Duraplasty with biosynthetic cellulose: an experimental study

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The search for the ideal dural implant has been one of the main challenges of modern neurosurgery. Research on dural replacement has been infrequently conducted during the last two decades. Since the use of lyophilized dura for duraplasty was introduced by Sharkey, et al., in 1958, very few new materials have been tested and generally accepted. Muscle fascia remains the most accepted autologous implant in general use.

To find a new synthetic dural substitute, we planned an experimental study using biosynthetic cellulose. This material has been used with good results as a dermal substitute in burn victims, as a dermal protection in varicose leg wounds, and as a hemostatic agent in experimental liver surgery. It is used in odontology to assist the healing of dental furcal lesions. Physically, biosynthetic cellulose resembles the dura mater; it is maleable, easy to handle, and inexpensive. The purpose of the current study was to evaluate the action of biosynthetic cellulose when implanted as a dural substitute that is exposed to intact and damaged brain. The antifibrotic effect of this membrane was also studied by means of extradural covering with cellulose.

Materials and Methods

Pure cellulose is obtained by the action of Acetobacter bacteria by means of an industry-protected process (Biofill, Produtos Tecnológicos, Curitiba, Paraná, Brazil). The blocks of cellulose are cut into small 50-μm-thick pieces that resemble paper films with a smooth surface.

The surgical aspects of the experiment were performed at the Laboratory of Experimental Surgery at the Medical School of the University of Blumenau, Santa Catarina. Histological analysis of the specimens was performed at the pathology department of the Escola Paulista de Medicina in São Paulo.

Animal Groupings

Thirty-two young mongrel dogs were divided into three groups according to the type of experiment.

Group I. This main experimental group was designed to study the effects of cellulose, used as a dural substitute, on the intact brain. The group was subdivided into four subgroups according to...
of 50-micron thickness was used in Subgroups A, B, and C with the exception that a 50-micron cellulose film was applied extradurally after completion of dura-plasty to evaluate the antifibrotic effect of biosynthetic cellulose.

Group II. Five animals underwent a right-sided 4-cm-wide parietotemporal craniotomy during which a 2-cm piece of dura was removed. A punctiform corticovascular lesion was created with a thin forceps. The bleeding was controlled by application of a 10-micron thick film of cellulose to the bleeding site. An additional 50-micron piece of cellulose was sutured as a dural substitute before replacing the bone and suturing the muscle and scalp. To assess the antifibrotic effect of cellulose, three Group II animals received an epidural application of cellulose; the other two animals did not and served as a control group. Group II animals were observed for 270 days before sacrifice and histological examination.

Group III. Six dogs underwent a small (1-cm diameter) bilateral parietal craniectomy. The left side of the dura was covered by a 50-micron piece of cellulose. The right side was left unprotected close to the temporals muscle for use as a comparison. Two animals were clinically observed for 40 days, two for 80 days, one for 60, and one for 120 days before sacrifice and autopsy (Fig. 1).

Macroscopic Investigation

All the dogs were killed by administration of thiopental until cardiac arrest occurred. Brain fixation was achieved with an injection of 10% formalin via the carotid arteries and optic foramina. The separation of the head and removal of the scalp and soft tissues completed the preparation. Photographic documentation of macroscopic findings was made immediately after death. The surgical field was carefully reopened. The dura mater was incised and inspected in one dog from each subgroup of Group I and one dog from Group II. In the remaining animals, the dura was left untouched to ensure good histological results. No macroscopic evaluation was performed in Group III.

Microscopic Investigation

The brains of the dogs were fixed in 10% formalin for 10 days and then decalcified with 5% formic acid. After paraffin inclusion, 5- to 10-micron slices were stained with hematoxylin and eosin, van Gieson’s, Mallory’s, Masson’s trichrome, and Humason and Lushbaugh dyes for silver fibers. The prepared tissue specimens were observed using direct and polarized light microscopy. The thickness of the implanted material was measured as well as the newly formed external and internal connective tissue membranes (neodura), which appeared at different exposure times. Thickness values were achieved using the average of five measures obtained at different sites of the preparation. Statistical analysis was performed using the two-tailed Student t-test, variant analysis, and Newman–Keuls and Dunnett tests.

Results

During the immediate postoperative period, somnolence and late recovery were only observed in the Pilot Group; this was attributed to brain swelling caused by inadequate cerebral manipulation at the beginning of the experiment. Convulsions or behavior modifications were not observed, whereas good healing in almost all surgical
wounds was noted. Infections were observed in two ani-
mals in Subgroup B of Group I, which did not receive
prophylactic antibiotic medication. No undesired clinical
symptoms accompanied the wound infections and the ani-
mals exhibited normal behavior.

Macroscopic Findings

An overall good acceptance of the implant was ob-
served in Groups I and II. Epidural scar formation was
detected in Subgroups A and C of Group I and in the two
animals in Group II that did not receive epidural protec-
tion with cellulose (Fig. 2 upper left). Despite the two
wound infections found in two animals in Subgroup B, the
implants were very well accepted. Six dogs in Subgroup B
and the three animals in Group II that did receive epidi-
ural protection with cellulose displayed good acceptance of
the implant with a clean appearance of the extradural sur-
face. Only the nylon threads could differentiate the site of
implant (Fig. 2 upper right and lower).

Microscopic Findings

The cortex was intact with no adhesions in any of the
animals in Groups I and II. In Group I dogs, no difference
in cortical appearance was noticed at the sites of cellulose
and fascia implantation. The site of corticovascular lesion
in Group II showed no adhesion and good pial regenera-
tion. The cellulose was enveloped by two newly formed
connective membranes. The externally situated membrane
was thicker than the internal one. In some prepared tis-
" FIG. 2. Postmortem photographs of the operative sites.
Upper Left: Group II, postoperative Day 270. This dog was
not given epidural protection with cellulose. Notice the in-
creased epidural scarring tissue (fb). The dura (d) is opened
at the implant site showing biosynthetic cellulose (c) covered
by very thin internal and thick external membranes. No adhe-
sions to the cortex can be demonstrated. No comparative fascia implant was included in this group of animals. Upper
Right: Group I, Subgroup B, postoperative Day 90. Photo-
graph taken at the removal of the epidural protective layer of

membrane and the clean appearance of the epidural space (es). Lower: Group I, Subgroup B, postoperative Day 90. Smooth surface of the epidural space after removal of the epidurally placed

implant (C). This apparent reduction in epidural scarring

 placed (F) and the milky appearance of the site of cellulose
(D = dura) could not be demonstrated microscopically.

Histiocytes and collagen tissue invaded the cellulose up
to Day 30, disrupting its structure and fragmenting its fil-
aments while accompanied by a low cell reaction (Fig. 3 lower). Lymphocytic–mononuclear histiocytic prolifera-
tion was the reaction usually encountered at the suture
sites, mainly during the first 6 months.

Polarized light proved very useful in demonstrating
changes in the structure of the cellulose. As a material that
is refringent to polarized light, cellulose has a typical mi-
croscopic appearance with compact light-refringent fila-
ments. When implanted and attacked by connective tissue,
the filaments loosen and separate from one another and
the cellulose’s structure becomes evanescent on direct and
polarized light. The thickness of the microscopic fibers
diminished with time as did the low cellular reaction
(Fig. 4).

The biological behavior of this membrane was analyzed
by measuring five different sites of cellulose and newly
formed external membrane on the microscopic prepara-
tions. The values were submitted to statistical analysis.

The data showed a statistically significant increase in thickness in both cellulose and neodura after 30 days, with a slower decrease in thickness between 30 and 270 days \( (p < 0.05) \).

In Group III animals no microscopic difference was observed in the connective reaction with and without epidural coverage. This microscopic result contradicts the macroscopic report: when observed with the naked eye, the animals that received epidural protection with cellulose showed diminution of the epidural fibrosis.

**Discussion**

**Regeneration of Dura Mater**

The dura mater evolves from the embryonal mesenchyma, which differentiates, forming the scalp, bones, dura mater, arachnoid, and pia mater. Fibroblasts are the most important cells of the dura mater. Various authors have studied the regeneration of this membrane.\(^44,62,92,102\)

The first concept espoused was as follows: if a traumatic or tumoral dural defect exists and the arachnoid is intact, the contact between local blood clot, arachnoid, and muscle elicits a cellular reaction, mainly on the part of fibroblasts and histiocytes, which regenerates the membrane.\(^92\) If the injury is adjacent to soft tissue (muscle, fascia, or scalp) a neodura is formed in approximately 7 days, even without duraplasty. The stability and high differentiation of pia-arachnoid epithelium is the reason for the lack of meningocerebral adhesions during the fibrotic step of dural healing.\(^32,92\) If the arachnoid is damaged, a biological process consisting of fibrotic–glial reaction begins 6 hours after injury. Originating from the dural edges and muscle, the fibroblasts adhere closely to fibrils and form firm adhesions due to neovascular proliferation.\(^35,80\) Based on his experiments, Keener\(^52\) concluded that only fibroblasts that originate from soft tissues (fascia, muscle, or epidural and subcutaneous spaces) regenerate the dura; when defects are adjacent to bone, dural healing is inadequate.\(^53\)
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Historical Aspects of Duraplasty

The first description of dural substitution has been attributed to Beach in 1890, who used gold foil to protect the brain of a head-injured patient with remittent epilepsy. Since Beach's description, more than 40 different types of materials reportedly have been used for that purpose. Several authors have reviewed this matter and arrived at conflicting findings. Their main goal was to find the ideal dural substitute. This ideal substance must be inert, nontoxic, noncarcinogenic, and impermeable to fluids; it must be able to hold sutures and not cause meningeval adhesions or increase infections; it must be easily handled and sterilized; and it must be inexpensive.

During the first three decades of this century, metal foils were primarily used, being advocated mainly for the treatment of corticomeningeal adhesions. The use of gold, silver, platinum, aluminum, nickel, stainless steel, and tantalum as foils for cerebral protection was discredited by the works of Chao and colleagues and Delarue, et al. These authors demonstrated encapsulation and intense cerebromeningeal adhesions that surpassed some good results when the metals were implanted.

The use of elaborate animal membranes began in 1898 and is still advocated by some surgeons. Egg (vitelline), 42,84 cargile, 80,84 and allantoic membrane, 32,34 catgut, 25,28,33 and sheep peritoneum 32 were abandoned mainly because of accentuated inflammatory reactions, corticomeningeal adhesions, and late absorption.

The first description of dural substitution has been attributed to Sacchi in 1893. This surgeon performed a round craniotomy, leaving the galea adherent to the bone flap; he removed a round fragment of damaged dura, turned the bone flap upside down, and without sutures, replaced the dural defect with the adherent galea. Since 1909 fascia lata and fascia temporals have been used with low complication rates.

The first autologous membrane implantation, reported by Caporale and De Bernardis, was attributed to Sacchi in 1893. This surgeon performed a round craniotomy, leaving the galea adherent to the bone flap; he removed a round fragment of damaged dura, turned the bone flap upside down, and without sutures, replaced the dural defect with the adherent galea. Since 1909 fascia lata and fascia temporals have been used with low complication rates.

The extremely accurate and elegant study by Penfield in 1924 and a later one by Glaser and Thienes promote fascia as a well-accepted implant. It induces an initial giant-cell reaction and phagocytosis, which resolve over time. After 1 year a total disappearance of fascia tissue is detected. When the brain is experimentally damaged, very thin and easily removable adhesions are formed. Favorable biological behavior and low infection rates justify the broad acceptance of fascia among neurosurgeons.

In the past, fat was used for nerve adhesion, cerebral protection, and duraplasty. The works of Keller and associates, which compared the effects of various synthetic materials with that of fat, demonstrated the small number of side effects that accompany use of this tissue as a dural substitute. Despite these authors' eloquent experimental and clinical demonstration, very few neurological units use fat for duraplasty.

During the 1950s Sharkey, et al., proposed the use of lyophilized cadaveric dura mater and Rosomoff ensured its routine use in most neurological clinics. Abbott and Dupree reviewed 170 cases of lyophilized dura transplanted between 1953 and 1969 and found 100% implant adaptation by clean surgery, 95% by potentially contaminated operations, and 75% by infected operations. Cantore and coworkers reported no adhesions to brain or medulla when transplanted lyophilized dura was sterilized by gamma rays and stored in alcohol. Recently the presence of acquired Creutzfeldt–Jakob disease has been reported in previously noninfected patients less than 1 year after they underwent lyophilized dura implantation.

A variety of synthetic materials has been advocated for duraplasty. Celluloid and cellophane, polynylene film, and polyvinyl sponge were experimentally tested, with the clinical use of polyethylene demonstrating good early results. Microscopically, it was determined that this substance is enveloped by internal and external connective tissue membranes and that there is thickening of the arachnoid without cortical abnormality. Other synthetic derivatives, such as Orlon and Vinyon N, cause fibrotic reactions and adhesions when the cortex is experimentally injured. Teflon was found to cause dense adhesions and was very soon abandoned.

Medical silicones were initially used in neurosurgery in 1967 and became very popular among neurosurgeons. Their use was indicated for duraplasty, repair of cerebrospinal fluid fistula, enlarging of the cervical canal after surgery for multiple cervical discs, reconstruction of the dural canal in cases of myelomeningocele, cerebral protection in brain surgery when reoperation was anticipated, and prevention of hypertrophic epidural healing after laminectomy. The enthusiasm for medical silicones vanished after descriptions of late hemorrhagic and inflammatory complications resulting from use of this material.

Absorbable and nonabsorbable mesh, such as polyglactin 910 (Vicryl) and Mersilene, were found to cause low complication rates but are not currently used. Polyurethane derivatives recently have been reported to prevent cerebral and epidural adhesions when tested experimentally.

Natural and semisynthetic substances have been used in cerebral protection since the end of the last century. In 1895, Abbe used rubber tissue implants in cases of meningocebral adhesions and epilepsy, which caused dense meningeocortical reactions. Human fibrinogen, fibrin, and Gelfoam films were experimentally tested and recommended for duraplasty. LaRocca and MacNab advocated epidural use of Gelfoam films to prevent epidural scarring after laminectomy. However, Kiviiluoto demonstrated that fat is superior as a preventative method for epidural scarring. Resorbable collagen membranes have also been used for duraplasty; they display low complication rates without the formation of neo-membranes, allowing the fibroblasts to use the collagen tissue as a scaffold for their proliferation inside the pores of the graft.

Cellulose as a Substitute

Putnam and Frantz mixed oxidized cellulose with thrombin and used it experimentally as a brain implant and dural substitute. After 6 weeks they found total reabsorption and minimal glial reaction without meningocele-
bral adhesions. These results have been confirmed by Ingraham and colleagues in experiments using monkeys. Oxidized cellulose, industrially treated, is currently used daily by neurosurgeons. We were unable to locate any additional comments on the neurosurgical use of cellulose in the literature, besides those already cited.

The decision to test biosynthetic cellulose for duraplasty was based on the absence of adverse reactions in response to exposure of skin, oral gum, and hepatic tissue to this substance. Fascia was chosen as a comparison tissue because of its broad neurosurgical usage, despite inadequate experimental demonstrations of its effectiveness. Other comparative implants, such as lyophilized dura mater and other "artificial" dura implants, were not used because of technical and economic reasons.

Clinical observation of the animals in this study during the postoperative period revealed good wound healing with an absence of convulsions or behavior disturbances. Macroscopic examination of the specimens was intriguing. There was an evident diminution of epidural scarring when cellulose film was applied extradurally to the Subgroup B animals, which were observed for 90 days. This scar formation, which was observed in Subgroups A and C dogs, which did not receive epidural cellulose protection, could be explained by contact of the temporalis muscle with the dura mater. Other authors have demonstrated the antifibrotic effects of fat,54,55,66 Gelfoam,82,93 silicone,57,61 and polyurethane derivatives.91 On macroscopic examination of the Subgroup B animals, we inferred an antifibrotic effect of cellulose. However, on microscopic examination of Group III animals, we did not find consistent results. Further models must be promoted for this demonstration.

The microscopic appearance of the grafts in our study was very similar to those reported by other authors using implants of other materials.18,21,40,69,85 The synthetic substance is enveloped by two connective membranes and some cellular reaction is evident, mainly at the suture sites. Predominant fibrotic reactions at the suture sites have been recognized since early papers on this subject, however, not suturing the grafts could cause cerebrospinal fluid fistula. The remarkable findings of our experiment were: the low foreign body reactions, the decrease of cellulose and neodural tissue thickness, and the absence of cortical adhesion even in the presence of damage. The partial disappearance of cellulose, without foreign body reactions or phagocytosis, was presumably due to dilution in organic alkalis, as Frantz had already estimated.41 There was no difference in biological behavior between cellulose and fascia when the cortex was intact. The abovementioned findings, added to cellulose’s physical properties, malleability and distensibility, qualify it as a suitable substitute for the dura mater. Additional long-term studies of this material must be performed to clarify some issues.

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