HUMAN AMNIOTIC MEMBRANE AS A HOMOGRAFT MATERIAL IN MYRINGOPLASTY

by

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ORL - Otorhinolaryngology

TM - Tympanic membrane

TF - Temporal fascia

HAM - Human amniotic membrane

CSOM - Chronic suppurative otitis media

HUSM - Hospital University Sains Malaysia

ABSTRACT

IN BAHASA MALAYSIA

Objektif

Kajian ini bertujuan menilai keupayaan graf amniotic membran manusia sebagai graf alternatif dari segi struktur anatomi dan fungsi untuk menutup gegendang telinga berlubang semasa pembedahan myringoplasty. Kita juga membandingkannya dengan graf fascia temporal manusia.

Kaedah

Kajian prospektif ini telah melibatkan seramai 50 kes yang telah dibedah bagi gegendang telinga berlubang di Hospital Universiti Sains Malaysia dari Jun 1999 sehingga Jun 2001. Dua puluh kes melibatkan penggunaan amniotic membran manusia dan 30 kes seterusnya mengunakan fascia temporal manusia. Pemeriksaan klinikal dan audiometri dijalankan 3 bulan selepas tamatnya pembedahan.

Keputusan

Dalam jangka masa 2 tahun ini, daripada sejumlah 50 telinga yang mengalami gegendang telinga berlubang, 28 daripadanya terdiri daripada pesakit lelaki dan selebihnya pesakit perempuan. Linkungan usia mereka adalah antara 10 hingga 70 tahun dengan usia purata 32.08 tahun. Kesemua pembedahan dilakukan oleh pakar bedah di jabatan ORL. Pembedahan dijalankan melalui 3 pendekatan iaitu melalui saluran telinga (permeatal), tepi telinga bahagian depan (endaural) dan belakang telinga (postaural) tetapi

teknik dihadkan kepada "underlay" atau "pop in through perforation". Keputusan selepas pembedahan dinilai dari segi struktur anatomi dan fungsi. Untuk menilai keputusan struktur anatomi, kita mengambil kira keberkesanaan dari segi penutupan lubang gegendang telinga sepenuhnya, penutupan separa, infeksi, rejeksi dan lateralisasi. Dalam kumpulan fascia temporal manusia, penutupan sepenuhnya dicapai dalam 17 (56.7%) daripada 30 kes. Antara 13 kes (43.3%) yang gagal, 6 disebabkan penutupan separa, 4 infeksi graf, 2 rejeksi graf dan satu graf lateralisasi. Di dalam kumpulan amniotic membran manusia pula, penutuppan sepenuhnya dicapai dalam 13 (65%) daripada 20 kes. Antara 7 kes (35%) yang gagal, 4 disebabkan penutupan separa, 2 infeksi graf dan satu graf lateralisasi. Pernilaian dari sudut fungsi pula menunjukkan perbezaan ketara dari segi purata bacaan ambang konduksi udara sebelum dan selepas pembedahan bagi kedua-dua kumpulan yang dinilai. Walau bagaimanapun, penutupan ketara jurang udara dan tulang hanya dapat diperhatikan dalam kumpulan membran amniotic manusia sahaja.

Kesimpulan

Penggunaan membran amniotic manusia adalah suatu kaedah yang mudah, selamat dan menjimatkan kos untuk merawat gegendang telinga berlubang. Ia memberikan keputusan yang baik dari segi struktur anatomi dan fungsi dan ia setanding dengan graf fascia temporal manusia yang luas dipergunakan pada masa sekarang.

ABSTRACT

Objective

To evaluate the efficacy of preserved human amniotic membrane as an alternative graft material in terms of its anatomical and functional outcomes in closing tympanic membrane defects during myringoplasties. We also compared it with the standard temporal fascia graft.

Methods

A prospective review of 50 cases operated for tympanic membrane defects in Hospital University Sains Malaysia from June 1999 to June 2001. Twenty cases used preserved human amniotic membrane and 30 cases used temporal fascia graft. Clinical and audiometric assessment were done 3 months postoperatively.

Results

During a period 2 years, a total of 50 ears with tympanic membrane perforations were operated, out of this 28 were males and the remaining 22 were females. The age range was between 10 to 70 years with a mean age of 32.08 years. The operations were carried out by senior surgeons in the department. The surgical approach was either permeatal, endaural or postaural but the technique was limited to underlay or pop in through perforation. Postoperative results were evaluated with respect to the anatomical and functional outcomes. To assess anatomic results, we took into account successful closures, presence of residual perforations, infections,

graft dislodgment and lateralization. In the temporal fascia group, successful closure were achieved in 17 (56.7%) of 30 cases. Among the 13 failures (43.3%), 6 were due to residual perforation, 4 infections, 2 graft dislodgment and one lateralization. In the human amniotic membrane group, successful closure were achieved in 13 (65%) of 20 cases. Among the 7 failures (35%), 4 were residual perforations, 2 due to infection and one graft lateralization. In the evaluation of the functional outcomes, both groups showed significant difference between pre and postoperative mean air conduction thresholds component of hearing. However, a significant closure of air-bone gap was only observed in the human amniotic membrane group.

Conclusions

Human amniotic membrane is a simple, safe and cost effective technique for repairing perforated tympanic membranes. It gives good anatomic and functional results and is comparable to the standard temporal fascia graft material so widely used today.

CHAPTER 1

INTRODUCTION

1.1 Literature Review

In current times various graft materials are available to cover the tympanic membrane defects arising as a consequence of otitis media. Among the autograft material used are temporal fascia, tragal perichondrium and vein. Some of other graft materials recorded in the literature include skin, fat, cheek mucosa, periosteum, dura, sclera, mucosa, etc.

The choice of graft material depends largely on the following factors. Criteria for ideal substances (Catalano & Conticello, 1969):-

1. Availability
2. Preservable
3. Manageable
4. Acceptable to the host

Recently a tissue bank (First in the country) was set up at our hospital University Sains Malaysia (USM). The readily available human amniotic membrane in this center has prompted us to conduct a study to determine it's efficacy in myringoplasties. The choice of preserved human amniotic membrane as a graft satisfies the criteria mentioned above with an added advantage of its low antigenicity (Trelford & Trelford-Sauder, 1987, Akle *et al*, 1981 and Matthews, 1981). It does not show allergic or immunologic reactions (Trelford & Trelford-Sauder, 1987 and Frootko, 1985). It also has antimicrobial property (Rao & Chandrasekharan, 1981 and Talmi *et al*, 1991),

but it carries one disadvantage of transmitting the diseases like HIV, Hepatitis B etc.

In hospital USM, amniotic membrane is obtained from donors undergoing normal vaginal delivery and caesarian sections. All donors are screened for Hepatitis B and C, Syphilis and HIV. They must also have no history of transmissible diseases like syphilis, gonorrhea, toxoplasmosis, cytomegalovirus and AIDS. The amnion will only be procured if the membrane ruptured in less than 12 hours before delivery.

There is no age limit for the donors. The procurement takes place in the labour room. Donor's written consent is essential for obtaining amnion. The amniotic membrane is then subjected to various cleaning processes and dried using laminar air flow and subsequently freeze dried to ensure better physical properties. Sterilization of these processed human amniotic membranes is done at Unit Tenaga Nuklear (UTN) in Bangi, Selangor using Gamma Irradiation-2.5 Megarads. The duration for valid usage of this Lyophilised and air dry amnion is of 1 year, however it is best used within the first 6 months of date of processing.

The use of human amniotic membrane in surgical practice is substantial. It is used as a biologic dressing for burns (Colocho *et al*, 1974), in the treatment of leg ulcers (Ward & Bennett, 1984) and for vaginal epithelialization (Trancer *et al*, 1979). In otolaryngology, it has been used for replacing nasal mucosa

affected by hereditary telengectasia for the control of epistaxis and in cases of regional flap necrosis (Zohar *et al*, 1987).

Amniotic membrane has also been used in tympanic membrane grafting. Lyons *et al.* (1971) has employed the use of 70% alcohol preserved amniotic membrane in a variety of otological procedures. In his series of traumatic membrane repair, homologous amniotic membrane was used in 35 cases with excellent results. However, in perforations of tympanic membrane from chronic otitis media, an 80% failure rate was reported in 20 cases in which homologous amniotic membrane was used. The only successful cases in this group were those which had very small perforations in dry ear. The reasons for this high failure rates was not elaborated by the author.

One report from Israel found amniotic membrane not useful for the repair of ear drum perforation (Zohar *et al*, 1987). In this series, 5 out of 7 patients undergoing tympanoplasties were found unsuccessful for graft take up. All the patients had medium to subtotal perforations and non-discharging ears. The amniotic grafts were cut to size, allowed to dry, and was placed with the mesenchymal surface to the flap on antibiotic impregnated gelfoam patches and covered by the tympanomeatal flap. Again, the reason of failures was not explained by the author.

However, Jalisi *et al.* (1991) from Pakistan showed the effectiveness of human amniotic membrane in closing perforations in tympanoplasties. In his study, human amniotic membrane was used as an homograft material to

close tympanic membrane defect in 10 patients with otitis media. Amniotic membrane was procured from donors undergoing caesarian section. These were seronegative for hepatitis, HIV and syphilis. Buffered formaldehyde at pH 7.4 was used as a preservative. The graft was well taken in 7 patients giving a 70% anatomical success. Closure in air-bone gap of 20dB was achieved in 5 out of 7 cases giving a functional success of 71.5%.

Today, the most commonly used autograft is the temporalis fascia, which provides a thin, pliable and sturdy graft material. In addition, the temporalis fascia is readily available, it can be harvested through the same incision and has a very low metabolic rate thus enabling it to withstand the relatively avascular environment of the middle ear cleft (Nazir & Ranit, 2000).

However, its main disadvantages are (Laidlaw *et al*, 2001):-

1) the need for expensive equipment in an operating room setting.
2) the microsurgical skills of the surgeon.
3) the donor site morbidity that results.

These disadvantages are especially important in developing countries, where the incidence of tympanic perforations is high but medical resources are often limited.

Myringoplasty using a tragal perichondrial graft has its own benefits: it is easier to manoeuvre, it is good quality graft and is more easily harvested. No

head bandage or postaural dressing is required, there is no visible scar, nor are there any sutures to be removed. Quraishi & Jones (1995) reported a success rate of 94% which is comparable to temporalis fascia, with an average duration of 38% less time required than in procedures involving temporalis fascia.

If the perforation is small, central and dry with no evidence of middle ear disease, more conservative measures, such as an office myringoplasty under local anesthesia, may be undertaken. In this situation various graft material has been used with great success. One such example is the use of fat as a grafting material in perforation less than 25% of the pars tensa (Mitchell *et al*, 1997). In this situation these procedure can be done as day-stay cases and there has been no reported differences in success rate when compared to inpatient myringoplasty (Mitchell *et al*, 1996).

More recently AlloDerm, an acellular human dermal matrix has been used for office myringoplasty with success (Saadat *et al*, 2001). It is a new biomaterial that serves as a connective tissue matrix, providing soft tissue support and coverage that becomes integrated into the implanted bed. AlloDerm is processed from human donor skin obtained from an approved tissue bank. Donors are screened for HIV, hepatitis B and C and syphilis. The various processing methods involved also increases its safety as a homograft material.

The human amniotic membrane used in this study also undergoes the similar process described above that enables us to provide a safe and effective graft material. The advantages conferred by using human amniotic membrane includes ease of tissue graft handling for complete encompassment of the margins of the perforation, ready to use storable packaging and no donor site morbidity. By virtue of low antigenicity, free availability, inexpensive and favourable biocompataility, human amniotic membrane can be useful for closing tympanic membrane perforations.

1.2 TYMPANIC MEMBRANE

1.2.1 Anatomy

.

The tympanic membrane separates the tympanic cavity from the external acoustic meatus. It is a thin, semi-transparent, nearly oval in form, and very obliquely placed, forming an angle of about 55 degrees with the floor of the meatus. Its longest diameter is downwards and forwards and measures from 9 to 10 mm while its shortest diameter is from 8 to 9 mm. The greater part of its circumference is thickened and forms a fibrocartilaginous ring which is attached to the tympanic sulcus at the medial end of the meatus. A normal tympanic membrane is depicted in Figure 1.

This tympanic sulcus is deficient superiorly to from a of notch two bands, the anterior and the posterior malleolar folds that are prolonged to the lateral process of the malleus from the ends of this notch. The small, somewhat triangular part of the membrane situated above these folds is lax and thin, and is named as pars flaccida or the Sharpnell's membrane. The remainder of the membrane is taut called pars tensa.

The handle of the malleus is firmly attached to the inner surface of the tympanic membrane as far as its center which projects towards the tympanic cavity; the inner surface of the membrane is thus convex and the point of greatest convexity is named umbo.

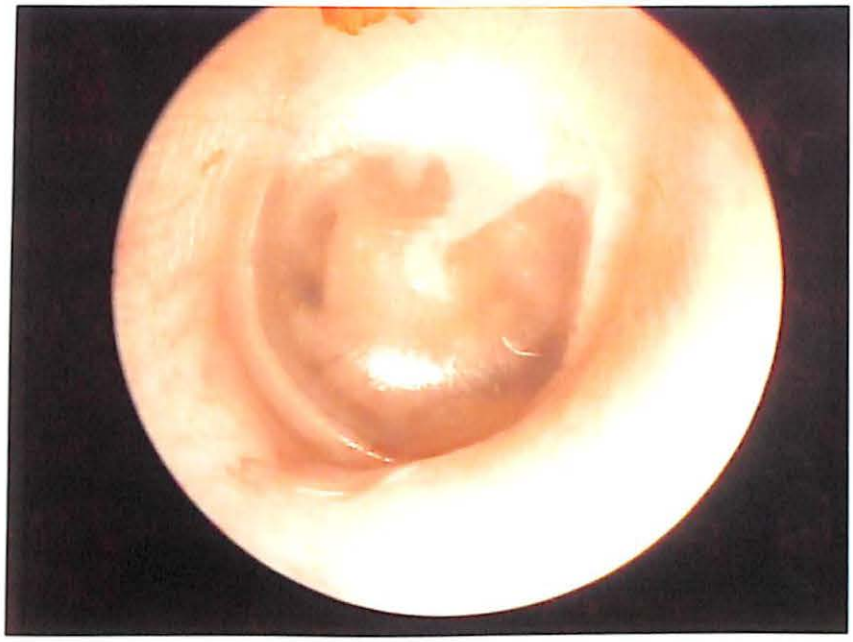

Figure 1. Normal tympanic membrane

1.2.2 Tympanic membrane in chronic suppurative otitis media.

Although the diagnosis of middle ear disease is based initially on the appearance and mobility of the tympanic membrane, findings in the eardrum may not always reflect pathologic conditions in the middle ear cleft. In silent otitis media, extensive inflammatory disease may exist beneath an intact tympanic membrane that shows minimal otoscopic signs (Paparella *et al*, 1980). Detailed electron microscopic descriptions of the human tympanic membrane have given a better understanding of its function, healing processes and response to the pathogenesis and pathology of otitis media (Sano *et al*, 1994).

The most notable histopathologic findings (Sade, 1979 and Sano *et al*, 1994) was an increase in the thickness of the pars tensa as a result of edema and fibrosis. The increase in thickness was greater in the submucosal layer owing to edema and dilated capillaries, as well as infiltration of plasma cells and polymorphonuclear granulocytes. An increase in thickness in the subepidermal layer was also observed, but to a lesser degree. Rich vascularization was seen in both the submucosal and the subepidermal layers. Inflammatory cells were localized to a larger degree in the submucosal layer than in the subepidermal layer.

In tympanic membrane from animal models of otitis media, tissue response to the inflammatory process occurred predominantly in the submucosal layer (Grotte *et al*, 1989).This finding further supports that regardless of the source

of inflammation, the inflammatory response of the pars tensa of the tympanic membrane was found in the submucosal layer. This is not surprising since this layer is highly vascularized (Andrew *et al*, 1982).

The tympanic membranes of rats with Staphylococcus aureus induced otitis media, the lamina propria was found hardly affected by or barely responded to the inflammatory stimulus (Grotte *et al*, 1989). The fibrous layer in the pars tensa of human temporal bones (Sano *et al*, 1994), however, showed great spaces in the framework of the fibrous layer owing to edema. In some cases these edema resulted in wavelike distortions between the fibrous bundles in the outer radial layer and also between typical collagen fibrils in the inner circular layer. Few inflammatory cells were found in the fibrous layer, and new vascularization was poor in this area in comparison with the submucosal and subepidermal layers. These findings may be the result of longer, more severe, and recurrent episodes of otitis media as compared with experimentally induced otitis media in animals.

Four histopathologic patterns of disease established in the inner and outer layers of the lamina propria in chronic otitis media (Sano *et al*, 1994):

1) the inner and outer layers (radial and circular fibers) present but contained some edema.
2) the outer fibrous layer present but a small inner layer remained.
3) the outer layer present but no trace of inner layer.
4) neither the inner nor the outer layer present.

In a scanning electron microscopic study of the tympanic membrane in chronic otitis media, the inner circular layer of the lamina propria was disorganized to a greater extent than the outer radial layer (Moller, 1981). There are some reasons why the inner circular layer is destroyed more than outer radial layer. The submucosal layer is the area in which most of the reactions associated with the inflammatory process take place and because the inner circular layer is located closest to the submucosal layer, the effect of inflammatory cells would be greater in this layer than in the outer layer.

Some other authors (Moller, 1981 and Paparella *et al*, 1985) have suggested that the breakdown of collagen might take place in the subepithelial layer where a large number of macrophages and fibroblasts are present. Since the inner circular fibers contain many more collagen fibrils than the outer radial fibers, fibrolytic reactions to collagen fibrils by inflammatory cells would have a greater effect on the inner layer.

It was therefore concluded that tissue response of the tympanic membrane in chronic otitis media is edema, followed by fibrosis of the submucosal layer (Sano *et al*, 1994). Edema in the fibrous layer create spaces between the fiber bundles and results in the degeneration of the fibrous network. Repeated inflammation causes more serious degeneration, such as the formation of non-elastic fibrous tissue and scar tissue, and may result in a loss of elasticity of the membrane. The pathologic changes that occur in the tympanic membrane and with persistent disease, will render the tympanic membrane

more vulnerable to perforations (Figure 2), retraction pockets and cholesteatomas later in life.

Figure 2. A central perforation in chronic suppurative otitis media

1.2.3 Histophysiology and cell biology.

The successful use of a large variety of tissues from different origin in myringoplasty is an intriguing problem and needs further explanation. To understand these observations one has to consider the condition of the tympanic membrane with a persisting perforation as a consequence of a healed chronic ear disease, as well as the course of events after covering the perforation with a graft.

In contrast to a traumatically induced perforation, a perforation resulting from past chronic middle ear disease does not usually heals spontaneously. In these cases the tympanic membrane is generally partly atrophic, poorly vascularized, and contain scar tissue. It has been known for years that etching of the rim of a perforation or removal of the scar tissue at the edge can stimulate the membrane to proliferate and narrow or even close small perforations. However, large perforations fail to heal with these techniques. Covering these perforations with tissue grafts is necessary for closing these perforations.

The general feeling is that the graft's function is as a scaffold for guiding the epithelium to migrate from the perforation edges that have been stimulated to proliferate by removing the scar tissue. However, a second and even more important role of the graft is that it gives mesenchymal cells and capillaries an opportunity to grow into and along the graft. In this way the epithelium which migrates along the graft may proliferate.

Bridging of the perforation by the epithelium is no longer exclusively dependent on a cell supply from distant proliferative centers as occurs with perforations which are not closed with a graft. After closure of the gap the mitotic activity decreases rapidly and a re-organization occurs. The lamina propria arises from fibroblasts migrating into and along the graft; it gradually becomes thinner by resorption of the graft. Grafts composed mainly of collagenous fibers, like fascia and tympanic membrane, have been shown to be highly resistant to resorption. Remnants of these tissues may persist for years.

No apparent differences have been found between the healing of a perforation with the use of autologous or preserved allogenous grafts. The conclusion seems to be justified that vitality of the graft is of no importance to the healing process. Although preservation has an effect on the antigenic properties of an allogenous graft, the way in which immunological interference occurs is not reported in most clinical cases and it remains an unresolved problem in allograft myringoplasty. Furthermore, the use of preserved grafts can be assumed to have an additional advantage in preventing early graft perforation as compared to fresh autologous tissues. While in fresh autografts the autolytic enzymes are an important factor in early graft destruction, such enzymes are inhibited or even destroyed by some of the currently used preservation methods.

1.3 MYRINGOPLASTY

1.3.1 Definitions and terminology.

Myringoplasty and Tympanoplasty are descriptive terms defining surgical procedures that address pathology of the tympanic membrane and of the middle ear respectively. Myringoplasty is an operative procedure used in the reconstruction of perforation of the tympanic membrane. It is assumed that the middle ear space, its mucosa, and the ossicular chain are free of active infection while undertaking this procedure.

Tympanoplasty is an operation performed to 'eradicate disease in the middle ear and to reconstruct the hearing mechanism, without mastoid surgery, with or without tympanic membrane grafting' (Committee on Conservation of Hearing of the American Academy of Ophthalmology and Otolaryngology, 1965).

1.3.2 Historical background.

Myringoplasty is one of the most common operation performed in otologic practice and Berthold (1878) in Germany successfully repaired a tympanic membrane with a full thickness skin graft and called the operation 'myringoplastik'. Initially, their methods were not widely accepted. Not until the 1950's, when Wullstein and Zoellner reintroduced it, did myringoplasty stir the interest of the otologic community. Wullstein (1956) and Zoellner (1957) revolutionized middle ear surgery by advocating reconstructive grafting of the chronically diseased ear through the use of full or split thickness skin grafts. These operations were designed to restore or conserve hearing and promote healing, after the exenteration of disease from the middle ear or mastoid.

During the 1950s, with improvements in surgical techniques, improved optics, and the origins of microsurgery, myringoplasty could be performed with greater safety and increased graft survival.

Since the earliest practice of myringoplasty in 1878, a wide variety of grafting techniques and materials have been used, including amniotic membrane, autologous mucous membrane of cheek, dura mater, tympanic membrane, cornea, periosteum, vein, connective tissue, adipose tissue, perichondrium and temporalis fascia. (Gibb & Chang, 1982)

Shea (1960) accidentally tore the tympanic membrane during a stapedectomy procedure and repaired the tear successfully with a free autologous vein graft

placed medial to the tympanic membrane, thus introducing the 'underlay' technique in myringoplasty. Ringenberg (1962) was the first to use free autologous fat grafts.

Chalat (1964) was the first to use tympanic membrane allografts in three patients with one success. As an alternative to tympanomeatal allografts for the repair of large perforations, Perkins (1975) introduced formaldehyde preserved autologous temporalis fascia graft. This substance greatly enhanced the tensile strength of the homograft drum, however, the detoxification process was time consuming and technically demanding.

Temporalis fascia is the most commonly employed grafting material as it is easily harvested through the same incision, thin, pliable and sturdy material (Nazir & Ranit, 2000) with reported success rates ranging from 88% - 95% (Smyth,1992, Vartiainen & Nuutinen, 1993 & Emmett, 1999).

Small perforations of the tympanic membrane may also be repaired by stimulating or enhancing reparative processes, by topical application of weak acids, like sodium hyaluronate (Stenfors, 1989) and basic fibroblast growth factor. (Vrabec *et al*, 1994)

1.3.3 Classification.

Wullstein (1956) created a classification scheme identifying five basic types of tympanoplasty at the Fifth International Congress of Otorhinolaringology in Amsterdam (Figure 3):-

- Type 1 is myringoplasty.

- Type II tympanoplasty is for tympanic membrane perforations with erosion of the malleus. It involves grafting on to the incus or the remains of the malleus.

- Type III tympanoplasty is indicated for destruction of the malleus and incus ossicles, but with an intact and mobile stapes. It involves placing a graft onto the stapes and providing protection for the round window.

- Type IV tympanoplasty is used for ossicular destruction including destruction of all or part of the stapes arch. It involves placing a graft onto or around a mobile stapes footplate.

- Type V tympanoplasty is used when the footplate is fixed. The Paparella modification of Type Va with fenestration of the horizontal canal, has largely been abandoned in favor of Type Vb with stapedectomy.

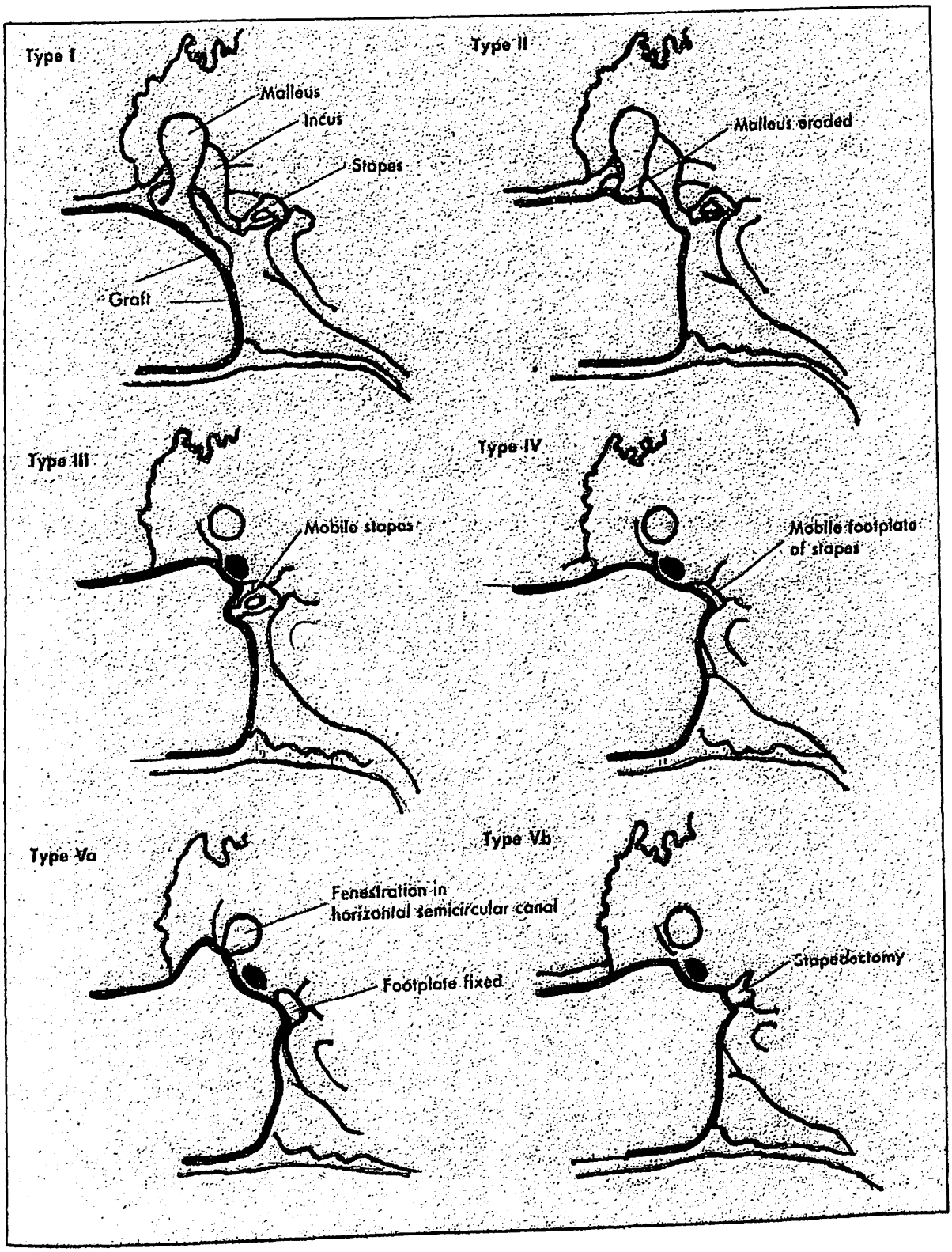

Figure 3. Wullstein's five tympanoplasty techniques

1.3.4 Indications for myringoplasty.

1) To improve hearing. This is indicated in hearing loss of less than 30 dB and conductive in nature. A greater hearing loss usually indicates an ossicular pathology.

2) To decrease the incidence of middle ear infection. The infection is intermittent in nature and often occurs following water contamination of the ear or coincidental with an upper respiratory tract infection. If the discharge is constant from a central perforation the surgeon should suspect coincidental disease in the mastoid air cell system or the paranasal sinuses.

3) Social indications. Patients, especially swimmers, may request repair of an ear drum to reduce the risks of infection.

1.3.5 Contraindications for myringoplasty (Glasscock, 1976).

Absolute

1) Uncontrolled cholesteatoma.
2) Malignant tumors.
3) Unusual infections.
4) Complications of chronic ear disease such as meningitis, brain abscess or sinus thrombosis.

Relative

1) Eustachian tube insufficiency or dysfunction.
2) Uncooperative patients.
3) A dead ear.
4) A better hearing or only hearing ear.
5) Elderly patients.
6) Young patients.
7) Cases of repeated failures.

1.3.6.b Approaches.

The approach to the ear can be permeatal (transcanal), endaural or postauricular.A permeatal approach is via the natural external auditory canal. It has the advantage of least disturbance to the patient but gives limited access and can be difficult in narrow ear canals, which may require drilling of the bone to achieve a satisfactory view. An external incision is usually needed to harvest graft material.

The endaural incision starts in the superior meatus, passes through the cartilaginous gap just posterior to the tragus and extends superiorly and posteriorly around the root of the helix. This incision gives good access to posterior perforations. Temporalis fascia graft material can be taken through the upper extension of the incision. Anterior perforations can pose a problem in terms of lack of access and at times bone must be removed from anterior canal wall for adequate exposure.

The postauricular approach gives excellent anterior access. The incision is made along the postauricular sulcus, then raising the posterior meatal skin together with the annulus and remnants of the tympanic membrane. The posterior view may be slightly restricted but can be improved by drilling bone away posteriorly from an area already denuded of meatal skin. Fascia is also easily accessible from the same incision.