Modern Cell Technologies in the Treatment of Patients with Tympanic Membrane Injury Inflicted by Mine Explosion

V. P. Tumanov, V. T. Pal'chun, A. A. Pomatilov, E. V. Polyakova, A. V. Zakharova, and V. N. Yarygin

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We evaluated the efficiency of transplantation of cultured human allofibroblasts onto tympanic membrane damaged by mine explosion in combination with Tampograss dressing (Paul Hartman). Transplantation of cultured human allofibroblasts was effective in 100% cases. Application of the film with fibroblasts onto perforation occupying from $^1/_4$ to $^1/_2$ of the tympanic membrane was more effective by 15% (by 59% in subtotal perforation) than tympanoplasty with amnion membrane. The mean duration of tympanic membrane restoration after spontaneous healing and amnionoplasty is virtually the same, while transplantation of allofibroblasts accelerated the process in comparison with other groups in perforation of any size; in subtotal defect the duration of tympanic membrane restoration was shorter by 14±1 days.

Key Words: *cell technologies; cultured human allofibroblasts; tympanic membrane perforation*

A specific feature of modern war conflicts and terrorist acts is wide use of mines and explosion weapons, leading to various injuries, including ENT injuries. The etiological factors of these traumas are extreme acoustic, vibration, and barometric exposure of organs and systems sensitive to these factors, surpassing the tolerance threshold of live tissue. Plastic surgery is often employed in these injuries. Unsatisfactory result of transplantation of various biological materials (partial or complete lysis of the graft) is a frequent problem of plastic surgery, including the interventions in otorhinolaryngology. This can result from active infectious process or from autoimmune rejection. Autoimmune rejection in myringoplasty is determined by changed antigenic composition of damaged tissues and hyperactivation of the immune system under the effect of many stimulators, which leads to inadequate reaction to the transplant. The search for universal plastic material is in progress [1,2,4,5,10]. By the present time the use of cultured human skin cells is a promising trend. Keratinocytes [9] and fibroblasts [8] are used mainly for the treatment of burns and wounds. Our studies confirmed high plastic characteristics of allofibroblasts and the possibility of their application in otorhinolaryngology [3,6,7].

We evaluated the efficiency of transplantation of cultured human allofibroblasts to the tympanic membrane damaged by mine explosion and combination of this treatment with application of Tampograss dressing (Paul Hartman).

MATERIALS AND METHODS

Twenty-seven patients aged from 18 to 57 with injuries to the hearing system were hospitalized at

Department of ENT Diseases, Therapeutic Faculty, Department of Pathology, Pediatric Faculty, Russian State Medical University; N. I. Pirogov Municipal Clinical Hospital No. 1, Moscow

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the ENT Department of Municipal Clinical Hospital No. 1. The time of admission depended on the severity of clinical status caused by combined injury (craniocerebral injury, burns, traumas of the limbs and chest) and was 1-12 days. The main complaints concerning the acoustic analyzer were poor hearing, obstruction and ear noise. All patients were thoroughly examined.

According to tonal threshold audiometry, conductive hearing loss with the bone-air interval of 20-35 dB was diagnosed in 38.7% cases, mixed hearing loss of different severity with the maximum elevation of hearing threshold by bone conduction for speech frequencies of up to 40 dB and at 4-8 kHz frequencies to 60 dB was diagnosed in 46.9% patients, and high-frequency neurosensory hearing loss with threshold air conduction increased to 45 dB at a frequency of 8 kHz was detected in 14.4% cases, impaired hearing was detected in the ear with intact tympanic membrane in all 7 cases (Figs. 1, 2). Tympanoplasty with different materials was carried out 3-5 days after hospitalization in 22 patients without signs of reactive inflammation in the tympanic area.

Patients of different age and gender with uni- and bilateral injuries to the membranes were observed. Transplantation of human allofibroblasts was used in 17 patients, plastic repair using chicken amnion in 9, in 6 cases the membrane was closed just with Carboxyl II film, and no plastic repair was carried out in 12 patients (Table 1).

Allofibroblast culture transplant was delivered from tissue culture laboratory on the day of operation in a special container (patent No. 2165765, license No. 1281/04). Legal and ethic aspects of the use of this modern technology are based on patent No. 2165765, license No. 1281/04, and decisions of the Academic Council of Ministry of Health of the Russian Federation and the Board of the Russian Academy of Medical Sciences. Bacteriological and virological tests were carried out in all cases. Fibroblasts were cultured in Eagle's medium with 10% fetal calf serum and 2% glutamine in a CO_2 incubator. Five to seven passages were made after development of islet growth. Cell inoculation density was $20\times10^3/cm^2$ and remained constant at all stages (Fig. 3). Allofibroblast culture was applied onto a thin (0.1 mm) transparent polymeric film. Fibroblasts were fixed on both sides of the film due to collagen monolayers, which provided stable position of the transplant without glue. The film with allofibroblast culture was applied onto the tympanic membrane.

The meatus and tympanic membrane were anesthetized with 1% ultrocaine in typical points. The outer surface of the membrane was deepithelialized around the perforation (5 mm from edges of perforation) or subtotally. A fragment of film with fibroblasts was cut out; its shape was the same as of the perforation, but the fragment was larger, so that to close the defect. The transplant was tightly fixed to the tympanic membrane with a gauze turunda. The tampon was removed on day 7 after cell transplantation, polymeric film carrying allofibroblasts was removed on day 21. Starting from day 7 no tampons were used; dry cleansing of the meatus was carried out daily. The treatment protocol included antihistamine, antibacterial drugs, and drugs for the treatment of neurosensory hearing loss. An important role in the treatment by transplantation of cultured human allofibroblasts was played by modern interactive dressing. We applied Tampograss dressing (an atraumatic turunda from hydrophobic polyester impregnated with autoemulgated ointment base). A specific feature of this dressing is that it provides elasticity of the membrane surface and

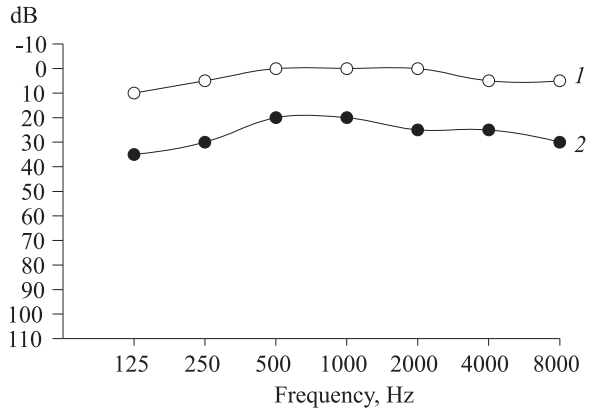

Fig. 1. Left ear audiogram of patient V. with disruption of the tympanic membrane caused by mine explosion. Here and in Figs. 2, 4, 5: *1*) sound conduction in the bone; *2*) sound conduction in the air.

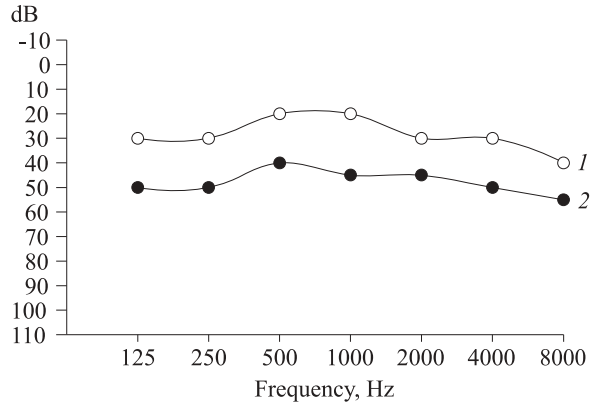

Fig. 2. Right ear audiogram of patient T. with severe acute middle air injury caused by mine explosion.

TABLE 1. Groups of patients

Parameter	Treatment method				Total
	AFP	CAP	SUT	no plasty	
Number of perforations	17	9	6	12	42
Complete closure of perforations	17	4	3	1	25
Method efficiency, %	100	44	50	8	56

Note. AFP: allofibroblast transplantation; CAP: chicken amnion plasty; SUP: synthetic film transplantation.

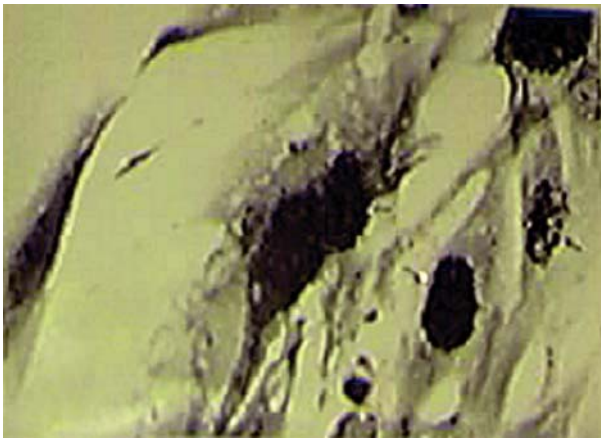

Fig. 3. Allofibroblast inoculation density of $20\times10^3/cm^2$ is constant at all stages (×250).

edges during all phases of its healing, prevents drying of the membrane, stimulates angiogenesis and epithelialization, does not stick to the tympanic membrane, does not damage the newly formed epithelium, allows oxygen access and good ventilation due to large-hole texture, and is permeable for exudate.

RESULTS

The results of application of immobilized fibroblasts on the perforation occupying $^1/_4$ to $^1/_2$ of the tympanic membrane were 15% better (in subtotal perforation 59% better) than after tympanoplasty with amnionic membrane. Comparative dynamic observation of patients for 12 months after transplantation revealed no re-perforations, adhesions, or formation of cholesteatoma, that is, the transplant provided stable dynamic results and promoted repair of the tympanic membrane.

According to tonal threshold audiometry carried out repeatedly after 1 year, the bone/air interval decreased to 5-20 dB in all patients in whom the tympanic membrane defect was closed (Figs. 4, 5). In other 18.4% patients, in whom perforation of the tympanic membrane did not close, hearing did not change. Hearing threshold decreased to 15-25 dB only for medium frequencies (500-2000 Hz), while for high frequencies (4-8 kHz) hearing threshold virtually did not change or decreased negligibly (by 5-10 dB), which led to the formation of stable high-frequency neurosensory hearing loss. Transplantation of cultured human allofibroblasts was effective in 100% cases. Positive results were observed in 44% patients treated by tympanoplasty with chicken amnion and in 50% patients in whom the perforation was closed by a synthetic film. Of the patients recovering without myringoplasty spontaneous healing of the tympanic membrane defect

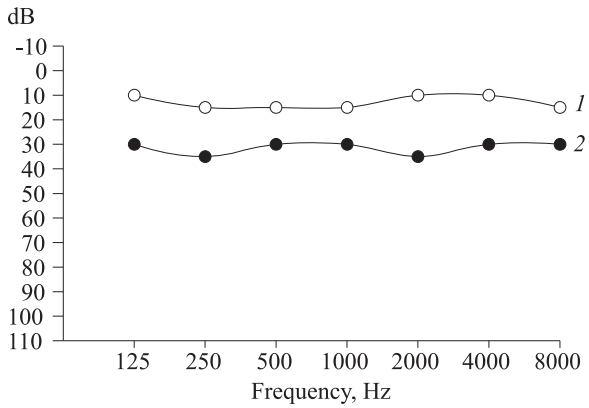

Fig. 4. Audiogram of patient E. with rupture of the tympanic membrane, caused by mine explosion, 1 month after treatment with chicken amnion.

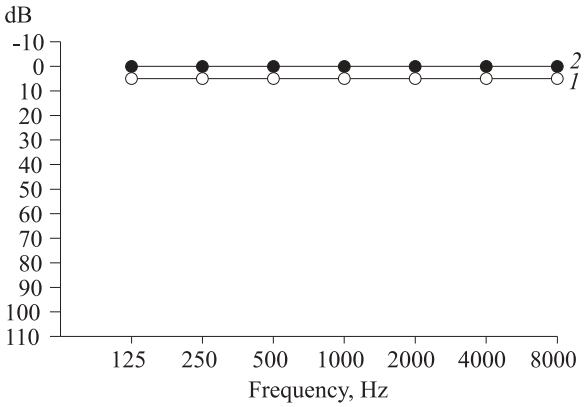

Fig. 5. Audiogram of patient I. with rupture of the tympanic membrane, caused by mine explosion, 1 month after transplantation of cultured human allofibroblasts.

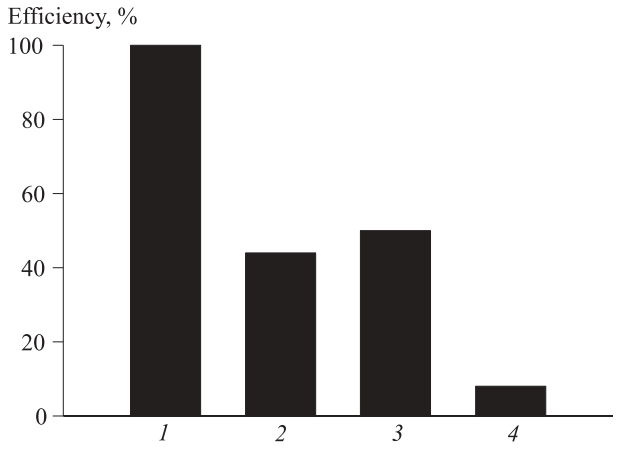

Fig. 6. Efficiency of using various methods of treatment of tympanic membrane injuries by mine explosions. *1*) allofibroblast transplantation; *2*) chicken amnion plasty; *3*) synthetic underlayer transplantation; *4*) no plastic repair.

was observed in only 1 case (10%). For the entire group of patients, tympanic membrane was restored in 56% cases (Fig. 6).

These data indicate that injury to the hearing system caused by mine explosion leads to anatomical and functional disorders and stable changes in the structures of the middle and internal ear. However, restoration of the tympanic membrane integrity after transplantation of cultured human allofibroblasts was more rapid and was observed in a higher percentage of patients than after treatment by other methods. Sound perception is changed significantly after mine explosion injury, and therefore all patients with such injuries should receive early drug and surgical treatment for preventing stable deterioration of hearing.

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