects of these two materials in the middle ear and the influences of BA on those effects using an in vivo model.

**Material and methods**

**In vitro study: image analysis**

GF and GS were cut into disks of 6.1 mm diameter and 4 mm thickness, immersed in 0.9% saline solution or 3% BA (pH 4.2, 20A (pHn 0.9% saline solution) for 24 h. Later, the specimens were placed in saline or BA depending on the group: group I, GF soaked in 0.9% saline; group II, GF soaked in 3% BA; group III GS soaked in 0.9% saline, and group IV was soaked in 0.9% saline; group II, GF soaked in 3% BA; group III GS soaked in 0.9% saline, and group IV was soaked in 0.9% saline; group II, GF soaked in 3% BA; group III GS soaked in 0.9% saline, and group IV was soaked in 0.9% saline. After 21 days, the animals were separated into four groups. The same surgeon performed all surgeries. The animal was placed supine on a board after induction of general anesthesia with intraperitoneal administration of xylazine hydrochloride (15 mg/kg) and ketamine hydrochloride (87 mg/kg). The extremities were then secured to the board and the neck area was extended and shaved. Under sterile conditions, a ventromedial incision was made in the skin, anterior and perpendicular to the mandibular arch to expose the fibromuscular tissue. A surgical microscope was used to observe the fine structures of the surgical field. The right tympanic bulla was approached by dissection of the connective tissues, submaxillary glands, and digastric muscle. Then, the mucosa was scraped with a periosteal elevator. A hole measuring 3 mm on average was created in the bulla with a diamond burr to expose all the middle ear structures. Posteriorly, the middle ear cavity was filled with pieces of GF or GS soaked in saline or BA depending on the group: group I, GF soaked in 0.9% saline; group II, GF soaked in 3% BA; group III GS soaked in 0.9% saline, and group IV was the surgical control group, characterized by simple opening of the bulla without introduction of any absorbable gelatin sponge. The integrity of the ossicular chain and tympanic membrane was preserved. The incision was sutured with an uninterrupted absorbable stitch, followed by intraperitoneal administration of a single dose of ampicillin/sulbactam for infection prophylaxis.

The rats were then allowed to recover from general anesthesia in a warmed cage. All animals were individually fed ad libitum and kept in an animal facility under controlled conditions of light until euthanasia was performed according to established international and institutional protocols of animal care and humane treatment of experimental animals. After 21 days, the animals were anesthetized using sodium thiopental and ketamine before euthanasia by cervical spinal cord section. Subsequently, bullas were carefully harvested and kept in 10% formalin solution for fixation for 24 h. Later, the specimens were placed in Osteomol® (nitric acid decalcifying solution that
combines ethylenediaminetetraacetic acid (EDTA) and formic acid, manufactured by Merck Laboratories, Darmstadt, Germany) for 1 day, then were embedded in paraffin and were cut according to standard histologic techniques before staining with hematoxylin and eosin. An expert pathologist, blinded to the experimental group, reported the histological findings, which were focused on the observation of the middle ear characteristics, taking into consideration the mucosa, the presence of absorbable gelatin sponge and inflammatory cells (polymorphonuclear cells, macrophages, lymphocytes, giant cells). In addition, the fibroelastic activity (defined as fibroblast proliferation in wounds), mucosal and submucosal thickening (Dogru et al [9]), presence of the packing, and presence of inflammatory cells within the packing were also considered. The pathologist evaluated 7–15 different slides per animal. Then, the number of slides per sample that showed inflammatory characteristics was counted and later divided by the total number of slides that were obtained per sample. This generated a number between zero and one (0–1) called ‘the inflammatory ratio’ and the severity of inflammation was reported as absent (0.00), mild (0.01 < 0.34), moderate (0.34–0.66) or severe (>0.66) according to this numerical result. For statistical analysis GraphPad Prims 5.0c (GraphPad Software Inc., San Diego, CA, USA) and JMP 11 from SAS (SAS Institute Inc., Cary, NC, USA) software for Macintosh were used. The results were processed in different ways to determine the impact of the variables. For a general comparison of all the histological parameters as a whole per group of study, a one-way analysis of variance followed by a post hoc Neuman–Keuls multiple comparison test were performed using the estimated inflammatory ratio. Later, for determination of the level of significance between the degrees of histologic changes in the middle ear cavities, the chi-squared test and Fisher’s exact test were used, as reported by Dogru et al. [9]. A p value < 0.05 was considered statistically significant. A sample size of 4 was calculated by using an alpha probability of 0.05, a power of 0.7, which is acceptable for animal studies, and an effect size of 95%.

In vivo study: hearing threshold assessment

Animals were sedated with a combination of ketamine (50 mg/ml) and xylazine (20 mg/ml) administered intraperitoneally in doses of 87 mg/kg and 6.5 mg/kg, respectively. Subcutaneous needle electrodes were placed in the vertex (positive), behind the right ear (negative), and under the chin (ground) for recording of auditory brainstem response (ABR). An in-house designed ABR amplifier with 20 k overall gain, 100 Hz high-pass, 1.5 kHz low-pass, and 60 Hz notch was used. The acoustic stimulus was presented monaurally by occluding the left ear with a saline-soaked cotton plug. Compression click stimuli of 100 presented monaurally by occluding the left ear w a speaker placed 17 cm above the rat’s head. Each threshold measurement consisted of a sequence of ABRs acquired at 200 s intervals and different sound intensity levels in steps of 5 dB in pseudo-random order, with two consecutive intensities not differing by more than 30 dB. The intensities ranged from 40 to 85 dB SPL.

The experiments were conducted inside a sound-attenuated booth (50 × 30 × 30 cm) with 35 dB attenuation above 1 kHz. The animal body temperature was maintained at 37.0 inte°C. ABR acquisition was performed with an NI USB-6216 (National Instruments Corporation, Austin, TX, USA) USB multifunction data acquisition module and a 25 kHz sample rate in 32 s epochs, giving ABR averages from 1600 windowed samples. The entire data acquisition and analysis process was performed under the Mathworks MATLAB® environment.

A novel method for the hearing threshold analysis was developed based on the linear regression of two ABR voltage-related variables: the RMS value and the maximum–minimum differential. The best estimate of the hearing threshold level was obtained from a weighted average of these two estimates (Figure 1). All animals underwent otoscopic evaluation before euthanasia. For statistical purposes, considering that audiomeric data do not have compliance with the required parameters for analysis of variance, we used nonparametric tests for analyses (quartiles and confidence intervals) and matched-pair analysis with multiple pair comparisons using the Wilcoxon rank test with a significance level of 0.05 for intergroup analysis.

Results

In vitro study: image analysis

The tridimensional image analysis in Figure 2A shows a marked degradation of GF by day 14, evidenced by a significant enhancement of the disk luminosity and the loss of the inner mass of the sponge. A much more significant degradation was observed for GS, which was totally degraded by the third day, with only an enrichment of bubbles within the remaining surrounding gel, and non-detectable gelatin by day 4. The random spikes represent the luminosity of the air bubbles and are considered to be random artifacts that did not affect the analysis of the disk itself.
Further image analysis using histogram of pixels seen in Figure 2B was performed. The red bars (left) represent the luminosity in terms of the number of pixels that are present in the disk, and the blue bars (right) depict the pixels from the background, also showing non-complete degradation of GF by day 14 characterized by a clear separation and differentiation of the red from the blue bars. A total degradation of GS is shown by the third day, represented by a disappearance of the red bars (disk) and the permanence of unchanged blue bars (background). This result was also corroborated by visual examination of the sample. The luminosity difference, the total area change, and the entropy were also analyzed and reflected a high index of degradation for GS when compared with GF, which was unchanged in those samples treated with BA, indicating that this molecule does not affect the gelatin absorption rate and that their degradation depends on the physical properties of each gelatin. The entropy is referred as the function of patterns of adjacent pixels that represents the internal pattern of the image in the study. GF shows an erratic entropy pattern that is related to a poor pattern of degradation as compared with GS, which showed a typical exponential function with a steady pattern of degradation by day 4 [7].

In vivo study: ABR thresholds

As expected, a conductive hearing loss was seen in all groups with a hearing threshold deterioration of 30.85 ± 6.27 dBSPL, from the first postoperative day, reaching a peak between the third and seventh day, followed by posterior hearing improvement, most pronounced in group III. In group III, two of the rats developed otitis media with an evident amount of fluid in the middle ear, which was correlated with their hearing deterioration by day 21 after having shown hearing improvement by day 14. Consistently low ABR thresholds were also obtained in this group across all measurements, as seen in Figure 3, but statistical significance (p < 0.05) was only reached by day 14, which was expected considering the fact that GS has a higher degradation rate and is totally reabsorbed by the third day. Finally, intergroup comparisons were performed, showing a statistically significant (p < 0.05) difference when
Figure 2. Image analysis: tridimensional (A) and histogram of pixels (B). (A1) GF + NS, day 1: complete integrity of the disk. (A2) GF + NS, day 3: partial degradation of the disk after 14 days. (A3) GS + NS, day 1: partial degradation of the disk after being soaked with NS. (A4) GS + NS, day 3: complete degradation by the third day. The randomly distributed blue spikes are bubbles. (B1) GF + NS, day 1: red bars are clearly separated from the blue bars, representing complete integrity of the disk. (B2) GF + NS, day 14: red bars are overlapping the blue bar representing partial degradation of the disk with residual material within the Petri capsule. (B3) GS + NS, day 1: the red bars are closer to the blue bars with some overlapping, representing partial degradation of the disk. (B4) GS + NS, day 3: no red bars are present, which is translated in a complete degradation of the gelatin sponge without remnants within the Petri Capsule by day 3. Visual corroboration was made. GF, gelfoam; GS, GelatiSpon gelatin sponge; NS, normal saline.
Figure 2. (Continued).
Figure 2. (Continued).
Figure 2. (Continued).
Figure 3. Postoperative auditory brain stem response (ABR) thresholds. *Statistically significant value (p < 0.05) is shown for those ears treated with GS soaked with saline on day 14. On day 21 this significance was lost. Two rats in this group presented with otoscopic finding of otitis media. BA, boric acid; BO, bulla open (control); GF, gel foam; GS, GelitaSpon gelatin sponge; NS, normal saline.

Comparing group II (GF + BA) and group III (GS + 0.9% saline, NS) (Table I).

In vivo study: post-surgical assessment

A general comparison of all the inflammatory ratios per group showed statistical significance (p < 0.05) when comparing group I (GF + NS) to group II (GF + BA), group III (GS + NS), and group IV (bulla open, BO control), as seen in Figure 4. Other comparisons were not significant, showing that the inflammatory ratios of those ears packed with BA-treated GF (0.27 ± 0.15 standard error, SE) and GS soaked in saline (0.35 ± 0.09 SE) are similar to each other and to the surgical control group (0.47 ± 0.16 SE). A higher inflammatory index was seen in saline-treated GF (0.63 ± 0.15 SE), while BA-treated GF and GS treated solely with saline had a much lower index. There was a surprisingly low cellularity in BA-treated GF, with a very low inflammatory response when compared with GS soaked with saline, even in the presence of the gelatin by day 21. No packing was present in any of the animals treated with GS soaked with saline by day 21. The middle ears in the surgical control group showed higher inflammatory ratios when compared with BA-treated GF and GS soaked with saline. Finally, two of the animals within this group that presented with the typical inflammatory findings of otitis media had pronounced mucosal thickening and gland hyperplasia.

Last, when analyzing the degree of inflammation based on the histological parameters within the middle ear cavities individually, the presence of the packing and the presence of inflammatory cells within the packing were statistically significant with p values of 0.0006 and 0.0015, respectively. However, the presence of inflammatory cells, fibroblastic activity, mucosal and submucosal thickening, and the overall inflammatory status were not statistically significant, as shown in Table II.

Discussion

Absorbable gelatin sponges have hemostatic properties and are indicated in surgical procedures for bleeding control when classic techniques are not effective or cannot be performed in a particular procedure [10]. As surgical techniques have developed across all specialties, absorbable gelatin sponges in otorhinolaryngology were initially used for epistaxis control but extra utilities were later developed such as its use as a material support for temporal fixation of stapes prostheses, as a substitute for the corneal lining enhancing the epithelialization and closure of tympanic membrane perforations, and as an adherence promoter of the tympanic graft to the tympanic remnant [11], and they have been also useful in achieving

Table I. Summary of audiometric data (ABR threshold in dBSPL) with multiple comparison test between groups using Wilcoxon rank test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median</th>
<th>25–75% interquartile range</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II (GF + NS/GF + BA)</td>
<td>50.03/46.98</td>
<td>42.25–51.95/39.58–51.33</td>
<td>0.01</td>
<td>37.76–54.68/40.02–52.40</td>
<td>p &gt; 0.05, NS</td>
</tr>
<tr>
<td>I/III (GF + S/GS + S)</td>
<td>50.03/41.48</td>
<td>42.25–51.95/35.93–46.43</td>
<td>5.08</td>
<td>37.76–54.68/35.71–46.56</td>
<td>p &gt; 0.05, NS</td>
</tr>
<tr>
<td>II/III (GF + BA/GS + S)</td>
<td>46.98/41.48</td>
<td>39.58–51.33/35.93–46.43</td>
<td>5.07</td>
<td>40.02–52.40/35.71–46.56</td>
<td>p &lt; 0.05*</td>
</tr>
<tr>
<td>II/IV (GF + S/BO)</td>
<td>50.03/52.85</td>
<td>42.25–51.95/37.25–59</td>
<td>–3.01</td>
<td>37.76–54.68/37.68–60.77</td>
<td>p &gt; 0.05, NS</td>
</tr>
<tr>
<td>II/IV (GF + BA/BO)</td>
<td>46.98/52.85</td>
<td>39.58–51.33/37.25–59</td>
<td>–3.02</td>
<td>40.02–52.40/37.68–60.77</td>
<td>p &gt; 0.05, NS</td>
</tr>
<tr>
<td>III/IV (GS + S/BO)</td>
<td>41.48/52.85</td>
<td>35.93–46.43/37.25–59</td>
<td>–8.09</td>
<td>35.71–46.56/37.68–60.77</td>
<td>p &gt; 0.05, NS</td>
</tr>
</tbody>
</table>

ABR, auditory brainstem response; BA, boric acid; BO, bulla open (control); CI, confidence interval; GF, gel foam; GS, GelitaSpon gelatin sponge; NS, not significant; S, 0.9% saline; SPL, sound pressure level.
*Statistically significant value.
temporary eustachian tube obliteration. Currently, besides their supportive and reconstructive utilities, absorbable gelatin sponges formed into small cubes can be deposited in the external acoustic meatus or in open tympanomastoidectomies to support tympanic grafts and help in the repositioning of the tympanomeatal flap [12,13]. Degradation patterns of absorbable gelatin sponges have been an important issue because gelatins behave differently if they lie on the top of a cavity or just on the surface of the tissue. We demonstrated with the in vitro findings obtained in this study that the structure of the gelatins plays a very important role in their degradation. If the collagen component has more cross-linkages, as is the case for GF, the degradation process in the control gel environment is very slow and predictably could last up to 6 weeks, which is well beyond the time frame of our analysis. In contrast, finely produced collagen such as the GS has fewer cross-linkages and a lower mass that will eventually degrade much more rapidly. BA is a weak acid that might be used in an in vivo model and it seems not to interfere with the degradation of absorbable gelatin sponges as might be inferred from the results of our in vitro model considering that, macroscopically speaking, the length of degradation of the gelatins did not vary after treatment with

**Figure 4.** Overall inflammatory status. The highest inflammatory ratio was seen in those ears packed with GF + NS, while the lowest inflammatory ratio was shown in the ears treated with GF + BA, even when compared with those treated with GS + NS, presumably due to the anti-inflammatory effects of boric acid (BA). Interestingly, the surgical control group showed a higher inflammatory ratio when compared with GF + BA and GS+NS, but less than GF + NS. BA, boric acid; GF, gelfoam; GS, GelatiSpon gelatin sponge; NS, normal saline. *Statistically significant value (p < 0.001).

**Table II. Ratios for histological findings.**

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Group</th>
<th>Inflammatory cells</th>
<th>Fibroblastic activity</th>
<th>Mucosa and submucosa thickness</th>
<th>Presence of packing</th>
<th>Inflammatory cells within packing</th>
<th>Overall inflammatory status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.20</td>
<td>0.27</td>
<td>0.17</td>
<td>Yes</td>
<td>0.43</td>
<td>Mild</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.00</td>
<td>0.83</td>
<td>1.00</td>
<td>Yes</td>
<td>0.83</td>
<td>Severe</td>
</tr>
<tr>
<td>3</td>
<td>GF + S</td>
<td>0.67</td>
<td>0.78</td>
<td>0.78</td>
<td>Yes</td>
<td>0.78</td>
<td>Severe</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.58</td>
<td>0.67</td>
<td>0.67</td>
<td>Yes</td>
<td>0.67</td>
<td>Moderate</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.11</td>
<td>0.06</td>
<td>0.17</td>
<td>No</td>
<td>X</td>
<td>Mild</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0.12</td>
<td>0.15</td>
<td>0.09</td>
<td>Yes</td>
<td>0.30</td>
<td>Mild</td>
</tr>
<tr>
<td>7</td>
<td>GF + BA</td>
<td>0.67</td>
<td>0.67</td>
<td>0.83</td>
<td>Yes</td>
<td>0.67</td>
<td>Severe</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>0.17</td>
<td>0.00</td>
<td>0.17</td>
<td>Yes</td>
<td>0.17</td>
<td>Mild</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>0.25</td>
<td>0.17</td>
<td>0.08</td>
<td>No</td>
<td>X</td>
<td>Mild</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>No</td>
<td>X</td>
<td>Moderate</td>
</tr>
<tr>
<td>11</td>
<td>GS + S</td>
<td>0.17</td>
<td>0.17</td>
<td>0.33</td>
<td>No</td>
<td>X</td>
<td>Mild</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>0.60</td>
<td>0.33</td>
<td>0.60</td>
<td>No</td>
<td>X</td>
<td>Moderate</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>0.67</td>
<td>0.50</td>
<td>0.50</td>
<td>No</td>
<td>X</td>
<td>Moderate</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>0.89</td>
<td>0.78</td>
<td>1.00</td>
<td>No</td>
<td>X</td>
<td>Severe</td>
</tr>
<tr>
<td>15</td>
<td>BO</td>
<td>0.17</td>
<td>0.00</td>
<td>0.33</td>
<td>No</td>
<td>X</td>
<td>Mild</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>0.33</td>
<td>0.22</td>
<td>0.22</td>
<td>No</td>
<td>X</td>
<td>Mild</td>
</tr>
</tbody>
</table>

Chi-squared test $p = 0.55$ $p = 0.31$ $p = 0.35$ $p = 0.0006^*$ $p = 0.0015^\dagger$ $p = 0.55$

BA, boric acid; BO, bulla open (control); GF, gel foam; GS, Gelita sponge; S, saline.

Inflammatory degree: absent 0; mild <0.34; moderate 0.34–0.67; severe >0.67.

*Statistically significant value ($p < 0.0001$).

†Statistically significant value ($p < 0.01$).
BA, but further molecular studies must be done to corroborate this hypothesis. Also, the in vivo histological findings support the in vitro results, considering that no packing was visible after day 21 in those samples treated with GS soaked in saline. Besides, the anti-inflammatory effects of BA were corroborated, showing less inflammatory ratios in those ears packed with BA-treated GF when compared with GF soaked with saline, being comparable to those treated with GS soaked in saline. We can speculate that BA crystals are present as long the absorbable gelatin sponge remains in the middle ear; we were not able to detect BA crystals within GF in this study.

Despite the benefits and contributions to the development of otologic surgery that occurred after absorbable gelatin sponges were widely used, some disadvantages were also found that might alter the success of particular procedures. These disadvantages are mainly determined by the inflammatory reactions they generate and the length of duration of the gelatin at the anatomic location where they are placed. For example, retro-tympanic adherences might be triggered by an increased inflammatory response due to the presence of the absorbable gelatin sponge in addition to the surgical manipulation itself, which could lead to failure of some middle ear procedures such as stapedectomy. However, untreated GF could trigger unregulated fibrosis that would make middle ear reconstruction less effective, as was suggested by the results observed in those ears packed with saline-treated GF.

When comparing absorbable gelatin sponges with the control groups, some studies demonstrated a lower healing rate and a greater level of osteogenesis in the middle ear of patients when absorbable gelatin sponge was used, as well as higher postoperative ABR thresholds, which are considered to rule out hearing relative to preoperative levels and those ears packed with GS soaked with saline did show a significant hearing improvement by day 14. However, considering that our in vitro study showed total degradation of GS by day 3, the delay in the recovery of hearing might be related to the length of the inflammatory response, which is usually cleared after the first week of the surgery. This result might point to the exclusion of ototoxicity after using this packing. However, considering the long period of degradation of GF prolonging the conductive hearing loss, further conclusions based on the ABR are not feasible.

A comparison study between GF, Sepragel® (Genzyme Company, Redgefield, NJ, USA) and Nasopore® (Polyganic, Bangalore, India) showed that the histologic appearance of GF-treated middle ear was characterized by more severe acute inflammation in the short term and prominent fibrosis in the long term [15]. Among the different types of absorbable gelatin sponges, GF is the more widely used. We found that inflammatory reactions for GF and saline were more prominent than for other groups and that the gelatin was still detectable through day 21. This result is relevant due to the extensive inflammatory infiltrate within the gelatin remnants. Although a positive correlation between the duration of reabsorption and inflammatory reactions could not be established in this study, we can speculate that this relationship can be an important factor when considering the use of absorbable gelatin sponges in middle ear surgeries. In contrast, we found very low rates of inflammatory reactions for GF treated with BA. This result could be explained by the antiseptic effect of BA, which likely lowers the amount of immediate mucosa in contact with GF by promoting a low pH micro-environment within the middle ear cavity. BA crystals could be retained in the complex structure of collagen cross-linkages, and persist while the GF is present in the middle ear. It was not possible to demonstrate this in the present study. Meanwhile, the GS was completely reabsorbed; together with our in vitro observation, we can predict that this degradation happens in a shorter time than for GF. While the rapid reabsorption could lessen the effects of inflammatory responses in the middle ear, such rapid rates of reabsorption could present a negative factor if middle ear reconstructive structures need to be stabilized for longer periods of time.

BA was considered as an object of study due to its therapeutic properties and its recognized use as an antiseptic. However, the effects of BA in the middle and inner ear have not been described in detail. Recent publications support the proposition that BA is not ototoxic if dissolved in distilled water or low-grade alcohol (40%) solutions [16–18]. This study lacks histological findings for ruling out a possible ototoxic effect and more extensive study will be required to determine whether or not BA has toxic effects within the cochlea by penetrating into the inner ear through the round or oval window, in addition to morphological studies of the organ of Corti to define the presence of effects on inner or outer hair cells.

Finally, GF treated with weak acids such as BA can be less expensive and a reasonable approach for preventing aberrant healing within the middle ear in those institutions where financial resources are limited, considering the high cost of GS.

To our knowledge, we present a first and novel approach of image analysis to study the possible
behavior of gelatin sponges when combined with other substances.

Conclusion

GS showed faster reabsorption than GF in vitro, and GS-packed middle ears resulted in a faster hearing recovery and less inflammation than the ears packed with GF soaked in saline. Soaking GF sponges with BA appeared to offset the inflammatory response of saline-soaked GF, making this inflammatory response comparable to that of GS-packed ears.

Finally, a reasonable method of pixel analysis can be used to determine the effects of different substances such as collagenases, antibiotics, and/or macromolecular crystals or substances in combination with absorbable gelatin sponges.

Acknowledgment

Dr Francisco Bruni MD and the Pathology Laboratory team at Clinica El Avila, for tissue processing and evaluation and Dr Vet. Manuel Moya at the Medical Faculty Animal House and Instituto Nacional de Higiene Animal House for animal care and procedures.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References