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Tympanic membrane structure analysis and tissue engineering

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Aim: To undertake electron microscopy and immunohistochemical studies of the normal human tympanic membrane as a basis to develop a tissue-engineered tympanic membrane. **Background:** The functional results of myringoplasty, using currently available graft materials, remains uncertain. A tissue-engineered tympanic membrane should theoretically improve outcomes. Successful tissue engineering of the human tympanic membrane requires an intimate understanding of the cytokeratin (CK) expression of the outer and inner layers, for identification of cells in cell culture, as well as the collagen components of the middle layer.

Method: Tympanic membrane structure was examined histologically using standard techniques of light and electron microscopy. CK expression of the outer and inner layers, was analysed by immunohistochemistry.

Results: Histology confirmed the three-layer structure of the tympanic membrane. The middle collagenous layer consists of outer radial and inner circular fibres, with parabolic fibres observed between them. CK 5, 10, 14, 16 and 19 are expressed in the outer layer. The outer epithelial layer consists of an outer keratinising stratum corneum with underlying stratum granulosum, stratum spinosum, and stratum basale layers. The thin inner mucosal layer consists of simple squamous or cuboidal cells. Positive staining of CKs 4, 7, 8, 18 and 19 occurred in the inner layer, with focal areas of CK 13 and 14. Collagen expression of the middle lamina propria is mainly types II and III, with type I to a lesser extent.

Discussion: CK expression of keratinocytes from the outer layer, and mucosal cells from the inner layer, enables us to test cell growth on different scaffolds and in different environments. We are progressing on our work to determine the collagen composition of the middle layer. There is good evidence to suggest that the middle layer provides the tension of the tympanic membrane and its loss results in a flaccid tympanic membrane, with impaired function. This knowledge is necessary for the development of an appropriate scaffold to support cell growth.