

Original Article

Combined approach for experimental Oto-neurosurgical procedures

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Abstract

Background: Experimental procedures will continue to be a key element while going through the learning curve in the use of the endoscope and minimally invasive procedures. We describe the technical procedure of an experimental approach to middle ear in New Zealand rabbits through external auditory canal and its relevance as an ideal model to study graft materials and serve as a training tool for potential applications in otoneurology.

Methods: A group of 28 adult New Zealand rabbits were subjected to an experimental myringoplasty, combining the transmeatal and retroauricular approach with endoscopic assistance and microsurgical technique. The different anatomical steps and systematization of the complete experimental procedure are described.

Results: An experimental approach to middle ear live model and basic anatomic description was successfully used, standardizing the ideal technique. The eardrum could regenerate with no complications and with functional preservation in all the myringoplasty cases. This strategy involves a safe combined approach to the tympanic membrane and others neurosurgical as transcochlear and translabyrinthic approaches and is useful as a test of other experimental procedures to evaluate biomaterials to repair the eardrum currently studied. This experimental myringoplasty model also facilitates functional tests such as impedanciometry and the endoscopic follow-up of the whole process.

Conclusions: The method described to perform an experimental myringoplasty (type I tympanoplasty) in a New Zealand rabbit is an option to be used as a basic model to study the behavior of the graft in the tympanic membrane. Also, basic concepts for the use of combined instrumentation are established in the treatment of eardrum lesions, as a refinement of the technical training application in microsurgery and assisted endoscopy in the transcochlear and translabyrinthic approaches and otoneurology areas.

Key Words: Endoscope, endoscope assisted surgery, experimental surgery, microneurosurgery, myringoplasty, otoneurology

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INTRODUCTION

Otoneurology has been one of the most useful endoscopic fields for diagnostic techniques and treatment. The regular use of the endoscope for diagnostic and exploratory purposes in the nose, pharynx, and external auditory canal is currently a conventional practice to obtain and document diagnostic information and case follow-up by means of instrumented exploration in outpatients.

In addition, it has been gradually integrated into therapeutic procedures using rigid endoscopes with a work channel, as well as for assisted endoscopic procedures to aid in microsurgical procedures. Such is the case of the sphenoid sinus and sellar region through the intranasal approaches and the cerebello-pontine angle,^[12] as well as in other keyhole options at several anatomical sites. There is little evidence for the use of middle ear endoscopy with experimental and therapeutic purpose.^[13] This approach is reserved for microsurgery using the translabyrinth route.^[3,15]

In every case, it is crucial to implement experimental activities in artificial models, animal models^[16] including rabbits,^[6] cadaver, and recently in virtual intelligent models, in order to develop endoscopic skills.^[10] However, experimental practice in animals will continue to be a key element going through the learning curve in the use of the endoscope and microscopy when such practice follows bioethical principles. In case of potential applications for otoneurology, its relevance is established in tympanoplasty, where few reports are currently found.^[1]

This work proposes the use of the New Zealand rabbit as an option for training in the use of the rigid endoscope through the ear canal, complemented with a retroauricular dissection, not only for diagnostic purposes but also as a therapeutic tool with the possibility of assisting in the microsurgical technique. Neither an anatomical atlas of the rabbit's ear was available to use nor impedance measurements were found in the literature for this purpose.

The experimental technique described here, once implemented as a basic experimental model, shall have great potential applications in future tympanoplasty procedures in humans.

MATERIALS AND METHODS

A group of New Zealand rabbits of approximately 2.5 kg (5 lbs) were kept under proper environmental conditions, water, and food *ad libitum*, and managed according to the technical and bioethical recommendations of the Official Mexican Standard ZOO-099, validated as a guideline for the proper care and handling of laboratory animals

according to international standards.

The rabbits were maintained in laboratory animal conditions with light and dark 12-h cycles at 22°C and 70% humidity.

The animals were divided into four different experimental tympanoplasty groups: intact, sham operated, muscle fascia repair, and the last one for testing an experimental polymer of chitosan specifically designed for the tympanoplasty.

This report only describes the experimental phase of standardization of the myringoplasty technique (type I tympanoplasty).

Anesthesia for the experimental procedure

The rabbits were sedated using 2 mg/kg xylazine intramuscular injection, allowing the animal to rest or be free to avoid stress. After 5 minutes, the anesthetics were given (intravenously with xylazine and diluted ketamine), and the rabbit could be kept asleep with spontaneous ventilation during the time necessary to explore the external auditory canal (EAC) and perform the procedure. This is a viable strategy where no intubation or anesthetic gases are needed to reach the surgical objective.

In all cases the oximetric concentration, heart rate and CO₂ was measured. After surgery, the rabbit had to be kept under surveillance and in the proper temperature until its total recovery. A single dose of enrofloxacin IM 4 mg/kg was delivered in the immediate postoperative period.

The rabbits were caged individually in the conventional environmental conditions described and given 10 mg of paracetamol in the drinking water daily during the first 5 days to relieve pain.

Endoscopic system

The EAC as well as the eardrum were explored, introducing the rigid endoscope such as the *Hopkins* scope that measures 2.4 mm in diameter and 18 cm in length. Also, a Striker microcamera model 888 videocamera was used, with 150 W light source, color monitor, and video recording system to document the basal condition of the eardrum. This same procedure can be used under sedation to evaluate the integrity and gradual repair of the eardrum during the time of the study [Figure 1].

Impedanciometry and acoustic reflexes

Impedance and acoustic reflexes were measured with *Interacoustics* model AZ26 equipment on all rabbits to check the integrity and function of the tympanic membrane (TM), once the cartilage of the acoustic meatus was sectioned exactly above the bone-cartilage junction along two-thirds of the ear canal diameter. The ear pressure measuring device was placed airtight in the EAC with a 4 mm olive.

RESULTS

The experimental myringoplasty model was successfully performed showing evidence of regeneration in all cases without complications such as scar or cholesteatoma. The description of the procedure follows as a technical note.

Microsurgical procedure

The initial step in this process is to shave the hair in the surgical area and subsequently wash with 11% iodopovidone. Sterile drapes are placed and 2% xylocaine is injected into the retroauricular area at a dose of 4 mg/kg.

A 2 cm retroauricular incision is made on the skin, along the natural fold of the ear, and the dissection is made by planes along the soft tissue of the superficial fascia and dorsal superficial scutuauricular muscle, which is sectioned in the same direction as the skin incision. Subsequently, fine blunt dissection is used working through the soft tissue to the tympanic part of temporal bone until the temporal muscle is found, separating its borders with the retractor. The temporal muscle and the extrinsic auricular muscles are separated around the tympanic part and dissected with fine blunt dissection as well [Figure 2]. Once the cartilaginous portion of the acoustic meatus is identified, the bony portion is dissected until the ear canal base is reached at the temporal part of the skull. In this step, care should be taken to avoid damage to the posterior auricular vein and artery.^[14] In the lateral portion of the external ear canal, the mastoid portion is found and dissected if the mastoid approach is used. Once the external ear canal is exposed in its two portions (cartilage and bone), the cartilage of the acoustic meatus is sectioned exactly above the bone–cartilage junction along two-thirds of the ear canal diameter [Figure 3]. The dissection at this level is important because impedance measurements and acoustic reflexes can be standardized using an anatomic dissection that results in an EAC of the same size. Next, the ear is retracted laterally and the eardrum will be visualized. This exposure allows to do the acoustic impedance tests and to inspect the EAC and eardrum. Finally, the membrane to be used in the tympanoplasty is placed in position over the perforation, attached to the remaining eardrum (*overlay technique*).^[4,5,8,7] Closure is by planes and Nylon 4-0 is used for the skin using separate stitches. The whole procedure is documented with video recording. The surgical procedure is performed with surgical microscope with 10× and 24× magnifications.

Endoscopic procedure

A 30° endoscope 30/1.8 mm is used for guidance. The myringotomy is done with a micro-knife through the external ear canal. It is possible to use the rigid 30° scope to assist during the microsurgical procedure to visualize the lateral borders of the perforation and the proper

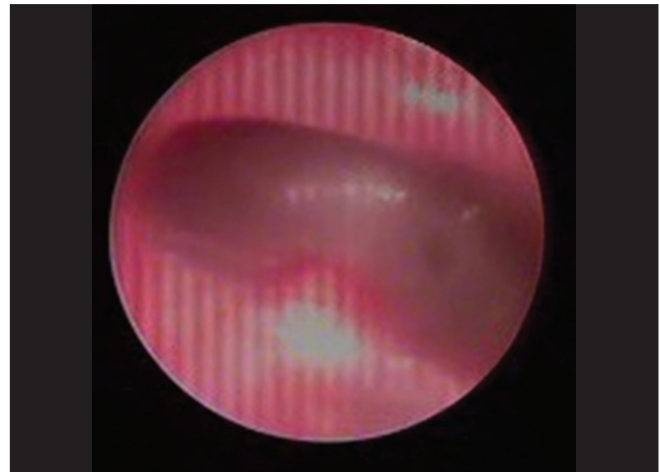


Figure 1: Visual endoscopy of a rabbit right tympanic membrane to evaluate the integrity and gradual repair of the eardrum during the time of the study

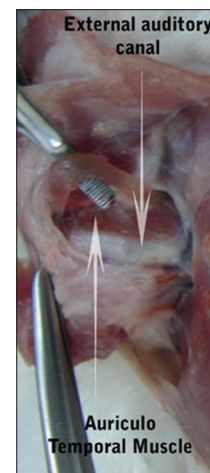


Figure 2: Dissection of the external auditory canal with intrinsic muscle

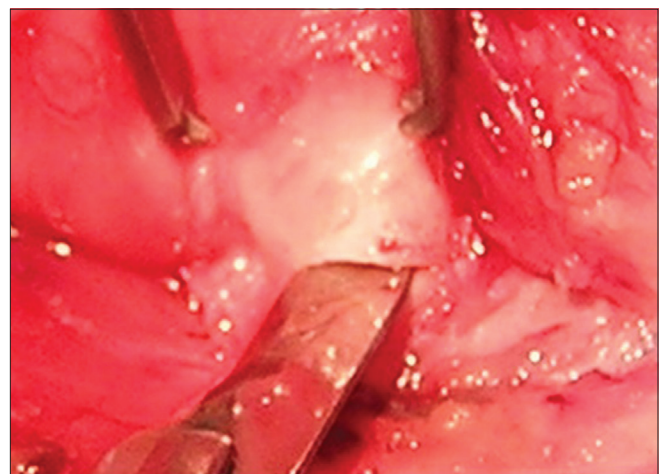


Figure 3: Exposure and sectioning at the base of cartilage from the external auditory canal

attachment of the membrane used for the tympanoplasty, with a Hartman micro-forceps.

Eardrum replacement technique using chitosan matrix or temporal muscle fascia

The excised eardrum is replaced with chitosan matrix of a larger size than the size of the perforation (overlay technique) to hold the borders over the remaining tympanic membrane and to keep the chitosan or fascia membrane in the proper position [Figure 4].

If the rabbit belongs to the standard tympanoplasty group, we use temporal muscle fascia taken from the same rabbit for repair (gold standard). In every case, a successful regeneration of the tympanic membrane was seen after 120 days, evaluated through endoscopic view and microscopic inspection of the thickness of the repaired membrane [Figure 5].

Eardrum retrieval

On day 120, under analgesia and anesthesia, the animals are euthanized to obtain the block sample of the complete tympanic membrane, considering the following steps:

Before circulation stops in the rabbit, a continuous drip with 2% paraformaldehyde and 0.5% glutaraldehyde in the EAC is given. Immediately after the rabbit is sacrificed, the eardrum is removed with surgery in a block, using a retroauricular incision and a transmeatal approach to be subsequently studied. Once obtained, the specimen is maintained immersed in a paraformaldehyde 4% fixating solution and 2.5% glutaraldehyde for 12 h, exerting pressure on the membrane to keep it flat.

A phosphate buffer solution and phosphate saline buffer are used for subsequent microscopic and ultrastructural analysis to follow the condition of the graft.

DISCUSSION

One of the end points of this paper is to create an original



Figure 4: Proper placement of chitosan membrane over the TM through the external auditory canal

technical procedure as the main part of an experimental model to evaluate the biological behavior of the eardrum using different techniques for the tympanic membrane repair (experimental tympanoplasty type I). A standard technique for this purpose has not been described in the literature. So far, the most common reports are in rats, but these rodents usually have spontaneous regeneration in the membrane after myringotomy. We believe that the rabbit is the ideal model because endoscopy can be used to check and follow the behavior of the different graft materials, with the additional advantage of functional evaluation through conventional techniques (tympanometry, acoustic reflex, etc.). Eventually, it will be possible to do a comparative study (as the second step of this experimental procedure) to evaluate the follow-up and structural quality of the regenerated eardrum and its function once it has been repaired. A live animal is necessarily required to do this study. As shown, all the eardrums regenerated when this procedure was used, showing different thicknesses of the tympanic membrane at 120 days after the repair with no evidence of granuloma scar or cholesteatoma. We found that the possibility of a cholesteatoma is more related to the material in the graft and not necessarily to the surgical technique. However, this paper is only an overview about the ideal experimental model. In the future, the structural and functional response of the graft will be tested *in situ*.

The second learning point with the use of this model is the development of surgical skills in the laboratory *in vivo* for microsurgical and endoscopic basic techniques. These educational objectives are not possible to achieve in virtual models, software, or other training resources. This basic procedure is not meant to be compared with the surgical training in cadaver and its benefits. However, the scientific value of live models will be a relevant part of the whole scientific and educational strategy, and not only as a hands-on training.

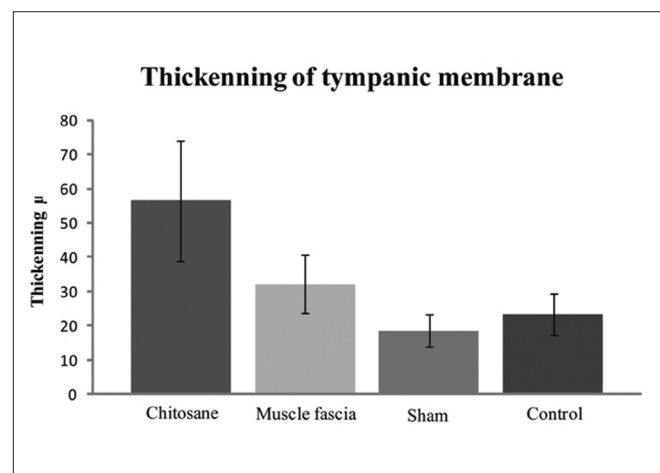


Figure 5: Thickening of repaired tympanic membrane after 120 days of myringoplasty, by different graft materials evaluated through the tympanoplasty technique described

One of the basic criteria in surgical training remains experimental practice not only in relation to the acquisition of skills and abilities, but also to the possibility of generating new knowledge according to the development of new technologies. It is feasible to optimize resources in new experimental models in otoneurology, which also allows us to evaluate implant to induce tissue regeneration *in situ*.

It is relevant to recall the importance that the multidisciplinary work between otorhinolaryngology and neurosurgery specialists has had, as it is the case of the transotic approaches to the cerebello-pontine angle.^[2] Altogether, we have learned from the drill of the temporal bone to the extradural approach through the middle ear, and the functional preservation of the facial nerve and the hearing and balance protection.

Today this collaboration remains and it should start from the basic training of the resident, with the aim of achieving the development of new technologies applied to surgery, new procedures with adequate capacity of resolution, and the maximal functional preservation.

That is why, experimental procedures such as this should be part of the elementary training procedures in otoneurology through approaches that combine effectively with endoscopic and microsurgical strategies for the treatment of pathologies affecting the cerebello-pontine angle.^[9,11]

These procedures will also induce the generation of new scientific knowledge in both areas, as it is the case of the application of implants that they favor the tissue regeneration membranes.

CONCLUSIONS

The method described for experimental approach to middle ear and myringoplasty in New Zealand rabbits offers an option for basic training in microsurgery and assisting endoscopy in the otoneurology and neurosurgical fields. It also establishes the basis for the application of combined instrumentation of eardrum lesions, and thus a potential further technical refinement

in its use. Functional analysis with impedanciometry and monitoring is possible in the follow-up of the membrane repair by means of endoscopy. It is a basic strategy in the otoneurological approaches for people in training. Additionally, this study allows testing other options in the analysis of biomaterials and their role in the repair process, which will be analyzed in a future report.

REFERENCES

1. Ayache S, Tramier B, Strunski V. Otoendoscopy in cholesteatoma surgery of the middle ear: What benefits can be expected? *Otol Neurotol* 2008;29:1085-90.
2. Browne JD, Fisch U. Transotic approach to cerebellopontine angle 1992. *Neurosurg Clin N Am* 2008;19:265-78.
3. Cannoni M, Pellet W, Kanaan M, Bisshop D, Pech A, Zanaret M, et al. Results of surgery of acoustic neuroma via translabyrinth and suprapetrous approaches. Development of a classification by House and Brackmann based on facial function. *Ann Otolaryngol Chir Cervicofac* 1990;107:81-100.
4. Glasscock ME, Kanok MM. Tympanoplasty a chronological history. *Otolaryngol Clin North Am* 1977;10:469-77.
5. Goodman WS, Wallace IR. Tympanoplasty--25 years later. *J Otolaryngol* 1980;9:155-64.
6. Hildmann H, Muller-Greven D. Behavior of the middle ear mucosa in cholesteatoma and following tympanoplasty. An experimental histological study in rabbits. *Arch Otorhinolaryngol* 1977;216:513-4.
7. Kim JH, Choi SJ, Park JS, Lim KT, Choung PH, Kim SW, et al. Tympanic membrane regeneration using a water-soluble chitosan patch. *Tissue Eng Part A* 2010;16:225-32.
8. Kartush JM, Michaelides EM, Becvarovski Z, LaRouere MJ. Over-under tympanoplasty. *Laryngoscope* 2002;112:802-7.
9. Kojima H, Tanaka Y, Yaguchi Y, Miyazaki H, Murakami S, Moriyama H. Endoscope-assisted surgery via the middle cranial fossa approach for a petrous cholesteatoma. *Auris Nasus Larynx* 2008;35:469-74.
10. Leung RM, Leung J, Vescan A, Dubrowski A, Witterick I. Construct validation of a low-fidelity endoscopic sinus surgery simulator. *Am J Rhinol* 2008;22:642-8.
11. Nuseir A, Sequino G, De Donato G, Taibah A, Sanna M. Surgical management of vestibular schwannoma in elderly patients. *Eur Arch Otorhinolaryngol* 2012;269:17-23.
12. Prat R, Galeano I, Iniasta J, Conde R, Alvarez-Garjito JA. Endoscopy-assisted surgery of the sellar region. *Neurocirugia (Astur)* 2008;19:501-6.
13. Presutti L, Marchioni D, Mattioli F, Villari D, Alicandri-Ciuffelli M. Endoscopic management of acquired cholesteatoma: Our experience. *J Otolaryngol Head Neck Surg* 2008;37:481-7.
14. Popesko P, Horák RV. Rabbit on: A colour atlas of anatomy of small laboratory animals. Wolf Publishing Ltd.; 1992.
15. Spiegel JH, Kessler JL. Tympanic membrane perforation repair with acellular porcine submucosa. *Otol Neurotol* 2005;26:563-6.
16. Veldman JE, Kuijpers W. Antigenicity of tympano-ossicular homografts of the middle ear: Analyses of immune response to viable and preserved grafts in animal models. *Otolaryngol Head Neck Surg* 1981;89:142-52.