Clinical application of a physically and chemically processed human substitute for dura mater

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Object. Allogenic human fascia lata used in neurosurgery as a dura mater substitute can be associated with the risk of virus and bacterium transmission and with a delay in its incorporation due to immunological and inflammatory reactions. The authors review their preliminary experience with a chemically and physically processed fascia lata graft.

Methods. Grafts that had been treated with solvent detergents, freeze-dried for conservation, and gamma irradiated (25,000 Gy) for sterilization were placed into 17 patients during neurosurgical procedures performed to treat brain tumors, cerebral malformations, trigeminal neuralgia, and posttraumatic lesions. The handling properties of the material, surgical wound features, and hematological parameters were evaluated. The average follow-up period was 23.8 ± 2.2 months (mean ± standard deviation). The handling properties and biocompatibility of these human dural substitutes were highly satisfactory and no major complications were observed. Postoperative computerized tomography or magnetic resonance images obtained in 13 patients revealed no abnormal findings at the site of fascia lata implantation. In one patient who underwent a second surgery performed 12 months after the initial operation, this dural substitute was found to have been recolonized by host fibroblastic cells and replaced by autologous collag enous tissue.

Conclusions. Human fascia lata that has been rendered safe by applying physical and chemical treatment is a fully biocompatible alternative to the dural graft materials currently available.

Key Words • dural substitute • human allograft • fascia lata

Among the various substitutes for dura mater that have been used in the past 100 years, collagenic connective tissue grafts (autologous, allogenic, or xenogenic) and/or synthetic materials (resorbable or nonresorbable) have been used successfully.11 Fascia lata meets almost all the criteria required for an ideal dural graft.31,38 Indeed, it can be used with minimal or no complications because it is relatively simple to obtain and is associated with no risk of prion transmission.40 During the last 40 years, fascia lata has been successfully used in duraplasty to repair dural defects in cranial neurosurgery (defects caused by tumor or trauma)48 or dural tears following spine surgery (tears created during procedures such as laminectomy or resection of a herniated vertebral disc).13

Human-tissue allografts remain associated with two major problems, however. First, such allografts can be associated with the risks of bacterium and virus transmission.5-7,9,14 Although the selection of a donor for tissue grafting is very rigorous, Deijkers, et al.,19 have demonstrated that organisms of low pathogenicity (mainly skin commensal organisms) can be cultured from graft material containing 50% tissue, whereas organisms of high pathogenicity (mainly contaminants from the gastrointestinal tract) can be cultured from graft material containing only 3% tissue. Leille and colleagues21 have estimated that the risk of virus transmission was 10-fold higher in cases of organ tissue transplantation than in those of blood transfusion. Hence, the most important preoccupation among administrators of tissue banks, in the last decade has been the reduction of this risk.

Second, clinical studies have shown that allograft tissue reconstructions tend to heal slower than repairs made using autograft tissue. Minami and associates25,26 found that the cells of tendon tissue are antigenic. Pinkowski and colleagues22,23 also have demonstrated that major histocompatibility antigens or human leukocyte antigens on the cell surface are strongly immunogenic, whereas antigens present in the extracellular matrix and collagen are weakly immunogenic.

To avoid previously encountered complications, we have developed a procedure in which a sequence of multiple chemical and physical treatments are conducted to reduce the immunogenicity of the graft material as well as the risk of agent transmission. We have previously demonstrated that this treatment does not alter the biological and mechanical properties that are essential for an ideal dura mater substitute (Fig. 1).13 Biocompatibility testing was used to evaluate the ability of the graft to be recolonized. The cell culture response on exposure to human allografts was estimated using indirect and direct methods. Major aspects of cell physiology, such as growth, metabolic activity (succinate dehydrogenase activity), membrane integrity (neutral red uptake), and absence of cell necrosis (lactate dehydrogenase activity in the culture medium), were investigated af-
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Fig. 1. Photomicrographs of fascia lata after the chemical and physical treatments. A: Chemical treatment destroys the cells by protein coagulation, nuclear acid precipitation, and membrane degradation, and leads to a low antigenic tissue. Goldner trichrome, original magnification × 250. B: Ultrastructural analysis of the tissue performed using confocal laser scanning microscopy shows well-aligned collagen bundles (arrow) and cavities between the fibers for cell recolonization. This demonstrates that the treated fascia lata can be considered an appropriate scaffold for cell growth. Acriflavine, bar = 50 μm.

Fig. 2. Photograph of a flat freeze-dried treated human fascia lata allograft, which has been sealed in a double-plastic bag and sterilized with gamma rays.

This retrospective study is the first to demonstrate that fascia lata can be subjected to strong physical and chemical treatment and remain compatible with human application.

Clinical Material and Methods

Preparation of Human Tissue Allografts

The fabrication of this treated fascia lata has already been described. Portions of fascia lata from selected donors were procured according to the common standards of the European Association of Musculo Skeletal Transplantation (Vienna, 1997). Donors were selected based on a review of their medical histories, including risk factors for subacute spongiform encephalopathies. Minimum serological testing, which included detection of HIV-1 and -2, human T-cell lymphotrophic virus, hepatitis B and C, and syphilis, was performed. Procurement of the tissue was accomplished in an operating room. All instruments and equipment used for procurement were sterilized. The fascia lata was washed in sterile physiological saline solution at room temperature before shipment to the tissue bank. There, the fascia lata was mechanically stripped of its external loose connective tissue, including adipose tissue, vessels, and nerves. The remaining tissue was cut into pieces and washed by pulse lavage. The chemical treatment plan developed by the University Tissue Bank included multiple steps. Briefly, pieces of tissue were extensively defatted in absolute acetone followed by immersion in ethanol. Next, prion inactivation was obtained by applying NaOH (1 N) at room temperature for 1 hour as recommended by the World Health Organization. A reduction in the immunogenicity of the tissue was obtained by protein coagulation, nuclear acid precipitation, and cell membrane degradation, which was accomplished using NaCl and H2O2. After each procedure, pieces of fascia lata were intensively washed using a continuous flow of distilled water. Allografts were freeze-dried, packed in a double-plastic bag, and sterilized by gamma irradiation at 25,000 Gy (IBA Mediris, Fleurus, Belgium). The allografts were then stored at room temperature (Fig. 2). This treatment led to the inactivation of viruses such as HIV and hepatitis B and C, and diminished bacterial activity.

Clinical Studies

The clinical use of the processed fascia lata in neurosur-
gical procedures was approved by the Committee of Medical Ethics, University Clinical Hospital and Faculty of Medicine.

The fascia lata was only used in scheduled operations. Patients with acute infection and those in poor overall health were excluded from participating in this study, as were pregnant or breast-feeding women. Before its use, the fascia lata was immersed for 5 minutes in a physiological solution to recover its flexibility. At the time of implantation, the fascia lata was trimmed and closed by suture (eight cases), glue (seven cases), or cupping (two cases). Bioadhesive agents such as fibrin glue were applied over the suture in three cases.

Postoperatively, a wound inspection and hematological examination were routinely and carefully performed. Computerized tomography scanning or magnetic resonance imaging was performed in 13 patients.

**Patient Population**

Between January 2000 and September 2000, 17 patients (nine male and eight female patients) ranging in age from 2 to 71 years (mean 44 years) underwent surgery to treat nine brain tumors, three cerebral malformations, three instances of trigeminal neuralgia, and two posttraumatic lesions. Follow-up periods in this trial ranged from 21 to 29 months (23.8 ± 2.2 months [mean ± standard deviation]) (Table 1).

**Results**

**Complications of the Grafting Procedure**

No tear was reported when the fascia lata was grafted. Postoperatively, no wound infection and no subcutaneous cerebrospinal fluid collection were reported.

The results of the hematological examinations are reported in Fig. 3. In nine patients a significant transient increase in the level of CRP was correlated in three cases with an increase in the number of white blood cells 1 week after the operation. In three patients, a slight increase in the CRP level was observed 1 month following the intervention. No other clinical signs or biological parameters (such as CRP level or results of a urinary analysis) of infection or inflammation were observed.

Seven patients displayed complications (for example, slight hypacusus, headache, and hemiparesia) after surgical intervention or radiotherapy. None of these complications was related to the fascia lata graft. Postoperative computerized tomography or magnetic resonance images obtained in 13 patients revealed no abnormal findings at the site of the fascia lata implantation. In no case was the fascia lata graft removed during a neurosurgical procedure in the follow-up period; however, 1 year after implantation, in one case the graft was removed during orthopedic surgery. In that case the treated graft had been placed in a spinal location following resection of herniated discs at L4–5. During surgery the graft was found to have been replaced by autologous tissue and there was only minimal adhesion to the underlying surface. Histological examination revealed duralike tissue composed of banded collagen, fibroblasts, and vessels (Fig. 4).

**Discussion**

A dural substitute should satisfy the following criteria: 1) be unable to induce a harmful foreign body reaction; 2) be
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free from any potential risk of known and unknown infections; 3) be capable of being recolonized by host tissue; 4) have mechanical properties similar to those of the natural dura mater, especially in flexibility and strength; and 5) be storable and readily available when needed.41

To date, no material has met all these criteria. The use of many nonabsorbable materials for artificial dural grafts, such as silicone, has demonstrated that foreign body reactions persist for a long time, leading to serious complications such as intracapsular hemorrhage from excessive neovasularization.1,8,27–29,34 Due to their mechanical incompatibility and inadequate degradation rate, bioabsorbable artificial dura mater materials have not been widely used in clinical practice. The use of human dura mater grafts has been rejected by neurosurgeons because of the risk of prion (Creutzfeldt–Jakob disease) transmission.19,24,37 Although Type I bovine collagen has demonstrated good cell attachment, it remains associated with the risk of bovine spongiform encephalopathy transmission.23,39

Advantages of the Treated Fascia Lata

The risks of virus, bacterium, and prion transmission associated with this treated graft remain very low because of the rigorous control used in the selection of donors and the treatment applied, which inactivates bacteria and viruses. Sodium hydroxide and ethanol, which are included in our treatment of the graft material, have been proved to inactivate viruses.16,35 Gamma irradiation at a level of 25,000 Gy, which is applied to the fascia lata, has also been shown to inactivate HIV and hepatitis C.6,10,17,20,36 The use of solvent detergents and gamma irradiation has been demonstrated to provide a significant reduction in bacterial activity.2,18,20,22

Because prion inactivation should become a priority for allografts, we applied NaOH (1 N) to the fascia lata following the recommendation given by the World Health Organization in 1992.3,4,12,40 At the end of our treatment, the fascia lata allograft presents no risk of conventional or nonconventional agent transmission.

This graft is a bioabsorbable composite sheet composed of well-aligned collagen bundles and cavities between the fibers for cell recolonization of the matrix (Fig. 1). Indeed, an in vitro study has already demonstrated that the treated graft is a fully biocompatible graft and an appropriate scaffold for cell recolonization.13 Our clinical study has shown that treated grafts do not induce an inflammatory reaction or a reaction caused by a lack of matrix recolonization. We have demonstrated that the treated fascia lata is replaced by autologous collagenous tissue. Use of this graft can minimize the potential risk of chronic foreign body reaction against nonabsorbable synthetic material.

The use of treated human fascia lata allografts in various cranial and spinal locations has proved this material to be a successful substitute for dura mater, with respect to its flexibility and performance. Its storage capacity and its availability in various sizes in our tissue bank has allowed surgeons to choose the most suitable graft for a specific surgical indication. Its use has proved to be advantageous.30

This graft material also has been used in orthopedic (for synostosis and to accomplish tenodesis), abdominal, and otorhinolaryngological surgical procedures. To date, the graft has been used in 33 cases for purposes other than as a dural substitute.

Conclusions

Our chemically and physically treated human fascia lata graft material has satisfied all the criteria required for a dural substitute and is, therefore, a good alternative in cases of elective surgery or repeated operations.

Disclaimer

None of the authors has any financial interest in the process described in this paper or in the fascia lata allograft that is produced.

Fig. 3. Graphs depicting the time courses of CRP concentrations (mg/dl; upper) and white blood cell (WBC) counts (lower) before the operation (preop), 1 week after the operation (postop), and during the chronic stage (chro, > 1 month postoperatively). Values are expressed as means ± standard errors of the means.

Fig. 4. Photomicrograph of the dural graft obtained at a repeated operation performed 12 months after the original procedure. The duralike tissue is composed of banded collagen tissue with spindle-shaped cells (fibroblasts). The original dural tissue is not recognizable. Hemalum and eosin, original magnification × 125.
References