The concept of an artificial tympanic membrane

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A review is given of the development of the concept of an artificial tympanic membrane. Starting with homologous tympanic membranes we compared biodegradable collagen materials (homologous and heterologous) and biodegradable synthetic materials, poly-glycolic acid, poly-lactic acid and poly- α -amino acids. As a non-degradable material microporous PTFE and bisphenol-A poly (carbonate) were investigated. It is concluded that either poly- α -amino acid or PTFE with poly- α -amino acid maybe a suitable material for an artificial tympanic membrane.

Keywords *myringoplasty homografts biomaterial allografts drum xenografts*

Attempts to close a perforated tympanic membrane date from 1640 when Marcus Banzer used pig's bladder as a prosthetic covering for this purpose. The alternative approach, namely myringoplasty, where it is hoped the grafted material will be incorporated into the new tympanic membrane has developed since 1950.

Moritz, Wullstein & Zöllner, the pioneers of myringoplasty, used free skin grafts but following the description of the use of temporalis fascia by Heermann¹ most otologists have used and still use this. The use of other materials, mainly of mesenchymal origin, occasionally autologous, not infrequently allogous (homologous) and sometimes even xenologous (heterologous) has been reported.

Exogenous xenologous i.e. non biological materials have only occasionally been attempted and then without success.^{2–4}

The idea of a tympanic membrane 'from

the shelf' still remains an attractive proposition for a number of reasons. It could reduce operating time, could have a predictable absorption rate, and could be used in those cases where homograft drums are advocated, i.e. congenital ears, atelectatic ears and subtotal perforations with loss of malleus. For subtotal or total perforations with loss of the malleus we tried homograft tympanic membranes.

At first the take rate or non-perforation rate of our ethanol fixed drums⁵ was encouraging (83% of 35 cases), but after 1 year or more the percentage declined to 55%. Moreover the remaining drums invariably turned into a poorly functioning tympanosclerotic mass.

In some cases, after an interval of 3 months to several years, we noticed a granular myringitis which responded temporarily to corticosteroid drops, only to be followed by a total perforation, indicating rejection.

We abandoned therefore the use of homograft drums and homograft material altogether, switching to fascia¹ or cartilage.⁶ In the meantime we looked for another solution to the problem of a graft material.

A graft material for a tympanic membrane should meet the following requirements: it should be thin though of sufficient mechanical strength, soft and pliable, it should allow epithelium to cross and adhere to the graft and it should outlive the regeneration speed of the migrating epithelium from the drum remnants, it should not evoke a severe inflammatory response nor should it be rejected by the host tissue.

In our opinion 3 approaches are possible. The first is the development of a synthetic biodegradable grafting material with a controlled rate of degradation, the second the application of non-degradable microporous synthetic material, which could function as a permanent reinforcement of a healed membrane by replacing the absent collagen middle layer, the third possibility is a combination of both of these. Apart from the above mentioned requirements a biodegradable material should not shed unwanted degradation products.⁷ To test these different materials a series of animal experiments was conducted.

Materials and methods

Three month old rats free from otitis media were used and under general anaesthesia the right tympanic membrane including the annulus and malleus were removed transmeatally.

Subsequently a myringoplasty was performed with a circular artificial membrane using the underlay technique. At the same time the same material was implanted subcutaneously on the back. Post-operatively and during the whole experiment strict aseptic precautions were taken to prevent mycoplasma infections and tetracycline was added (1 mg/100 gm/24 h)

to the drinking water. After intervals varying from 1 week to 1 year, 5 rats per material per sacrifice time were chosen at random and killed.

Subcutis of the back and both temporal bones were removed, fixed, decalcified, embedded and sectioned. Sections 7 μ m thick were prepared in such a manner that the external auditory meatus was cut lengthwise in a craniocaudal direction and the sections were stained with Azan.

The only dog experiment (mongrel dogs > 15 kg) was done in a similar fashion although only 2 dogs per material per sacrifice time were killed and no tetracycline was fed.

None of our animals developed otitis media.

The histological examination was performed by a pathologist who had no knowledge of the material used. Attention was focused on repair activity, inflammation, foreign body reaction, fibrosis, hyalinization, adhesions and the thickness of the resulting membrane. In this way we compared autologous fascia, homologous drum, homologous collagen, heterologous collagen, heterologous amnion, proplast, poly-(D,L-lactic acid), poly-(glycolic acid), poly-(β -benzyl-L-aspartate-co-L-leucine) 50/50, microporous poly (tetrafluoroethylene) and bisphenol-A poly (carbonate) as tympanic membrane graft.

Results

BIODEGRADABLE SYNTHETIC DRUM

Like others,⁸⁻¹⁰ we have tried, by 'chemical engineering',¹² to purify and reconstruct collagen membranes but with no success.¹¹ This idea which seems sound, since the collagenous structure is the most important part of the drum,¹³ did not give good results in our animal experiments. In the human, collagen as a facial nerve tube has been shown to be satisfactory¹⁴ though this material did not do well in tympanic membrane replacement in man.¹⁵ Collagen as a biomaterial is still poorly understood¹⁶ and it could be that our

techniques of purifying did not meet the required standards.

A second type of material we have used belongs to the group of synthetic biodegradable polymers.¹⁷ In this group the poly- α -amino acids and the poly- α -hydroxy acids represent two important classes of accepted biodegradable polymers.¹⁸⁻²² In the period 1955-1970 poly-glycolic acid, poly-lactic acid and co-polymers of glycolic and lactic acid were developed and extensively investigated.²³⁻²⁵ These polymers are degraded by hydrolysis releasing glycolic acid and lactic acid as harmless degradation products.¹⁷ The rates of degradation have been established surviving up to 12 months after implantation in soft tissue.²⁶ Polymers of glycolic acid and co-polymers of glycolic acid and L-lactic acid are well known as synthetic resorbable suture materials.^{23,24,27} The synthesis and properties of poly-(D, L-lactic acid) has also been reported.²⁸⁻³⁰ Grafts prepared from poly-[Asp (OBz)⁵⁰ Leu⁵⁰], i.e. a 50/50 random co-polymer of β -benzylaspartate and L-leucine, were tested in two consecutive animal studies with rats and details are reported elsewhere.³¹ The results show that these materials behave identically when implanted subcutaneously or as a tympanic membrane replacement. This makes the often heard remark of differences in immunological behaviour between the middle ear and other sites a questionable one.

Poly-glycolic acid grafts were not satisfactory because of a rapid disintegration of the graft into sharp needles. The two other materials gave satisfactory results in rats as did poly-[Asp (OBz)⁵⁰ Leu⁵⁰] in mongrel dogs. In dogs the poly (D, L-lactic acid) did less well than in rats giving a more marked inflammatory reaction in both the middle ear and subcutaneously.

NON-DEGRADABLE, MICROPOROUS GRAFTS AND DOUBLE LAYER GRAFTS

Non-degradable microporous grafts which are intended for a permanent reinforce-

ment of a healed ear drum have to fulfil to a certain extent the same requirements as mentioned above with respect to their synthetic biodegradable counterparts. There are, however, important differences, the main one being that they must remain completely 'inert'; neither their chemical structure, nor their inherent mechanical properties must change after implantation. During the healing process they must become incorporated into the newly formed tympanic membrane, preferably by the ingrowth of tissue or by a covering of tissue on each side. Eventually the grafted material must function as a permanent reinforcement of the healed membrane. The ingrowth of tissue, including collagen fibres, into a microporous material is governed by at least two parameters: the pore diameter and the degree of porosity. Too small a pore diameter will prevent tissue from growing into the pores whereas a low degree of porosity, i.e. the absence of a large number of intercommunicating pores will obstruct the nourishment of ingrowing tissue. It is difficult to establish the ideal pore diameter and degree of porosity.³²⁻³⁷

Nowadays a considerable number of microporous membranes prepared from different polymeric materials is commercially available, e.g. filtration membranes or spunbonded nonwovens. Considering only the pore diameter and the degree of porosity, several of these commercial membranes appear to be potential candidates for the application as permanent tympanic membrane graft.

Most of these membranes, however, have been prepared from polymeric materials, which are either not sufficiently 'inert' themselves, e.g. cellulose triacetate, or which contain additives which are toxic to the human body, e.g. non-medical grade poly-vinyl-chloride.

A suitable commercially available microporous membrane¹⁵ is one made from bisphenolpoly-carbonate (Nuclepore®) having a thickness of 8 μ m, a porosity of 10% and a pore size of 10 μ m. Another

material is PTFE which has been recognized as an almost perfectly 'inert' and is used as a vascular prosthesis. Some experimental microporous PTFE membranes having intercommunicating pores were tested as reinforcing tympanic membrane grafts. Of these one membrane had a thickness of 13 μm , a porosity of 95% and a pore size of 10–15 μm . The other membranes were thicker (25–500 μm) and had considerably smaller pore sizes (maximum 2 μm).

From an experimental study in rats we obtained the following information. Both the bisphenol-A poly (carbonate) and the thinnest PTFE membrane evoked no adverse or only a very mild tissue reaction. Tissue had clearly grown into and through the pores of the latter membrane whereas the small sized pores of the other PTFE membranes were found free of tissue and are therefore unsuitable. At the same time the surfaces of these membranes were not completely covered with tissue. Moreover, the thickest PTFE membranes sometimes gave rise to moderate inflammatory reaction.

The thinnest PTFE membrane which was successfully applied had one serious drawback, that is, because it creased and curled up so easily, it was very difficult to manipulate in the ear. It was therefore decided to use this membrane in combination with biodegradable poly-(D,L-lactic acid) as a 'double layer' graft in a second animal study with rats.³¹ The microporous PTFE discs, implanted in combination with biodegradable poly (D,L-lactic acid) film as a double layer graft evoked hardly any adverse tissue reaction, neither did the poly- (D,L-lactic acid) film. The PTFE discs became a real part of newly formed eardrum or of the subcutis after degradation of the poly-(D,L-lactic acid) layer. Connective tissue covered both sides of the membrane and had grown into the pores. This observation is in full agreement with the results obtained by Winter³⁵ who used a similar PTFE membrane as a transcutaneous implant.

The other non-degradable implant we

tried in the second animal study with rats³¹ was the rather stiff microporous bisphenol-A poly-carbonate). The tissue reaction was also very mild and the grafts were surrounded by a very thin capsule of fibrous tissue. However, contrary to what was observed in the case of the PTFE membranes, hardly any tissue had invaded the pores. It turned out that a pore size of 10 μm was too small to allow the ingrowth of tissue. At first sight the small difference between the pore size of the bisphenol-A poly (carbonate) membrane and the PTFE membrane can hardly explain why tissue had grown abundantly into the pores of the latter membrane. It is, however, plausible that the combined effects of the pore patterns, the degree of porosity and the material resilience resulted in the observed difference between the ingrowth of tissue into the bisphenol-A poly (carbonate) membrane and into the PTFE membrane. The thinnest PTFE membranes, this time impregnated with poly-(D,L-lactic acid) instead of being applied as a double layer graft, were also investigated in dogs. After degradation of the poly-(D,L-lactic acid) the micropores PTFE grafts were nicely incorporated into the newly formed tympanic membranes by the ingrowth of tissue which was also observed with the implants in rats. However, the degradation of poly-(D,L-lactic acid) evoked a more pronounced tissue reaction in dogs than was observed in the middle ear of rats.

Conclusion

The functioning of several potential synthetic biodegradable eardrum grafts and non-degradable, microporous eardrum grafts was examined both visually and histologically in three consecutive experimental studies with animals. After the first and second experimental study in rats, poly-(D,L-lactic acid), poly-glycolic acid and poly-[Asp (OBz)⁵⁰ Leu⁵⁰] were selected as potential synthetic biodegradable grafting materials, whereas a micro-

porous PTFE membrane showed potential for the reconstruction of a missing fibrous middle layer of an eardrum.

After the third experimental study with dogs only the synthetic biodegradable graft prepared from poly-[Asp (OBz)⁵⁰ Leu⁵⁰] and the porous PTFE membrane were finally selected for a possible clinical trial. The PTFE membrane was implanted as a composite microporous PTFE—poly-(D,L-lactic acid). This biodegradable polymer, however, is no longer considered as a suitable grafting material because of the marked adverse tissue reaction it evoked in dogs. Therefore, if clinical trials will be performed with microporous PTFE, it is proposed to use this membrane as a composite graft with poly-[Asp (OBz)⁵⁰ Leu⁵⁰].

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