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Wound healing of mucosal autografts for frontal cerebrospinal fluid leaks – clinical and experimental investigations*

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SUMMARY

Wigand introduced in 1981 the concept of applying autogenous free mucosal grafts for small to middle-sized cerebrospinal fluid (CSF) leaks of the frontal skull base. This operative technique has proved to be successful in clinical use. However, the details of wound healing of the free graft and the host area of the skull base are largely unknown.

We conducted a series of animal experiments using 21 rabbits. Standardized CSF leaks of the frontal skull base were created and then closed using free autogenous grafts from the nasal septum. Twenty specimens could be evaluated after different postoperative time intervals by means of conventional microscopy of histological serial sections. In addition, we carried out a small series of special clinical postoperative observations following routine sinus surgery for polypoid mucositis on our patients, applying free mucosal autografts to the intact frontal skull base.

The autogenous free mucosal transplants underwent a rapid process of histological remodelling. All grafts showed a reduction in size of about 1/5. The respiratory epithelia mostly disappeared postoperatively. The main histological feature consisted of a fibrous transformation of the graft, starting 8 days postoperatively.

The presented experiments and observations lay the foundation for optimizing the operative technique and the postoperative care of our patients suffering from CSF leaks.

Key words: autogenous mucosal graft, CSF leak, endoscopic surgery, skull base defect

INTRODUCTION

Endonasal surgery has opened up a broad range of indications in recent years due to the routine use of the endoscope and/or the microscope. Besides surgery for chronic sinusitis, the repair of small or middle-sized cerebrospinal fluid leaks (CSF leaks) at the frontal skull base has become an established indication for optically guided, endonasal microsurgery. Different surgical techniques (single layer vs. double layer closure; overlay vs. underlay technique) and grafts (septal mucoperichondrium, turbinate mucoperiosteum, fasica, muscle, fat, cartilage, bone; free grafts vs. flaps) are available for defect closure (Hosemann 1996, Terrell 1997).

Failure of the surgical intervention for CSF leaks may cause severe complications like meningitis or brain abscess. Based on this fact, comparative studies of the different surgical concepts are desirable. Keeping this goal in mind, however, fundamental examinations of the pathophysiology and morphology of each kind of repair should be undertaken.

Wigand introduced in 1981 the concept of a single-layer closure for frontal CSF leaks using autogenous free mucosal grafts (Wigand 1981). The skull base defect is exposed via the endonasal route with the aid of the endoscope and the mucosa around the leak is cleared microsurgically. An autogenous mucosal transplant is applied to the defect as an onlay graft using fibrin glue. The inferior turbinate usually serves as the donor area. Wigands method has proved to be successful in clinical observations (Wigand 1990; Hosemann et al., 1991; Gjuric et al., 1996). The basic mechanisms of pathophysiology and morphology, however, are largely unknown.

We have carried out a series of animal experiments on wound healing of free autogenous mucosal transplants for closure of frontal CSF leaks using rabbits. In order to overcome limitations in the positive evidence of animal experiments, we additionally subjected a small group of patients to specific clinical observations. Following, the most important results are presented.

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By analyzing wound healing of free autogenous mucosal transplants we lay the foundation for future optimizing of the size of the graft, the location of the donor area and the specific postoperative aftercare of our patients.

MATERIALS AND METHODS

a. Animal experiments

A total of 21 rabbits were anesthetized with ketamine (50 mg/kg i.m.) and xylazine (4 mg/kg i.m.). The frontal skull base was exposed using an external access by translocation of the nasal bones. Under the microscope the cribriform plate was cleared from the mucosa and smoothed down by means of a diamond drill. Following, a 2 mm i.d. perforation of the skull base was performed with the drill 0.5 cm lateral to the midline. The exposed dura was incised with a sickle knife leading to CSF leakage. The bony defect was closed by a square piece of free autogenous mucosa (0.5 cm in length) with the help of fibrin glue. The nasal septum served as the donor area. The graft was subsequently covered with a layer of gelatin and the operative site was closed. All animals were injected once with 100,000 I.E. benzyl-penicillin (i.m.) intraoperatively.

After different postoperative periods, ranging from 2 days to 12 weeks, the animals were killed (post-op. time interval [days]: 2; 4; 7; 14; 21; 28; 42; 56; 70; 84; two specimens each). The skull base specimens were gathered and decalcified. The tissue was carefully cut in the frontal plane (4-5 μm thick sections at an equal distance of 100 μm) and the sections were subjected to conventional histological staining (hematoxylin-eosin). In total, 800 histological sections from 20 specimens were evaluated by light microscopy (approval granted by the governmental animal care and use committee - No. 211/2531.3-10/91).

b. Clinical observations

Nine patients suffering from chronic-diffuse polypoid pansinusitis were subjected to a standardized observation. During routine ethmoidectomy according to Wigand (1990), the bone of the frontal skull base was inevitably exposed in individual areas following the removal of polyps. These mucosal defects were carefully recorded in a special diagram. At the end of the surgical intervention, an autogenous piece of mucosa originating from routine excision primarily of the dorsal attachment of the middle turbinate was applied to the denuded area of bone. The mucosal graft was fixed with fibrin glue and was covered with a sheet of gelatin. The length of the graft was carefully noted. There were no CSF leaks in this patient group and no patient suffered from any other severe disease. All patients had given their informed consent to the observation.

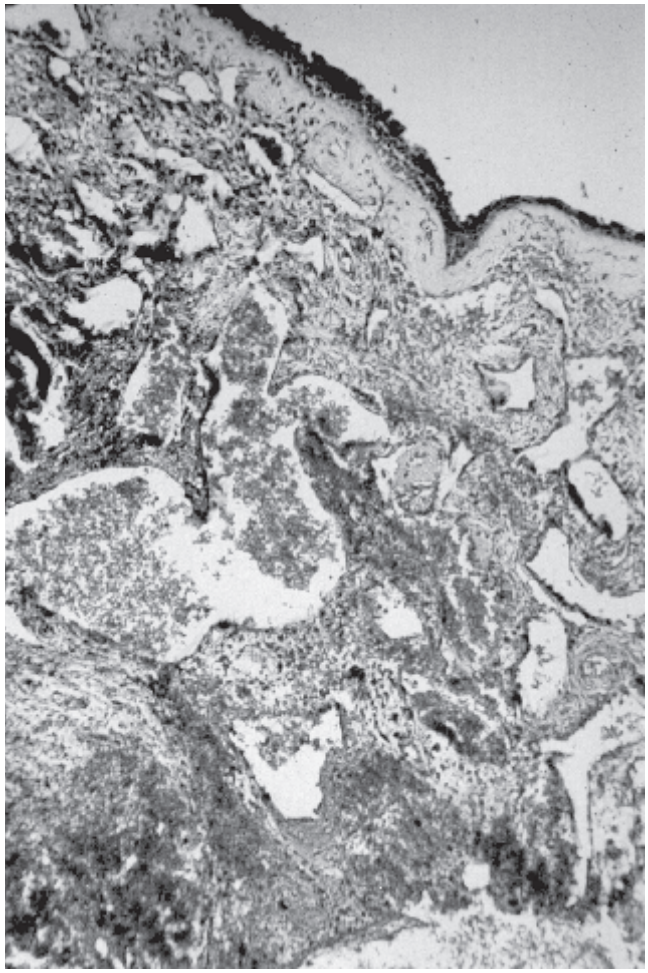

Figure 1. Cross-section of a free mucosal graft applied to the frontal skull base (2 days after surgery, human material). The surface is covered by an altered respiratory epithelium with focal metaplasia. The lamina propria shows congestion of blood vessels in addition to areas of interstitial hemorrhage.

During regular postoperative examinations the mucosal graft was removed after different time periods, ranging from 2 to 20 days (Table 1). The length of the graft was measured a second time. The piece of mucosa was subjected to routine histological serial sectioning and staining (hematoxilin-eosin). In total, about 300 histological sections were evaluated by light microscopy.

RESULTS

a. Animal experiments

The respiratory epithelium of the mucosal transplants disappeared completely after only 48 hours. Blood vessels were congested and the lamina propria was infiltrated by a significant number of inflammatory cells (neutrophil granulocytes, lym-

Table 1. Distribution of time intervals and measurements of 9 autogenous mucosal transplants.

Post. -op. time interval (days)	2	4	6	8		10	12		20
Number of specimens examined	1	1	1	2		1	2		1
Original length of transplant (mm)	6,7	8,8	8	4,2	5,2	9,8	11,2	15	4,3
Length post. -op. (mm)	5,5	6,5	6	3	4,5	9	10	12	3,3
Reduction in length post. -op.	18%	26%	25%	29%	13%	8%	11%	20%	23%

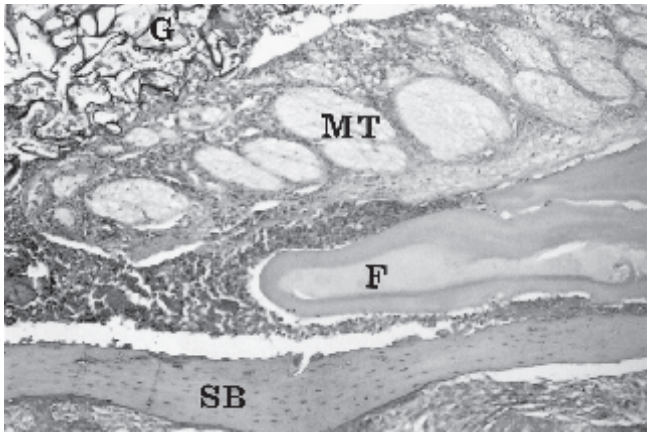

Figure 2. Histological section of the skull base with a free mucosal graft applied (4 days after surgery, animal experiment). Purulent inflammation leads to cellular infiltration of the undersurface of the graft. MT: mucosal transplant; F: fibrin glue; SB: skull base; G: gelatin.

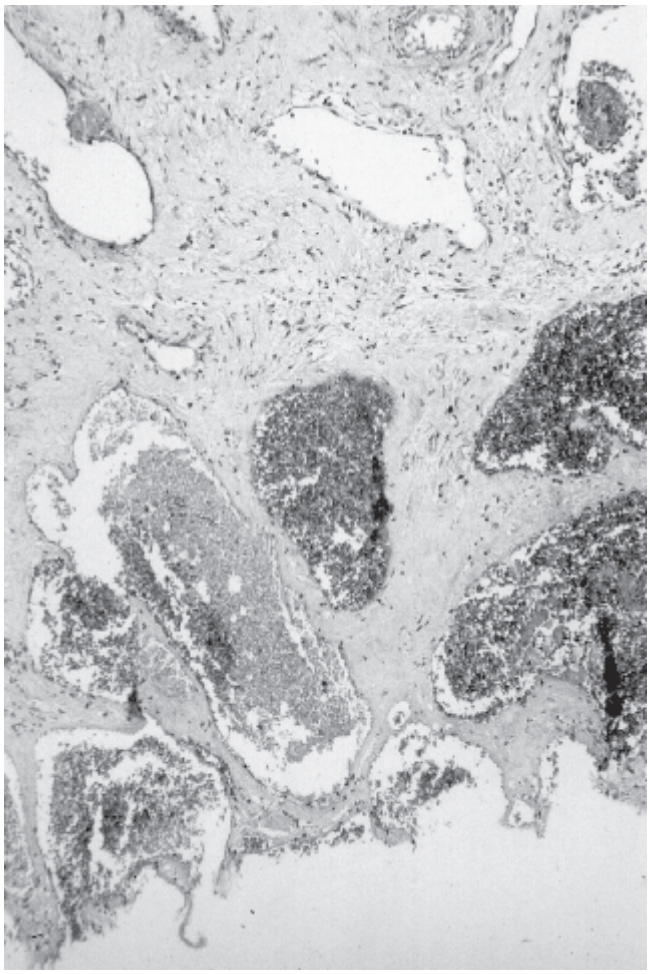

Figure 3. Detail of the basis of a free mucosal graft 12 days after surgery (human material). New blood vessels are seen entering the graft from below.

phocytes, plasma cells) and macrophages/histiocytes. The basal layer of perichondrium formed an obvious barrier for cellular transmigration.

Four days postoperatively the cellular infiltration had generally increased. In the case of purulent local infection, inflammatory cells were undermining the edges of the graft and gradually working their way along the underlying bone (Figure 2). The bone

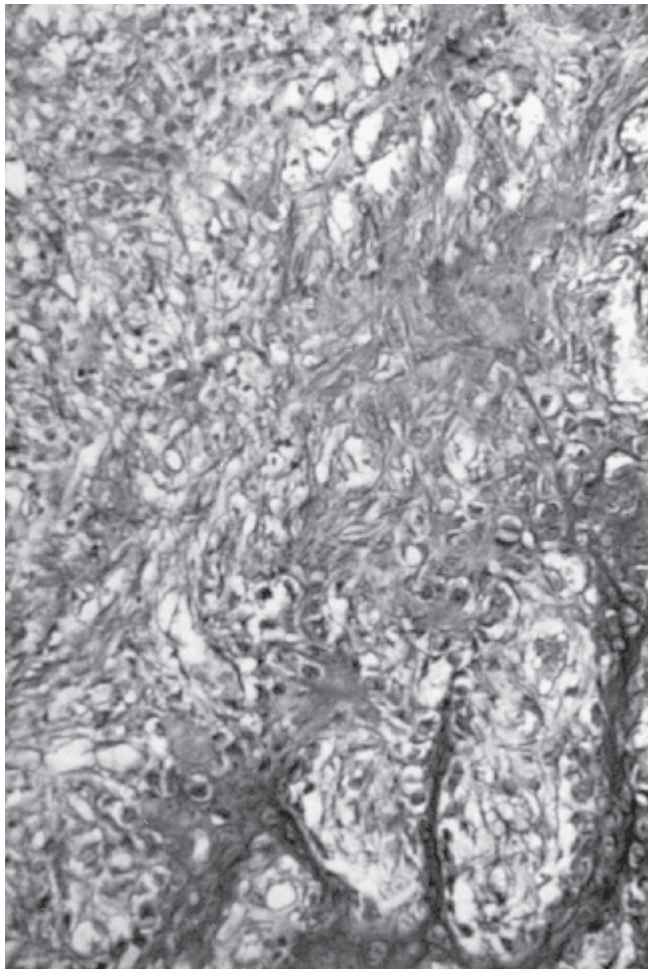

Figure 4. Histological section of a free mucosal flap applied to the frontal skull base (2 weeks after surgery, animal experiment). The lamina propria is dominated by immature fibrous tissue and beginning osteogenesis.

tissue itself showed initial signs of proliferation in the area of the CSF leak.

One week postoperatively the graft had considerably increased in thickness.

Two weeks postoperatively the original texture of the lamina propria had changed and immature fibrous tissue became the predominant feature (Figure 4). Capillaries were sprouting out of the bone of the host site and were invading the transplant. In the area of the bone defect obvious osteogenesis was seen.

Three weeks postoperatively the fibrous transplant was scattered with capillaries. The graft thickness increased again irrespective of the diminished number of inflammatory cells. The osteogenesis peaked, closing the former defect with immature bone.

Six weeks postoperatively the osteoneogenesis had decreased. However, some bony spiculae were even infiltrating the former transplant.

Eight weeks postoperatively the outline of the transplant could no longer be exactly identified by microscopy. The graft had been completely transformed into scar tissue with intercalated bony trabeculae. Some colonies of immature respiratory epithelia were enclosed giving rise to small cysts. These cysts increased in number over the next two weeks.

Twelve weeks after surgery the new bone tissue had maturated occupying large areas of the former transplant. Due to proliferation of mesenchymal tissue, the process of reepithelialization was mostly incomplete. Besides the small epithelial cysts mentioned above, some seromucous glands were observed among the transplanted tissue.
Throughout the entire postoperative period the meninges and the brain tissue did not show any major tissue reaction with the exception of one specimen (4th postoperative day).

A significant osteoneogenesis in the area of the maturating wound at the frontal skull base appeared to be a prominent histological finding. First signs of osteoneogenesis were observed at the edges of the bony perforation already 4 days postoperatively. At the same time, the original respiratory epithelium on top of the mucosal autografts got lost completely. Following, the increasing hyperostotic reaction revealed a specific pattern encasing the fibrous remnants of the autografts. The process of osseous integration of the transplant started after 3 weeks; it was best seen 6 weeks postoperatively, however. The skull base defect was closed completely by new bone growth already 4 weeks postoperatively. Significant local infection occurred in 3/20 animals. There was no general correlation between the local infection and the hyperostotic wound reaction.
The special feature of wound repair in rabbits favouring osteoneogenesis has been confirmed by other observers (Forsgren et al., 1993). Human wound healing differs in this aspect. Because of this fact, we designed an additional clinical study in our patients.

b. Clinical observations
All the host sites and grafts were void of macroscopic purulent inflammation. Relevant necrosis of the transplants could not be observed.
Two days after surgery the epithelium on top of the graft was mostly fragmentary, showing nothing but spots of single-layered basal cells. In some areas metaplasia gave rise to stratified epithelium (Figure 1). Hidden in mucosal folds some remnants of regular respiratory epithelium could be detected. The predominant histological features of the lamina propria consisted of congested vessels and intersitial hemorrhage combined with infiltration by lymphocytes and plasma cells.
Four days postoperatively the respiratory epithelium was preserved in only a few areas of the graft surface. Underneath, mucous glands showed signs of increasing degeneration. Ingrowing immature fibrous tissue started to change the texture of the lamina propria. The dilated vessels were occluded by thrombi.
Six days postoperatively and later the grafts were already fixed tightly to the skull base and could only be removed from the host area by considerable force. The majority of specimens showed marked atrophy of glands. The respiratory epithelia completely disappeared with the exception of two grafts revealing spotted epithelial covering and partly preserved glands (10th day and 12th day after surgery, respectively). Significant reepithelialization starting from the neighbouring mucosa of the operative field could not be detected in the follow-up period.
Eight days postoperatively and later fibrous transformation of the graft became the most prominent histological feature. The second important change in texture consisted of obvious neovascularization originating from the host area (Figure 3). The lamina propria was infiltrated by inflammatory cells.
Twenty days postoperatively the graft was completely transformed by fibrous tissue.
All grafts were reduced in size postoperatively to a similar extent. This process of retraction and scarring began in the first days of the postoperative time period (Table 1). The individual measurements are listed in Table 1.

DISCUSSION

Endonasal surgery for CSF leaks using mucosal transplants was first performed by Hirsch in 1952. However, general use of endonasal operative access for repair of small defects in the frontal skull base was not generally established until the introduction of special optical devices like the endoscope and/or the microscope. Utilizing optical aids for endonasal surgery, frontal skull base defects may be widely exposed with minimal additional risk to the patient. The mucosa around the defect can be cleared causing minimal additional trauma. The leak may be patched by applying microsurgical techniques (Mattox and Kennedy 1990; Hosemann et al., 1991; Gjuric et al., 1996; Weber et al., 1996). The present investigation is confined to smaller defects which are closed sufficiently by soft tissue alone. If the bony defect is larger than 1 cm in width, a bone or cartilaginous graft should be additionally inserted over the bony edges in order to prevent herniation of cerebral tissue.
The different techniques and materials currently in use have been primarily investigated by clinical follow-up studies, although most examined only a moderate postoperative time period (Terrell 1997). However, insufficiency of the transplants may only show up after a long time delay, resulting in meningitis or even brain abscess. These facts raise relevant questions related to pathophysiology which are still open to discussion: what is the normal time course of morphological changes in the free autografts? Does the respiratory epithelium covering autogenous mucosal grafts offer an advantage over simple mesenchymal autogenous grafts? Do the specific features of the host site (bony contact surface, area of missing support with damaged meninges) have an influence on wound healing?
The technique of Wigand (1990), which applies single – layer, autogenous free mucosal flaps, has several advantages over other techniques for smaller defects: the operative procedure takes minimal additional time by harvesting the graft only in the exposed operative field. In most cases, the graft is easily obtained from the tissue which has to be excised routinely during the operative approach. A free mucosal graft may be placed very precisely. There is no fear of displacement of the graft as with pedicled septal flaps which is caused by scarring of the pedicle (Dodson et al., 1994). Placement of a lumbar drainage is not necessary as a rule.
We have analyzed fundamental morphology by carrying out a series of animal experiments. As we took particular interest in

the histological details of taking and remodelling of the autogenous free mucosal graft, we did not design control groups of different treatment modalities. The specific reliability of autogenous free mucosal grafts could be proved by histological serial sections of specimens covering the postoperative time period from 2 days to 12 weeks. In order to overcome restrictions in the transferability of animal experiments, we additionally went back to standardized clinical observations. We accept the draw-backs resulting from the small sample number and the individual anatomy and pathology of our patients. A fundamental point of these observations, however, is represented by the repeated measurement of the graft size. Wound healing of the paranasal sinus mucosa does not seem to depend on the geometry of the wound area (Hosemann et al., 1991). Due to this fact we analyzed reduction in size by measuring only one dimension of our rectangular grafts.

In summary, several conclusions listed below may be drawn from the animal experiments and the observations of our patients. We hope that in the future comparative investigations of the wound healing process based on other operative techniques for CSF leaks will be presented in the literature.

CONCLUSIONS

The single layer closure of small to middle-sized frontobasal CSF leaks by use of autogenous free musocal overlay grafts according to Wigand (1981, 1990) has proved to be a reliable and secure operative technique. Necrosis or displacement of a transplant is unlikely to occur in the absence of local infection.

The autogenous mucosal transplant undergoes a relatively quick histological remodelling in the course of wound healing. One week after surgery the proliferation of fibroblasts appears to be the most important histological feature. Maturation gives rise to a fibrous transformation of the graft. Respiratory epithelia of the autogenous mucosal grafts mostly disappear postoperatively. Reepithelialization likely takes much longer than three weeks. The meninges participate in the normal reparative inflammation only to a small degree.

A reduction in size of about 20 % postoperatively has to be taken into account for the free mucosal grafts. Shrinking begins during the first days after surgery. Because of this fact, a 1/4 oversized piece of mucosa should be chosen for defect closure.

The mucosal transplant adheres to the host area of the bony skull base with remarkable strength within only 6 days after surgery. This effect is caused by the beginning of ingrowth of blood vessels out of the underlying bone. Nasal packing usually seems to be superfluous after this time. The same applies to a possible lumbar drainage (which in principle is necessary only in exceptional cases). Antibiotic medication is recommended for the time period mentioned.

Local purulent infection may interfere with the taking of the free mucosal graft. The existence of a dense basal connective tissue layer in the transplant (periosteum or perichondrium) offers additional resistance to the purulent infiltration.

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