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The effects of different packing materials on healing and hearing after trauma to middle ear mucosa, an experimental study in rats

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ABSTRACT

Purpose: To compare the performance of Spongostan, Otopore, Spongostan soaked with dexamethasone and Spongostan soaked with Hyaluronic acid (HA) as middle ear packing material after mucosal trauma.

Methods: Twenty rats were divided into 4 groups. In control group (group 1), the middle ear cavities of animals were bilaterally packed with Spongostan; in group 2, with Otopore; in group 3, with Spongostan soaked with dexamethasone; and in group 4, with Spongostan soaked with HA. Auditory brainstem responses (ABRs) were performed preoperatively and 1 and 6 weeks postoperatively. Histological analyses were performed to evaluate the inflammatory reaction and wound healing in the middle ear cavity.

Results: ABR recordings demonstrate that threshold level changes from baseline were minor in Otopore and Spongostan soaked with dexamethasone packed ears. Threshold levels were higher in the Spongostan and Spongostan soaked with HA packed ears compared with both Otopore and Spongostan soaked with dexamethasone packed ears. Histological analyses showed that Spongostan caused inflammation more intense than Otopore and Spongostan soaked with dexamethasone. Residual material at postoperative week 6, new bone formation and adhesion were common in the Spongostan group compared with other groups. Fibrosis was more common in Spongostan group compared with other groups but the difference was not significant.

Conclusion: Otopore appears to be safe and effective for use in otologic surgery. The inflammation, adhesion and new bone formation decreased when Spongostan was used with steroid or HA, when compared to Spongostan alone.

1. Introduction

Different middle ear packing materials (MEPMs) are used for procuring the support of graft material placed near the middle ear (ME) during surgery, creating an air-filled ME cavity and accelerating the mucosal recovery in case of mucosal damage. Thus, medialization of the graft materials prevented, and instead, it is retained in correct position. In addition, those materials do not require removal, and they offer advantages in terms of cost-effectiveness [1,2].

Spongostan™, an absorbable gelatin sponge (AGS) material, is the most frequently used MEPM. However, studies have shown that it tends to induce an extensive inflammatory response resulting in fibrosis, adhesion, or new bone formation, and they have toxic effects on the inner ear hair cells [3–6]. Consequently, researchers performed studies to discover materials that would lead to better wound healing. Hyaluronic acid (HA) is a biocompatible, non-ototoxic material [7]. Several studies have shown that the materials that contain HA significantly reduced the incidence, extent, and severity of postoperative adhesions [4,5,8]. Otopore™ is an absorbable MEPM that is fully synthetic, inert, and highly biodegradable. Dogru et al. [9] reported that Otopore might resulted in less inflammation and fibrosis compared to AGS. Glucocorticoids reduce inflammation in the ME mucosa in otitis media [10]. The aim of this study is to demonstrate the effects of various absorbable MEPMs (Spongostan, Otopore, Spongostan soaked with dexamethasone, and Spongostan soaked with HA) on mucosal healing and hearing, using electrophysiological and histopathological methods.

2. Material and methods

The local ethical committee for animal experiments approved this trial (decision no. 0018, May 13, 2015). The study was performed in the Laboratory of Animal Experiments. Twenty healthy adult male Wistar Albino rats with a weight of 200–350 g were used in the study. The animals were divided into four groups, each consisting of five rats. We used Spongostan (Ethicon, USA) in the control, and Otopore (Stryker, USA; Otopore group), Spongostan soaked with dexamethasone

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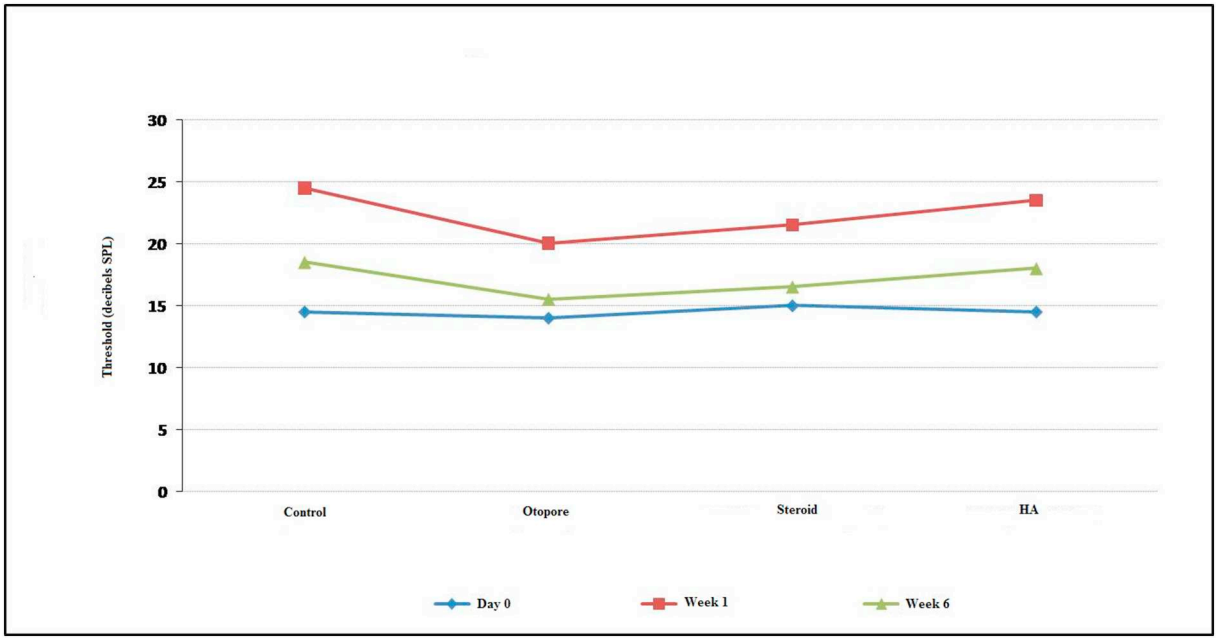

Fig. 1. Audiometric results. Auditory brainstem response (ABR) hearing threshold changes for each of the four groups ranging from Day 0 through postoperative Week 6.

(Onadron® 5 ml, I.E. Ulagay, Turkey; steroid group), and Spongostan™ soaked with HA (Healon® 2.3%, 0.6 ml, 25 g, Abbott Medical, USA; HA group) in both ears of the study groups. We followed the principles of the Declaration of Helsinki. The rats were accommodated at 25 °C in the light for 12 h and in the dark for 12 h in an environment where they could freely find food and water. The noise level was below 50 dB. Packing materials (PMs) were placed in both ears of the rats on Day 0, and electrophysiological measurements were performed using Auditory Brainstem Response (ABR) (Day 0, Week 1, Week 6) under general anesthesia. General anesthesia was employed by administering intramuscular 40 mg/kg ketamine HCl (Ketalar®, Eczacibasi, Turkey) and 5 mg/kg xylazine HCl (Rompun®, Bayer Vital, Germany). The drugs were mixed in one syringe under sterilized conditions, and they were injected into the right quadriceps femoris muscle. All the rats completed the study.

2.1. Auditory assessment

The rats' ears were examined under the microscope (Zeiss, OPMI 9, Germany) under general anesthesia on Day 0, and debris and plugs were removed from the external auditory canal to see the normal tympanic membranes (TM). The ABR test was performed using the Eclipse 25 ABR System (Interacoustic, Denmark). Basal auditory thresholds were obtained via ABR prior to surgery, to exclude the rats with hearing loss. The electrodes were placed as follows: ground line on the lower part of the forehead, positive line on the upper part of the forehead, one negative electrode on the left ear's mastoid, and the other negative electrode on the right ear's mastoid. Attention was paid to keep the cables away from the recording device as much as possible during the test, and effort was made to maintain the electrode–skin impedances below 5kΩ. Insert earphones (ER-3A) were used during the tests.

Click stimuli were given in the ABR test. At least two readings were taken for each ear at the same stimulus power. The threshold value was found, starting from 80 dB (dB), by lowering the level by 10 dB decrements if the threshold was exceeded, and 5 dB decrements when the values were close to the threshold. The threshold level was defined as behavior at the lowest repeatable power level that can be observed visually. The repeatability of behavior was tested by creating at least two traces for each reading, and the threshold was verified. A 90 dB sound pressure level (SPL) stimulus level was used when behavior could not be obtained at 80 dB SPL. The ABR threshold was defined as the lowest power level at which wave III of ABR can be observed. Following the intervention of TMs, ABR readings were repeated at weeks 1 and 6, and the findings were compared with the basal ABR recordings.

2.2. Surgical procedure

At the initial stage of the study (Day 0), the TMs of all the rats were perforated with the help of a myringotomy blade (with a larger size than the material to be placed in the ME and a central position) under general anesthesia under the microscope, after determining the threshold values with ABR. Blood that accumulated in the ME was aspirated using a cottonoid and thin aspirator to achieve a dry environment. The size of packing materials was standardized for each group (1 mm long x 1 mm thick). Being careful to preserve the integrity of the ossicular chain, all packing materials were gently placed in the ears of each animal in all groups through perforation, until reaching the level of perforation: Spongostan for the control group, Otopore for the Otopore group, Spongostan soaked with dexamethasone for the steroid group, and Spongostan soaked with HA for the HA group. Surgery was performed in both ears of the animals.

2.3. Histopathological assessment

The animals were sacrificed under general anesthesia, following the last ABR test, at week 6. The right and left temporal bones were removed and soaked in 10% formalin solution for 24 h, they were then soaked in formic acid for 48 h for decalcification. Five micrometer-thick sections were cut from paraffin-embedded blocks with a microtome, and the sections were placed on slides. The samples were stained with hematoxylin & eosin, and the specimens were examined by an experienced pathologist at 40 and 100× magnifications under a light microscope. The severity of inflammation was rated semi-quantitatively as: none/low (+), moderate (++), or extensive (+++), based on the cell (neutrophil and lymphocyte) infiltration observed in the inflammation zone and stain intensity. Development of fibrosis, new bone formation and the presence of adhesions with adjacent tissues observed

in the inflammation area during mucosal healing were rated as absent (0) or present (1). Residual material in the ME cavity was rated as absent (0) or present (1).

2.4. Statistical analysis

The results are presented as descriptive statistics (mean ± standard deviation [SD], median ± SD, frequency, ratio, minimum, and maximum). The normal distribution of data from each group was confirmed using the Kolmogorov–Smirnov normality test. Kruskal–Wallis test was employed to compare the groups for quantitative data. Mann–Whitney *U* test was used to detect the group causing the statistical difference, and compare the data that did not show a normal distribution. The Friedman test was employed for intergroup comparisons of ABR data, while the Wilcoxon signed-ranks test was used for binary evaluation. The Fisher–Freeman–Halton test was used for comparing qualitative data, and Fisher's exact test and Yates' continuity correction test (Yates corrected chi-square) were used for binary comparisons. Number Cruncher Statistical System (NCSS) 2007 and Power Analysis and Sample Size (PASS) 2008 statistical software (Kaysville, UT, USA) were used for analysis of data. A p-value < 0.05 was considered as statistically significant.

3. Results

3.1. ABR results

Fig. 1 depicts mean ABR threshold changes for each of the four groups at Day 0, Week 1 and Week 6. The mean ABR thresholds did not show any statistically significant difference among the groups ($p = 0.902$ for Day 0, $p = 0.101$ for Week 1, and $p = 0.094$ for Week 6).

In the control group, there were statistically significant differences in the ABR thresholds among Day 0, Week 1, and Week 6 ($p = 0.001$). There were statistically significant increases in ABR thresholds at Week 1 compared to Day 0, and at Week 6 compared to Day 0 ($p = 0.004$ and $p = 0.024$, respectively). It was noted that ABR thresholds decreased at Week 6 compared to Week 1 ($p = 0.011$).

In the Otopore group, there were statistically significant differences in the ABR thresholds among Day 0, Week 1, and Week 6 ($p = 0.001$). The increase in the ABR thresholds at Week 1 compared to Day 0, and decrease in the ABR thresholds at Week 6 compared to Week 1 gained statistical significance ($p = 0.003$ and $p = 0.014$, respectively). There was no statistically significant difference in the ABR thresholds between Day 0 and Week 6 ($p = 0.083$).

In the steroid group, there were statistically significant differences in the ABR thresholds among Day 0, Week 1, and Week 6 ($p = 0.001$). The increase in the ABR thresholds in Week 1 compared to Day 0, and decrease in the ABR thresholds in Week 6 compared to Week 1 were statistically significantly ($p = 0.004$ and $p = 0.008$, respectively). There was no statistically significant difference in the ABR thresholds between Day 0 and Week 6 ($p = 0.083$).

In the HA group, there were statistically significant differences in the ABR thresholds among Day 0, Week 1, and Week 6 ($p = 0.001$). The increase in the ABR thresholds at Week 1 compared to Day 0, decrease in the ABR thresholds at Week 6 compared to Week 1, and increase in the ABR thresholds at Week 6 compared to Day 0 were statistically significant ($p = 0.004$, $p = 0.015$, and $p = 0.008$, respectively).

3.2. Histopathological results

Residual material was observed in six ears in the control group, and no residual material was observed in the other groups. The difference was statistically significant ($p = 0.001$; Table 1).

New bone formation was observed in five ears in the control group, two ears in the Otopore group, and one ear in the steroid group. No bone formation was observed in the HA group. The difference in the bone formation ratios among the groups was statistically significant ($p = 0.048$). Binary comparisons showed that new bone formation ratio was significantly higher in control group compared to the HA group ($p = 0.003$). The differences of the new bone formation ratios in the other groups were not statistically significant ($p > 0.05$).

Fibrosis formation was observed in six ears in the control group, two ears in the Otopore group, two ears in the steroid group, and one ear in the HA group. There was no statistically significant difference among the groups ($p = 0.105$).

Adhesion formation was observed in seven ears in the control group, one ear in the Otopore group, five ears in the steroid group, and six ears in the HA group. The adhesion formation ratios showed significant differences among the groups ($p = 0.043$). The binary comparisons showed that the adhesion formation in the control group was significantly higher than that in the Otopore group ($p = 0.020$). There were no statistically significant differences in the other binary comparisons among the groups in terms of adhesion formation ($p > 0.05$).

Moderate inflammation was observed in two ears, and extensive inflammation was observed in eight ears in the control group (Fig. 2). In the Otopore group, there was no/low inflammation in nine ears, and extensive inflammation in one ear (Fig. 3). In the steroid group, no/low inflammation was observed in seven ears, moderate inflammation in one ear, and extensive inflammation in two ears (Fig. 4). Finally in the HA group, there was no/low inflammation in two ears, moderate inflammation in one ear, and extensive inflammation in seven ears (Fig. 5). The inflammation ratios showed significant differences among the groups ($p = 0.001$). The binary comparisons of the groups showed that the level of no/low inflammation was significantly higher in the Otopore group compared to the control and HA groups (p = 0.001 and $p = 0.005$, respectively). The no/low inflammation ratio in the steroid group was significantly higher than the control group ($p = 0.003$) and HA group ($p = 0.070$), however the difference between steroid and HA

Table 1
Histologic findings in the control, Otopore, steroid and HA groups. Last column indicates the comparison of histological findings between groups.

		Control (n = 10)	Otopore (n = 10)	Steroid (n = 10)	HA (n = 10)	[d]p
Residual material at 6 weeks	Absent	4 (40%)	10 (100%)	10 (100%)	10 (100%)	***0,001*** **
	Present	6 (60%)	0 (0%)	0 (0%)	0 (0%)	
New bone formation	Absent	5 (50%)	8 (80%)	9 (90%)	10 (100%)	***0,048*** *
	Present	5 (50%)	2 (20%)	1 (10%)	0 (0%)	
Fibrosis	Absent	4 (40%)	8 (80%)	8 (80%)	9 (90%)	***0,105***
	Present	6 (60%)	2 (20%)	2 (20%)	1 (10%)	
Adhesion	Absent	3 (30%)	9 (90%)	5 (50%)	4 (40%)	***0,043*** *
	Present	7 (70%)	1 (10%)	5 (50%)	6 (60%)	
Inflammation	None/mild	0 (0%)	9 (90%)	7 (70%)	2 (20%)	***0,001*** **
	Moderate	2 (20%)	0 (0%)	1 (10%)	1 (10%)	
	Severe	8 (80%)	1 (10%)	2 (20%)	7 (70%)	

[d] *Fisher-Freeman-Halton Test* ** $p < 0.01$ * $p < 0.05$.

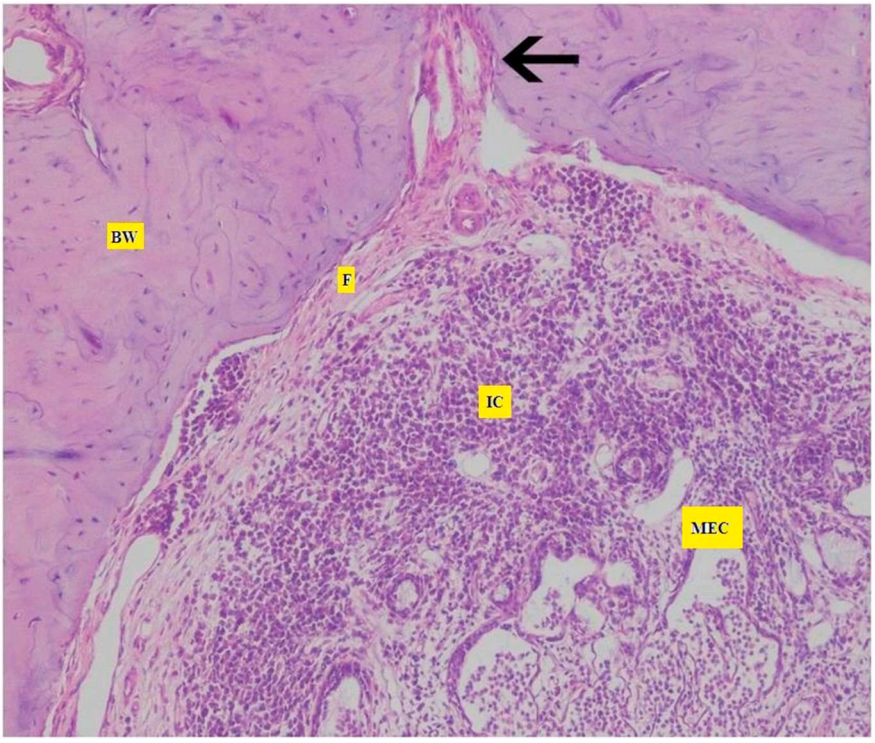

Fig. 2. A cross-section of the middle ear cavity of a Spongostan-packed rat (control group) that had severe inflammation at postoperative week 6. The arrow points to the site of mucosal trauma and adhesion findings that are observed in this area. Intensive inflammatory cells and fibrosis (F) are observed in the middle ear cavity. (Original magnification X100). BW indicates bullar wall; MEC, middle ear cavity and IC, inflammatory cells.

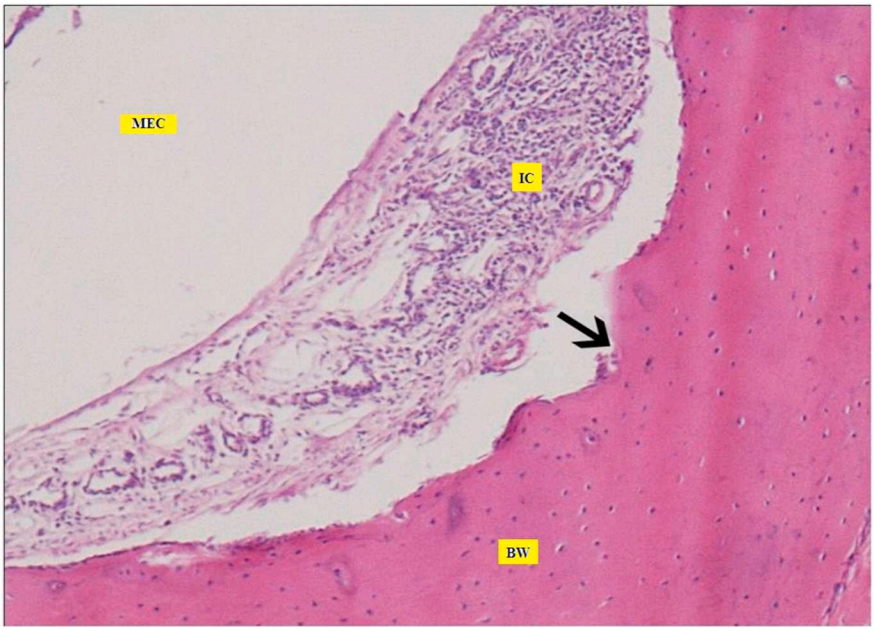

Fig. 3. A cross-section of the middle ear cavity of a Otopore-packed rat that had mild inflammation at postoperative week 6. The arrow points to the site of mucosal trauma without adhesion findings. (Original magnification X100). BW indicates bullar wall; MEC, middle ear cavity and IC, inflammatory cells.

groups was not significant. The inflammation ratio in the control group was significantly higher than the ratios of Otopore and steroid groups ($p = 0.005$ and $p = 0.025$, respectively). The extensive inflammation ratio in the HA group was higher than the steroid group, but the difference was not statistically significant ($p = 0.070$). The extensive inflammation ratio in the HA group was significantly higher than the Otopore group ($p = 0.002$).

4. Discussion

Formation of adhesion and fibrosis is not rare following otologic surgical procedures such as tympanoplasty and tympanomastoidectomy. Those conditions may cause hearing loss, TM retraction, and graft failure. The ME membrane is highly responsive to trauma and infections [11]. Therefore, PMs put into the ME cavity are as important as the graft materials used for repairing TM perforations. Those materials must be biocompatible, and they should not lead to any mucosal inflammation. The PMs are used for maintaining the graft material's stability during the healing process [12]. As mentioned above, the ideal PMs should have minimal effect on ME ventilation and mechanics.

Spongostan has been introduced as a hemostatic agent by Correl and Wise [13]. Subsequently, it has been used as in tympanoplasty to

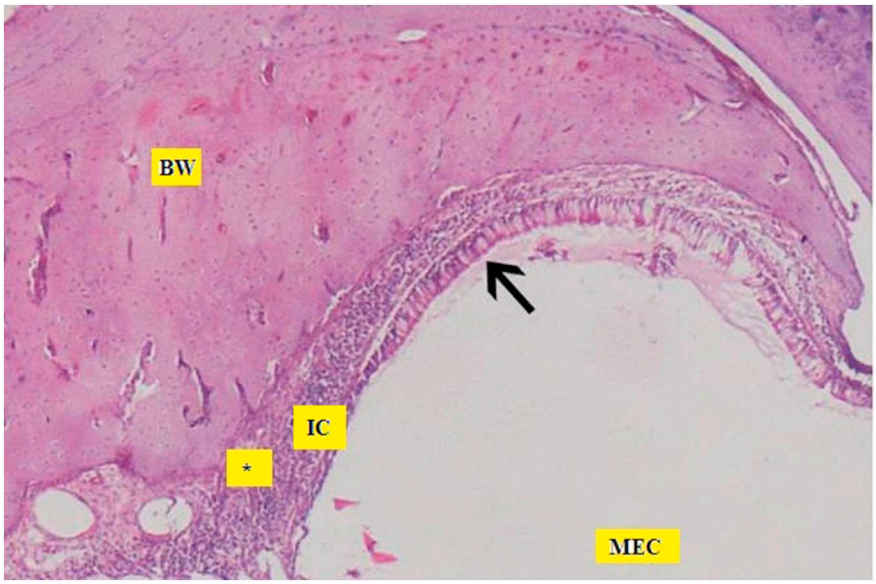

Fig. 4. A cross-section of the middle ear cavity of a Spongostan soaked with dexamethasone-packed rat that had mild inflammation at postoperative week 6. The asterisk indicates the site of mucosal trauma. In the area indicated by the arrow, inflammatory cells located beneath the ciliated pseudostratified columnar epithelium are observed. (Original magnification X40). BW indicates bullar wall; MEC, middle ear cavity and IC, inflammatory cells.

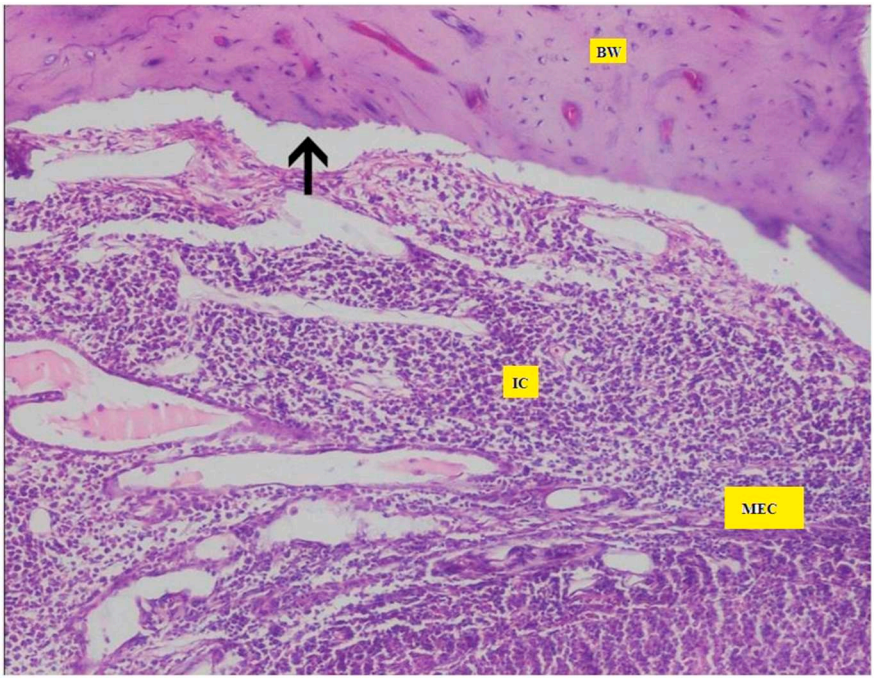

Fig. 5. A cross-section of the middle ear cavity of a Spongostan soaked with dexamethasone-packed rat that had severe inflammation at postoperative week 6. The arrow points to the site of mucosal trauma without adhesion findings. Intensive inflammatory cells are observed in the middle ear cavity. (Original magnification X100). BW indicates bullar wall; MEC, middle ear cavity and IC, inflammatory cells.

support TM grafts and the ossicular chain [13]. Following studies showed that the AGS should ideally be used in intact ME membranes, otherwise contact with damaged MEM might lead to fibrosis [14].

Although the ideal PM has not yet been discovered, studies have shown unfavorable effects of the AGS and their impact on the success of otologic surgery. An experimental study showed that fibroblast invasion developed on AGS caused connective tissue formation on the round window [15]. In an experimental study, scanning electron microscopy showed hair cell reduction and severe morphological defects in ears packed with AGS [7]. Thus, we aimed to investigate the effects of various absorbable materials on mucosal healing and hearing, to find a better alternative to AGS.

Otopore is a recyclable, synthetic polyurethane foam. It is phagocytosed by macrophages and drained through the Eustachian tube to the nasopharynx [16]. Further, it has been shown that this material causes less inflammation and fibrosis compared to AGS [9]. An experimental animal study comparing the effects of AGS and Otopore showed that there was an increase in the ABR thresholds at low frequencies in the AGS group. In this group, osteogenesis developed at more extensively while normal hearing and normal mucosal healing was observed with Otopore [16].

Corticosteroids are considered to reduce the adverse effects of AGS due to their anti-inflammatory effects. Kiris et al. put AGS soaked with levofloxacin plus dexamethasone into the ME cavities of study group, and saline-applied AGS into the ME cavities of the control group in an experimental study on pigs following damage of ME membranes. They showed that fibrosis and foreign body reaction were less in the study group [13].

HA is an extracellular polysaccharide with a molecular weight of 2–4 million Daltons, and it is usually found in epithelial, neural, and connective tissues [17]. Clinical and experimental animal test models have shown that HA, which is frequently used in ophthalmic surgery, has no ototoxic effect [18]. A study comparing the effects of AGS and esterified HA after ME mucosal damage showed that the esterified HA

had the same beneficial effects on healing. Conversely, fibrotic thickening and inflammatory cell infiltration were observed in the AGS group [4]. In another study, the effects of HA and AGS on healing and hearing were compared with the control group. A postoperative hearing evaluation was carried out with ABR, and it was found that hearing improved faster in HA group compared to the AGS group. More residual material was found in the AGS group, and new bone formation was not observed in the HA group [8].

The absorption of AGS in the human body is complete after approximately 4–6 weeks. However, in one study, the authors claimed that this duration could extend up to 3–9 months. They suggested that AGS did not disappear; instead, it penetrated the surrounding soft tissue, and was covered with mucosa [13]. In our study, we found residual material in the control group in Week 6. No residual material was found in the other groups at the end of the study. These results suggest that the absorption period of AGS is longer than 4–6 weeks, and absorption takes longer when AGS is used alone. In contrast, when used with a steroid or HA, the absorption was completed within 6 weeks. More extensive inflammation caused by AGS may have a role in this setting.

In our study, more inflammation was observed in the HA and AGS groups compared to the other groups. The inflammation was the least in the Otopore group, followed by the steroid group. Hence, we suppose that the inflammatory effects of AGS may be reduced by adding steroids, or prevented by using Otopore as a PM.

The control group showed more adhesion formation compared to the other groups. We observed adhesion formation in 1 of 10 ears in the Otopore group, and 7 of 10 ears in the control group. The adhesion formation ratio was significantly higher in the control group than in the Otopore group. Our results showed that AGS stayed in the ME cavity longer than the other PMs. This finding shows that, as the material stays longer in the ME cavity, it may cause more inflammation, the inflammation it caused may trigger adhesion formation in the ME cavity.

New bone formation may be encountered as an undesired process during inflammatory reactions. In our study, new bone formation was observed in 5 of 10 ears in the control group. In contrast, we did not observe any new bone formation in the HA group. Less new bone formation in the steroid, Otopore, and HA groups may suggest lack of a foreign body reaction to those PMs.

We did not find any significant difference among the groups in terms of fibrosis formation. Nevertheless, more ears formed fibrosis formation in the control group. We examined fibrosis formation at Week 6, but this process could still be continuing after this time. Therefore, examination of fibrosis after a longer period may give more accurate results.

The auditory effects of the PMs were evaluated in our study. There was no significant difference among the groups in terms of the ABR thresholds on Day 0. Likewise, the ABR thresholds did not show any statistically difference among the groups at Weeks 1 and 6. Therefore, we can say that there was no difference among the groups' hearing levels by the end of Week 6. However, the measurements showed statistically significant differences among the weeks when the ABR thresholds of each group were compared week by week. The increase in the ABR thresholds in the Otopore group between Day 0 and Week 1 was found to be significantly lower than the increases in the control and HA groups, which might be the result of the fact that the degradation of Otopore is faster than AGS. Moreover, since less residual PM in the middle ear cavity causes less conductive hearing loss, the changes in the ABR thresholds are lower compared to AGS. Additionally, pathological changes in the ME mucosa such as inflammation, new bone formation, adhesion, and fibrosis might have led to conductive hearing loss and affected ABR thresholds. In contrast, chemical ototoxicity to the inner ear might have led to damage of the hair cells and caused sensorineural hearing loss. Although our results showed no statistically significant difference among the groups in terms of the ABR thresholds, a limitation of our study is the absence of scanning electron microscopic findings that might have shown the morphological and quantitative changes in the inner ear hair cells.

The results of our study may offer evidence to support the hypothesis claiming that the negative effects of Spongostan on ME mucosa regeneration may be diminished when Spongostan is used together with steroid or HA, rather than used alone, although the beneficial effect would still be less than that of Otopore. However, it is still not known whether extensive inflammation, fibrotic changes, or adhesions arising in the ME mucosa would require revision surgery in the long term.

5. Conclusion

We demonstrated the positive effects of Otopore on ME mucosal regeneration and healing when compared to the other materials studied. The inflammation, adhesion and new bone formation decreased when Spongostan was used with steroid or HA, when compared to Spongostan alone. In addition, we showed that there was no difference between the PMs in terms of their effects on hearing.

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