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Configuration of experimentally produced cholesteatoma by transplantation of a free skin graft

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Abstract

Residual cholesteatoma was experimentally produced in guinea pigs by transplanting a free skin graft into the middle ear bulla. In group A, the graft was placed on the mucosa after scratching the surface with a pick, while in group B, it was placed on the bone surface following removal of the mucosa and drilling with a diamond burr. The group A procedure was conducted on the left ear and the group B procedure on the right ear in 12 guinea pigs. The animals were sacrificed at two, four and eight weeks after transplantation. In all 12 ears of group A, the graft kept its original flat shape, resembling an open type residue. In eight of 12 ears of group B, the graft grew forming a squamous pearl, while in the remaining four ears it retained a flat shape. The difference in configuration is thought to be due to the amount of granulation around the graft.

Key words: Cholesteatoma; Skin transplantation; Guinea pig

Introduction

Residual cholesteatoma occurs as a consequence of the growth of a fragment of matrix left inadvertently in the middle ear at the time of cholesteatoma surgery. Surgical findings in revision or second-look operations have revealed that it takes either one of two configurations; a squamous pearl (closed type) or an open type. A squamous pearl represents an epidermal cyst containing epithelial debris, while the open type is characterized by a circumscribed flat epithelium with epithelial debris on it. At present, it is not known what is responsible for the difference in the shape of residue. This paper shows the procedures needed to re-create the two respective shapes of residue in an animal model and to discuss the backgrounds of the difference in shape.

Materials and methods

Twelve guinea pigs, weighing 400 to 600 g, with normal tympanic membranes, were used for this experiment following our institutional guidelines regarding animal experiments. They were anaesthetized by intraperitoneal administration of sodium pentobarbital (30 mg/kg). A local subcutaneous injection of one per cent lidocaine was given supplementally. A free skin graft was harvested from the posterior auricle and trimmed to 2 × 2 mm of full thickness graft. Then, a retroauricular skin incision was made and the dorsal otic bulla was opened. In group A, the skin graft was placed through the opening directly onto the intact middle ear mucosa after scratching the surface with a pick. In group B, a graft bed was prepared via the opening by carefully removing a part of the mucosal lining and drilling the bone surface with a diamond burr. The graft was then transplanted onto the abraded bone surface with its subepithelial plane facing the bone. No special measures such as use of Gelform or fibrin glue were used to fix the graft. After completion of these procedures, the opening of the bulla was closed with dental cement and the wound sutured. In all animals, the group A procedure was conducted on the left ear and the group B procedure on the right ear.

The animals were sacrificed at two, four and eight weeks after the transplantation procedures for histological study. Under deep anaesthesia with sodium pentobarbital, the temporal bones were removed en bloc. They were then fixed in 10 per cent buffered formaldehyde and embedded in paraffin after decalcification in a mixture of aluminum chloride, 35 per cent hydrochloric acid, 99 per cent formic acid and distilled water. Serial sections were prepared and subjected to haematoxylin and eosin staining for light microscopic observation.

Results

In all 12 animals, the grafts survived in the dorsal otic bulla, without causing contamination by bacterial infection. Inflammatory reaction to grafting was

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TABLE I
OUTCOMES OF THE SKIN GRAFTS 2, 4 AND 8 WEEKS AFTER TRANSPLANTATION
NO. OF EARS

	2 weeks		4 weeks		8 weeks		
Shape of graft	Closed type	Open type	Closed type	Open type	Closed type	Open type	Total
Group A	0	4	0	4	0	4	12

localized in the dorsal otic bulla, with the other parts of the middle ear remaining unaffected.

Table I summarizes the results of the present study. In all ears of group A, in which the graft was transplanted onto the scratched mucosa, the graft retained its original flat shape with production of epithelial debris. The configuration resembled an open type of residual cholesteatoma. In group B, in which the graft was placed on the exposed bone surface, a squamous pearl was formed in eight of 12 ears. The size of pearl and volume of epithelial debris inside were prominent in ears long after transplantation. In the remaining four ears, the graft retained its original flat shape in the middle ear, as was seen in the specimens from group A.

Figure 1 shows a typical example of a group A specimen, in which the graft retained its original flat shape at four weeks post-transplantation. Skin appendages such as hair follicles and sebaceous glands survived without atrophy. The middle ear mucosa was lost and substituted by granulation at the site of grafting, while the rest of the mucosa in the bulla remained undisturbed. Accumulation of epithelial debris was seen on the surface of the graft.

Figure 2 shows a representative group B specimen, in which a squamous pearl was formed inside the otic bulla at four weeks post-transplantation. The pearl containing a large amount of epithelial debris was encapsulated by granulation rich in connective tissue. It was also surrounded by new bone regenerated following the surgical procedure. The original mucosal lining was totally substituted by the granulation.

Discussion

Although many experimental studies have been conducted to produce an animal model of cholesteatoma, mechanisms pertaining to the incidence of different shapes of residue have not yet been confirmed. Abramson *et al.* (1975) succeeded in producing a squamous pearl in guinea pigs by transplanting a free skin graft onto the exposed bone surface of the middle ear followed by irritation with talc. In an experimental study in cats, Jackson and Lim (1978) transplanted a free skin graft onto the scratched mucosa of the middle ear without using an irritant and found that the graft retained its original flat shape with labile junction to the surrounding mucosa. None of their cases showed squamous pearl formation. Vennix *et al.* (1994) performed a similar experiment in rats, and demonstrated that half of the grafts became squamous pearls while the rest were incorporated into the middle ear mucosa retaining the original flat shap. They further reported that extensive growth of the graft occurredwhen conjugated with bacterial infection.

In the present study, two shapes of residual cholesteatoma could be produced in animals by differing the surgical procedures for preparation of the graft bed. The granulation formed around the graft appears to play an important role in formation of a squamous pearl (Figure 3). As removal of the middle ear mucosa and drilling of the bone surface act as irritants on the graft bed, growth of the granulation was more prominent in group B than in group A. When involved with granulation, the graft tended to curl with its epithelial surface inside until the margins of the graft united on both ends.

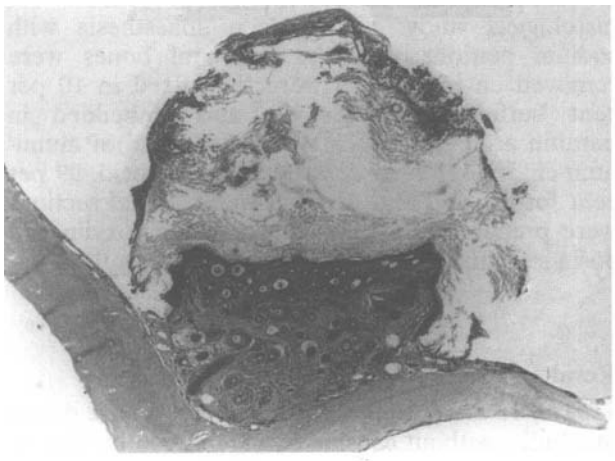

FIG. 1
Graft retaining its original flat open shape. Group A specimen at four weeks after transplantation. (H & E; × 10).

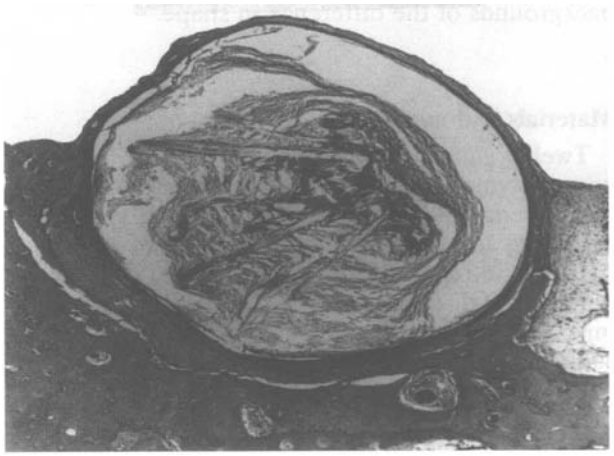

FIG. 2
Graft forming squamous pearl. Group B specimen at four weeks after transplantation. (H & E; × 10).

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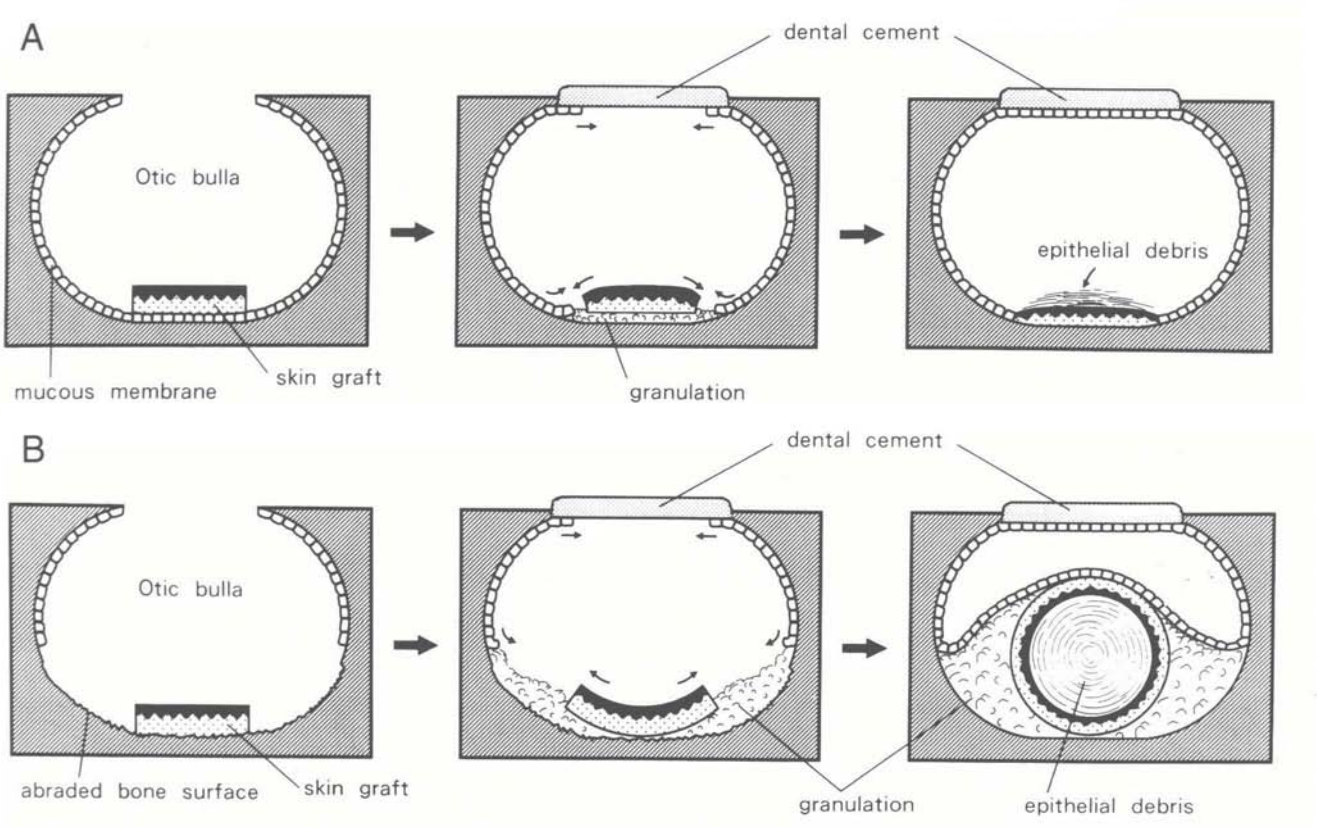

FIG. 3

Mechanisms of formation of an open type residue and a squamous pearl (closed type residue) in our animal model. (A) The graft retained its original flat open shape when placed directly on the intact middle ear mucosa after scratching the surface. (B) A squamous pearl was formed in the middle ear when the graft was placed on the abraded bone surface.

Macrophages and lymphocytes infiltrating the granulation produce significant amounts of growth factors such as interleukin-1 (Ahn *et al.*, 1990; Schilling *et al.*, 1992) and transforming growth factor-alpha (Schulz *et al.*, 1993) which facilitate proliferation of the graft epithelium. Capillaries rich in granulation also facilitate growth of the epithelium by providing a sufficient blood supply. Consequently, epithelium with involvement of granulation grows consistently to form a squamous pearl. When the post-operative granulation reaction subsides, growth of the pearl is no longer possible. Then, the pearl may be covered by regenerated mucosa. In contrast, when the graft is placed on the scratched mucosa, granulation is minor and limited to the mucosal surface just under the graft, resulting in conservation of the original flat shape. Due to the short follow-up period in the present study, we cannot be certain whether the grafts which retained their original flat shape will eventually develop into squamous pearls.

In conclusion, we consider that the shape of residual cholesteatoma is determined mainly by the granulation around the epithelial remnant. Granulation facilitates not only growth of the epithelium by stimulation with cytokines but also allows formation of a squamous pearl by involving the epithelium.

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