COMPARATIVE ELECTRON MICROSCOPE STUDY OF HOMOGRAFT OSSICLE PRESERVATION TECHNIQUES

Thesis submitted in partial fulfillment of the doctoral degree in

Otorhinolaryngology

Ain-Shams University, Cairo Egypt

by

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1992

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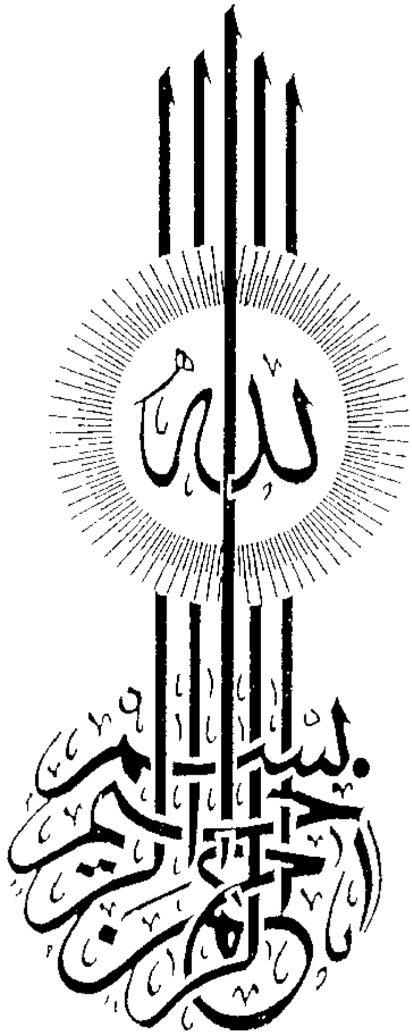
بسم الله الرحمن الرحيم

USE EAR INSTITUTE

July 1, 1992

A.A.M.Sarwat, M.D.
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El Mirghany, Heliopolis
Cairo, EGYPT

RE: Hassan Ahmed Wahba, M.D.

Dear Doctor Sarwat:

This is to inform you that Dr. Hassan Wahba has completed the practical and written portions of his research project on "Comparative Electron Microscope Study of Homograft Ossicle Preservation Techniques." His thesis is now ready for defense.

It is with regret that we see him leave. He is a very diligent, enthusiastic and competent individual. He has been one of our most active and productive fellows, and has shown himself to be a dedicated, tireless researcher with a quick grasp of problems, and an astounding facility with the scanning electron microscope. His friendliness and sense of team-spirit was appreciated by all. It was a pleasure having Dr. Wahba with us.

Sincerely,

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INTRODUCTION

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Glasscock & Kanok (1977) stated, "An open mind must be kept to all new ideas so that the goal in the control of ear disease must be to return the ear to its natural state."

Smith (1980) mentioned that ossicular reconstruction techniques are difficult to assess except in long-term follow-up studies. Underlying ear disease, especially cholesteatoma, increases the difficulty of determining the best method of ossicular reconstruction. Thus, the ear surgeon faces a dilemma: what type of material to use for ossicular reconstruction, alloplast (synthetic material) or transplanted tissue, and if the latter, orthotopic (derived from a like anatomical site) or non-orthotopic.

Smith (1980) adds that results of ossicular reconstruction with synthetic materials, cartilage struts, and non-orthotopic bone have not been entirely satisfactory. The great variation in surgical techniques of middle ear reconstruction and the multitude of plastic and metal prostheses available for ossicular replacement testify to the difficulty of this type of reconstruction and to the lack of uniform results. Extrusion, displacement, loss of continuity, and fixation are common recurrent problems of ossicular chain reconstruction with these materials.

Plester (1977) histologically studied 500 surgically removed ossicles during tympanoplasties. Seventy five percent showed pathology of the entire ossicle by microscopic examination. *Plester* concluded, "One should never use the patient's own ossicle for repositioning in ossiculoplasty."

techniques involve the use of acids, alkalies, salts of heavy metals, alcohol, ether, and organic solvents. All techniques must yield a sterile, morphologically intact end product.

Urist & Strates (1971) mentioned that the bone implant not only functions to consolidate and bridge the defect; i.e., it acts as a scaffold for the process of osteoneogenesis; but it also has a bone induction potential, i.e., the ability to stimulate host mesenchymal cells to differentiate into bone cells. This bone induction potential is inherent in the bone matrix, and it is thought to depend on certain proteins, the so-called bone morphogenetic proteins (BMP). Such proteins, although themselves not collagen, are firmly bound to the three-dimensional collagen network of the calcified tissue. BMP may be destroyed, with concurrent loss of bone induction potential.

In Egypt, chronic suppurative otitis media particularly due to cholesteatoma, is a common ear problem, that continuously challenges the otologic surgeon. After the condition is treated, and when no residual or recurrent disease is present, the problem of reconstruction of the ossicular chain is and will remain a primary concern.

Because the collagen matrix is architecturally important in preserving the morphological form of the ossicle, this study is a comparative analysis of the ultrastructure of the collagen matrix and its spatial arrangement. The ultrastructural spatial arrangement is the foundation for the occurrence of osteoneogenesis. And because antigenicity of the bone implant is not crucial to failure of implantation, as noted by *Feenstra (1977)* and *Gagnon*

(1979), the focus of this study is the collagen matrix and the possible changes that might occur due to the different techniques of preservation, and duration of preservation. *Feenstra (1977)* stated that all biological material, such as homograft ossicles, must be dated by realistic expiration dates (shelf-life). *Plester & Steinbach (1977)* also noted a relationship between the duration of preservation and osteoneogenesis: the longer the preservation period, the slower the bone replacement. This could be due to changes in the collagen matrix framework of the ossicle. The present study will use electron microscopy in an attempt to detect changes of the collagen matrix.

Microscopic examination of histologic specimens is a valuable method to obtain biologic information. The development of phase microscopy attained the limit of resolution of the light microscope, about 0.25 μ. This limit, imposed by the wave length of light, precludes adequate resolution at magnifications greater than 1000. Increasing knowledge, made evident that there were definitive structures smaller than those seen under the light microscope. With better resolution and higher magnification, the detailed form of these structures could be studied. The electron microscope, which uses a beam of electrons instead of light waves, allows such a detailed study. For transmission electron microscopy, histological preparations must be extremely thin and free of artefacts to allow free, "clean" penetration of the beam without scattering of the electrons *(Duvall & Santi, 1980)*.

Since the introduction of the scanning electron microscope in the mid 1960s, it has been widely applied to the study of the ear. Three-dimensional views of the ultrastructural level have added a new understanding of this complex organ. These new views bridge the gap between the understanding of the ear gained by light microscopy and by transmission electron microscopy. Unlike conventional transmission electron microscopy, the scanning electron microscope can be used to examine unsectioned biologic tissue *(Lim, 1980)*.

In this study we used scanning and transmission electron microscopic techniques to observe changes at the level of the collagen matrix in a large number of auditory ossicles harvested from cadavers. The specimens were preserved for various intervals over a 24-month period. Four different techniques of preservation were used: alcohol 70%; autoclave at 121°C for 20 minutes, then preserve in alcohol 70%; cialit 1:5000; and buffered formaldehyde 4% pH 7. Those methods were chosen because of their ease for later application to homograft tympanoplasty.

The ultimate aim of the study is to determine the best preservation technique and the optimum duration of preservation.

REVIEW OF THE LITERATURE

ANATOMY OF AUDITORY OSSICLES

Developmental Anatomy:. *Anson & Donaldson (1973)* described the developmental anatomy of the auditory ossicles:

The small ear bones, most of which are refashioned from the tissue of the mandibular and hyoid arches (Meckle's and Reichert's cartilages), are first formed as cartilage "models." Two portions have separate origins: the anterior process of the malleus, which forms independently and early in membrane bone; and the vestibular portion of the base (footplate) of the stapes, which is primordially a part of the otic capsule.

Growth is rapid. Within three weeks the ossicles have increased their overall dimensions by three times. At the 9-week stage, the general configuration of the ossicles resembles that in the adult. Just three months later, in the 15-week specimen, the ossicles have attained maximum size as chondral elements, a clear forecast of the adult morphology.

Since the three ossicles are derivatives of the branchial arches, uniformity in developmental pattern would be expected in their growth and histogenesis. Such, however, is not the case. Whereas the stapes sacrifices a large fraction of its bulk, the malleus and incus attain relative solidity (although canalized by numerous vascular channels). Thus, unlike the stapes, both the malleus and incus retain the general form of the cartilage "model."

Bone formation, begins at the 16-week stage in the malleus and incus, and at 19 weeks in the stapes. In the latter stage of bone formation, periosteal bone is present in the head and in the proximal part of the manubrium of the malleus. On the incus, the periosteal shell is spreading from the body to the short and long processes. In the stapes, ossification occurs at the basal end of each crus. Endosteal bone is being formed beneath the periosteal lamina of the malleus and incus. Meckle's cartilage undergoes conversion into a ligament, and the anterior process, until now free, is attached to the bone of the head of the malleus. Ossification continues at such a rapid pace that the ossicles, except for permanently cartilagenous areas, are essentially adult skeletal elements in the 25-week fetus.

The malleus and incus in a 16-week-old fetus begin to change from cartilage to bone. In a 20-week-old fetus, two major steps in the progress of ossification may occur: vascular buds invade the ossicle preparatory to the formation of endosteal bone; or such bone is being formed internal to the layer of periosteal bone.

In 20 & 24-month-old specimens, both periosteal and endosteal layers are present. Endosteal bone may appear as interconnected spicules or as a true lamina applied internally to the periosteal layer.

The structure of the malleus and incus in the adult ear is relatively solid. They look like a typical long bone but in miniature, but the marrow cavity of the diaphysis is replaced by vascularized endosteal bone.

In the embryo of 8 weeks, the future stapes is a chondral annulet. Its cartilage grows during the next two months changing from annular to stapedial (stirrup) form. The single ossification center appears in the 18-week specimen. Spreading along the crura toward the head and across the base (footplate), the bone forms a periosteal "collar" with scattered spicules of endosteal bone internally. No sooner is an osseous shell completed than a process of erosion begins to render it foraminous. Resorption of bone progresses at so rapid a rate that, in the 24-week fetus, the capital, crural, and basal parts are continuously open towards the obturator foramen. Periosteal bone may persist as a crista stapedis on the base. Finally, endosteal bone remains only as a thin lamina on the internal, or obturator, surface of the head and base.

As a result of these changes, the stapes of the adult is a fragile ossicle with a thin bilaminar base. Cartilage retained from fetal life is carried across the vestibular surface to the fenestral margin of the tympano-stapedial syndesmosis. The cartilage of the fenestra is continued into the vestibular aperture of the fissula antefenestram, where cartilage is frequently encountered in the late fetal and early postnatal stages and may even persist in the adult.

In the terminal step in osteogenesis, blood vessels enclosed by the newly formed bone occupy vascular channels that are circumferentially arranged. Other blood vessels cross the base in the mucous membrane, ascend to the head of the stapes, then continue at the submucosal level along the ossicular chain to the tympanic membrane.

<u>**Morphology of auditory ossicles:**</u> *Anson & Donaldson (1973)* detail the morphology of the ossicles and their ligaments and muscles:

The tympanic cavity contains three small, movable bones: the malleus or hammer, the incus or anvil, and the stapes or stirrup (fig.1).

The auditory ossicles extend like a chain across the tympanic cavity as a functional connection between the tympanic membrane and the vestibular (oval) window. The outermost ossicle is the malleus, firmly attached to the tympanic membrane; the innermost, the stapes, is fixed in the vestibular window and directly contacts the fluid perilymph of the internal ear. Between the malleus and stapes lies the incus. The three bones are bound together by articulations and are connected by ligaments to the walls of the ear cavity. This compound osseous system acts like a bent lever converting the vibrations of the tympanic membrane into intensified thrusts of the stapes against the perilymph.

The malleus: is the largest auditory ossicle. It lies farthest laterally and forward. Its upper part, the head (capitulum mallei), occupies the recessus epitympanicus. The head of the malleus is thick and club-shaped and presents on its posterior and medial surfaces an elongated saddle-shaped articular surface for the body of the incus. The head is separated by a constriction, the neck (collum mallei), from the handle of the malleus (manubrium mallei). The handle is a thin, bony rod, oval in cross-section, which tapers towards the tip, where it terminates in the form of a spatula. On the lateral margin it is covered by cartilage. The lateral surface gives rise to the stria mallearis and is covered medially by the mucous membrane of the tympanic cavity. The handle forms an angle of about 130 degrees with the head of the malleus. The size of its angle with the horizontal plane varies with an average of 45 degrees. Above and to the lateral side, the handle of the malleus continues into the

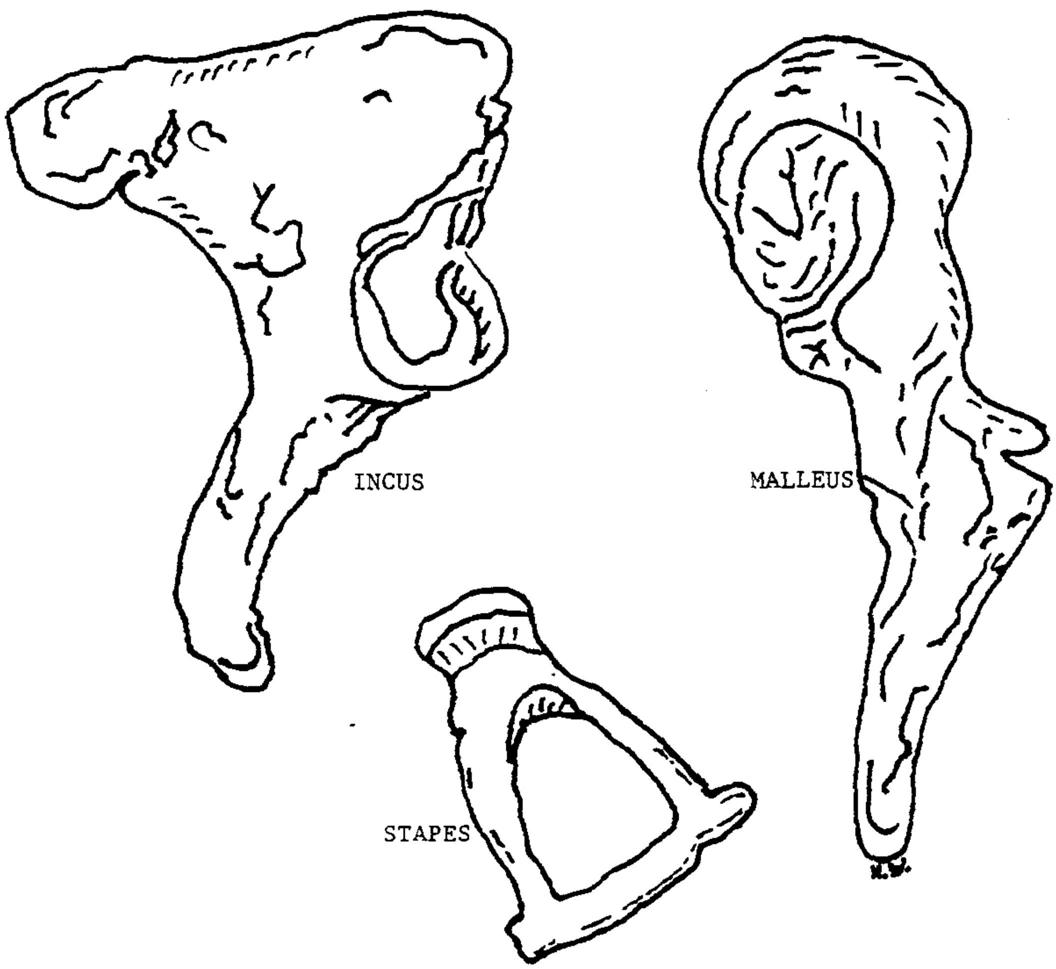

Fig.(1): The auditory ossicles.