Scientific Documentation

Lyoplant®

For the replacement of connective tissue structures in neurosurgery

Editor:
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Research and Development

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Lyoplant®

Introduction

A water-tight dura seal is a basic principle of cranial neurosurgery. If it is not possible to produce a watertight seal by suturing it becomes necessary to use replacement materials. The surgeon would certainly use autologous tissue for preference, but this is frequently impossible so that then alternatives become necessary.

The ideal replacement material for covering these defects is biocompatible and as it is degraded it should be replaced by endogenous connective tissue, that is as similar as possible to the structures of the dura mater. The replacement material also requires to be generally available, capable of being modelled into shape and yielding a cerebral spinal fluidtight closure. A new biological material produced from bovine pericardium, Lyoplant®, has been available for this purpose since the beginning of 1994.

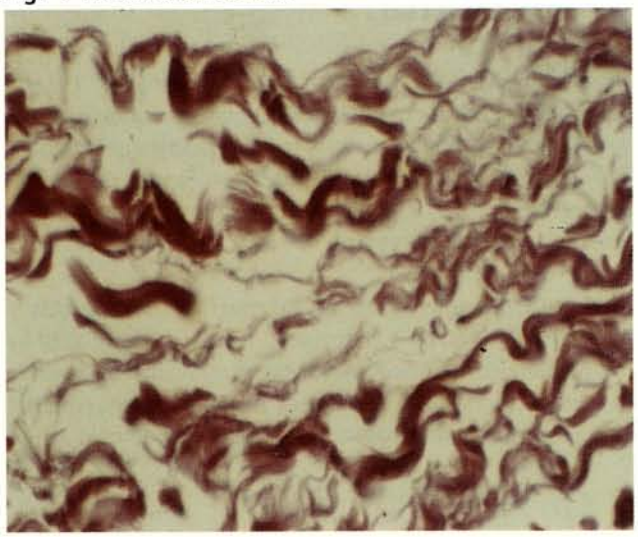
The suitability of Lyoplant® as a dura replacement has been confirmed in animal experiments and clinical tests. Histological investigations have revealed that, on replacement of Lyoplant® with endogenous connective tissue, trajectory-like rows of fibres are produced which are very similar to the normal tissue.

Characterization of the Medical Product

Lyoplant® is a pure collagen implant obtained from bovine pericardium. The special preparation procedure frees Lyoplant® from noncollagenous components, e.g. enzymes, lipids and noncollagenous proteins.

The gentle freeze-drying process ensures maintenance of the loose fibrous structure of Lyoplant® which provides optimal healing conditions after implantation.

Fig. 1 Micro-architecture



Lyoplant® made of bovine pericardium, is a single-layered membrane of connective tissue with the collagen fibres running in three dimensions. Lyophilization causes formation of a mesh with large communicating pores (220 x).

Production

The raw material (bovine pericardium) is subjected to a multi-step mechanical and chemical preparation process:

Bovine pericardium	
↓	
Mechanical treatment and cleansing	→ Removal of adhering fatty and connective tissue
↓	
Chemical treatment	→ Inactivation of enzymes and potential pathogens
↓	
Lyophilization	→ Freeze drying
↓	
Cutting to size	→ Cutting into various sizes
↓	
Packaging	→ Sealing in double peel packs
↓	
Gas-sterilizaton	
↓	
LYOPLANT®	

Sterilization

Lyoplant® is sterilized with ethylene oxide.
The sterilization process is so arranged that the residual quantity of gas is always below the limit of I ppm required by the German Federal Health Authority.

Safety with respect to transmission of zoo-anthroponoses

Lyoplant® is produced from bovine pericardium. In order to reduce the possible risk of a transmission of BSE (bovine spongiform encephalopathy) the following measures are taken:

1. Pericardium is a tissue that is regarded as safe
2. New Zealand, which is BSE free, is the country of origin of the pericardium
3. Lyoplant® is treated with caustic soda solution

Toxicology/Function Testing

Toxicological Test

Bearing in mind that it is a biological (xenogenous I) material produced from bovine pericardium the toxicity of Lyoplant® has been investigated in the following toxicological tests:

- Implantation test in the rabbit
- Cytotoxicity test
- Local intracutaneous compatibility test (intracutaneous test) in the rabbit
- Acute systemic toxicity test in the mouse
- Sensitization test in the guinea pig
- Haemolysis test on human erythrocytes

Summary of the results
Lyoplant® fulfils the criteria for nontoxicity in the aforementioned toxicological tests.

Testing in animals

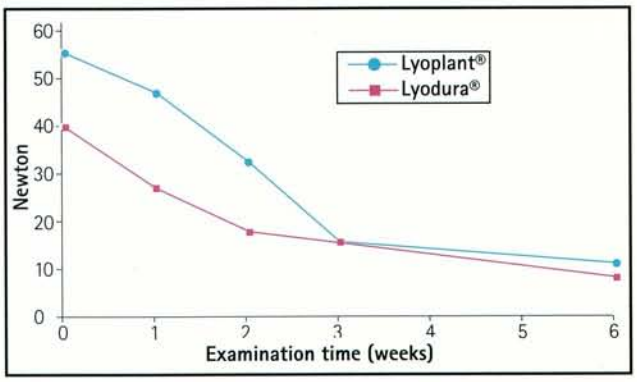
Testing Lyoplant® for biodegradation/ tear strength measurement and biocompatibility (rats)

Purpose of the test: The study involved the comparative testing of the tissue reaction and the reduction in tear strength of gas-sterilized Lyoplant® after s.c. implantation in rats, up to six weeks after implantation. The reference material was Lyodura®.

Investigations were made of:

- local inflammation reactions (erythema, oedema)
- cell reaction (granulocytes, macrophages, fibroblasts)
- macroscopic and microscopic degradation behaviour
- tear strength measurements (TSM)
- histology

Diagram: Tear strength measurement



Evaluation and discussion
The degradation behaviour of Lyoplant® is very similar to that of Lyodura®. The loss of strength at various times only differs at the start. From the third week on, the results in this investigation were comparable. The Lyoplant® samples exhibited neither immune nor inflammation reactions. There were no foreign body reactions. Eosinophile granulocytes were not observed.

Functional testing (dog) and tear strength measurement

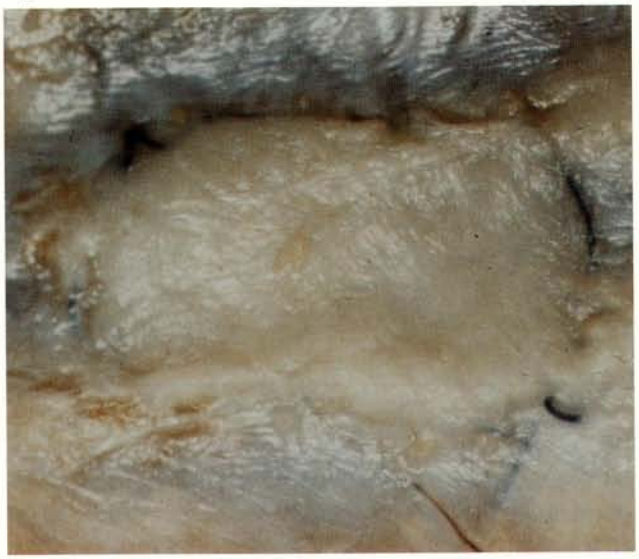
Purpose of testing
The study involved testing the tear strength (TSM) of and the tissue reaction to gas-sterilized Lyoplant® after functional implantation in dogs as dura replacement.

The individual investigations were:

- function (sealing)
- local inflammation reaction (erythema, oedema)
- tear strength
- histology

Results
The head wounds healed without complication. There were no infections, fistula formations or implant colliquations. Disturbances of the general condition were not observed. Irritation of the central nervous system, in particular a tendency to spasms, was not observed. The animals were sacrificed after 1, 3, 7 and 12 months.

Fig. 2:



The implanted Lyoplant® sample has also integrated without irritation, with an almost continuous junction with dura. Most of the sutures are likewise masked by a neomembrane. (IT 1 year).

On post mortem examination it was found that the head wounds had healed without irritation, with inconspicuous scars and little suture residue. After 1 month a few implants were still inflamed and red in places (= cell infiltration), after 3 months they were virtually nonirritated.

Lyoplant® and Lyodura® together with the endogenous dura mater in the area of the trepanation window, were firmly united with the underside of the M. temporalis. Both 1 month and 3, 7 and 12 months after implantation Lyoplant® was so well integrated that it was scarcely distinguishable from autochthonous dura. Immune reactions were not observed. Adhesions with the surface of the brain that might have been caused by the implant have not been observed at any time. At no time was calcification observed.

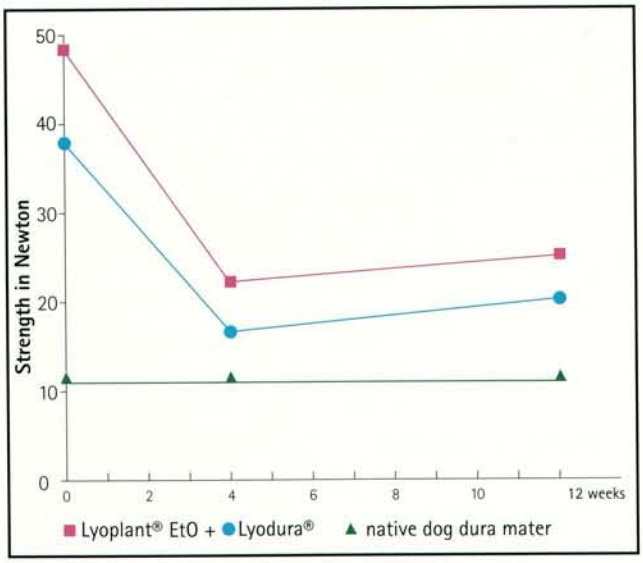
Measurement of tear strength (TSM)

The initial tear strength (0 value) of Lyoplant® was almost 30 % higher than that of Lyodura® implants. After one month all tear strengths approached those of native dog dura mater, when the Lyodura® and Lyoplant® implants did not differ from each other.

After 3 months a slight increase could be observed again in the strength of 21.5% for Lyoplant® and of 25.5% for Lyodura®.

Fig. 5:

(Functional implantation in dogs)



Diminution in tear strength of various bio-implants

Discussion and summary

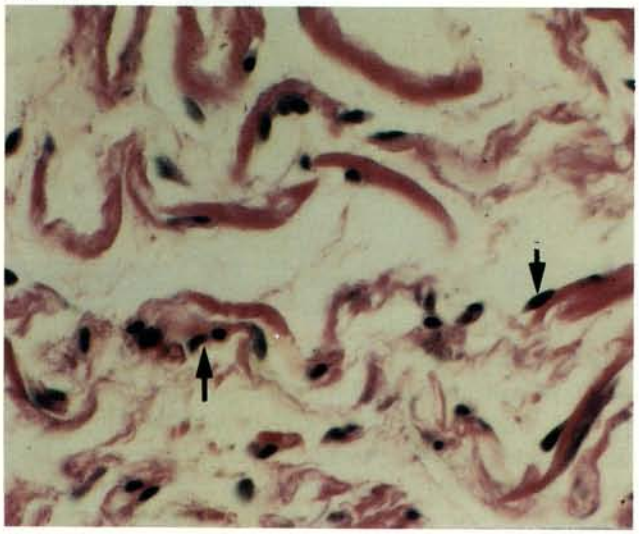
All Lyoplant® implants organized without complication. The macroscopic and microscopic inflammation reactions were very slight. Immune or foreign body reactions were not observed.

The strength behaviour of the implants is of interest. It appears that, no matter what the initial strength, after I month the tear strengths of all implants approach very closely to that of the native dura mater of the recipient.

Histological findings

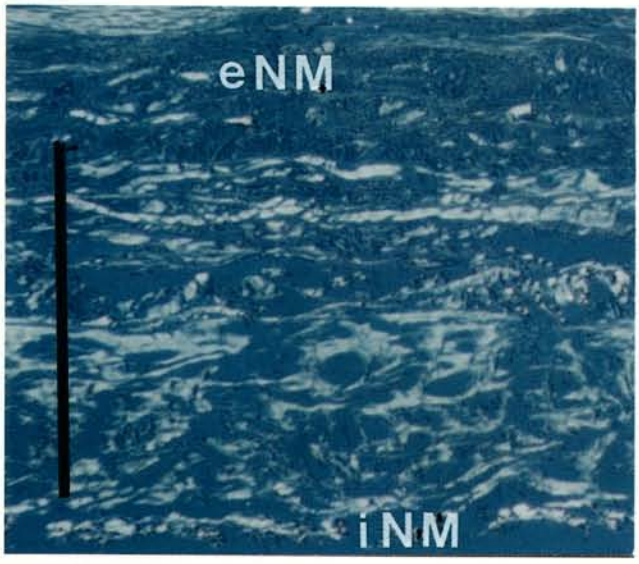
Vitality and organization of Lyoplant®

Fig. 3a:



The loosely-structured but relatively thin Lyoplant® is revitalized within 3-6 weeks. The fibrocytes that have immigrated (arrows), however, do not produce collagen at first, but contribute to the intermediary metabolism of the implanted fibres at a molecular level. (IT 6 weeks, 360 x).

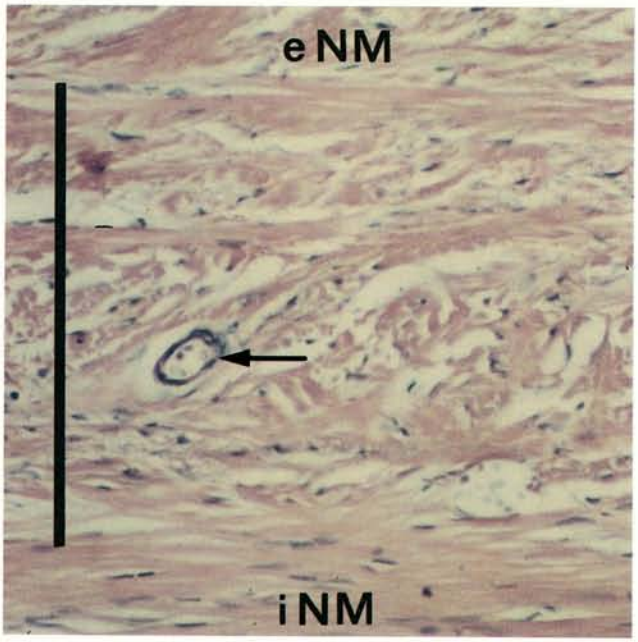
Fig. 3b:



Here, too, polarized light shows preservation of the micro-architecture of the implanted Lyoplant® (bar). The inner neomembrane (iNM) is very thin, the outer one (eNM) is easily distinguishable from the muscle fascia (F). (IT 6 months, 55 x).

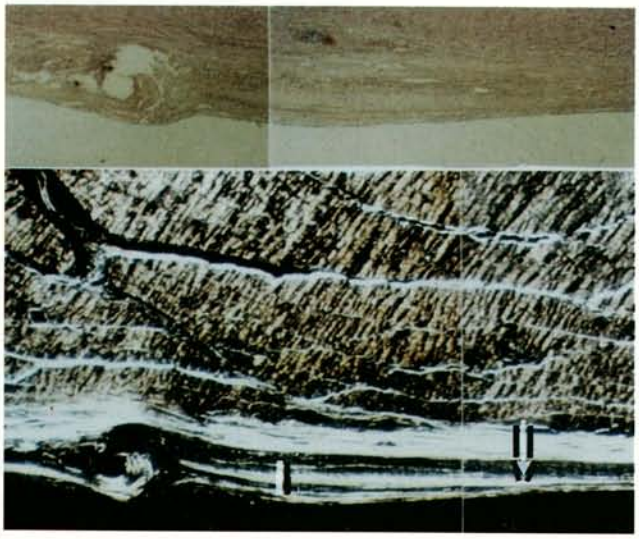
Tissue compatibility and biostability of Lyoplant®

Fig. 4a:



The Lyoplant® (bar) is fully integrated between the neomembranes (iNM). Capillaries growing into it (arrow), support the implant with nutrients. Replacement of the implanted tissue with the recipient's own collagen may occur. (IT 1 year, 130 x).

Fig. 4b:



The Lyoplant® implant (I) is also fully integrated as a thin layer of connective tissue and has fused to form a single entity with the neomembranes and the muscle fascia. Since one layer of the collagen fibres runs crossways, there is no birefringence under polarized light. (arrow, lower picture. Upper picture under normal light). (IT 18 months, 15 x).

Clinical Investigations

Introduction

The aim of this study has been to check how far Lyoplant® can be employed in neurosurgical head operations and in the spinal region. The target criteria were handling properties during the operation, immediate postoperative complications and long-term toleration.

Patients

A comparative and randomised study of Lyoplant® and Lyodura® was carried out. The present interim evaluation includes a total of 116 patients (59 Lyoplant® 57 Lyodura®, whereby 47% of the cases were male and 53% female. The average age of the patients was 54 years (range 24-82 years). In order to provide evidence concerning late complications and to be able to evaluate a constant patient population only those patients, whose operations were at least 6 months prior to the deadline for the interim evaluation, were included.

Results

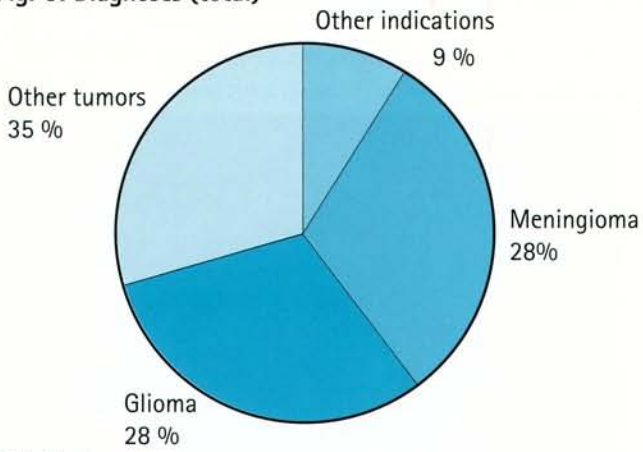
Indications

Lyoplant® and Lyodura® were primarily employed for tumours (n = 107) and also for a further 9 indications, such as aneurysm and other vascular malformations, for a cerebrospinal fluid fistula, a trigeminal neuralgia and a hydrocephalus. Figure 6 shows the distribution of the indications. Total removal was possible in 58% of the 107 tumours.

Operation time

Major interventions were involved, as is shown by the mean operation time of 300 minutes (120-900 minutes).

Fig. 6: Diagnoses (total)



Risk factors

In all, 60 patients entered the study with one or more risk factors. The most important risks such as age (26%), overweight (24%), hypertension (10%), cardiac infarct (3%) were approximately equally represented in the two treatment groups. The distribution of the risk factors between the two groups of patients was very nearly balanced with 49% amongst the Lyoplant® patients and 56% amongst the Lyodura®-treated cases.

Handling and safety of Lyoplant®

The suturing, cutting and modelling properties, thickness, stiffness, sealing properties against cerebrospinal fluid and the suture tear out strength were all scored by the operator on a polytomous or dichotomous scale. The results are presented in Table 1. It can be seen that the handling properties of Lyoplant® were scored more favourably by the operators than those of the reference material Lyodura®. The safety parameters cerebrospinal fluid seal and suture tear out strength were reported in 100% of the cases for both implants.

Table 1: Evaluation of the implant

	poor		moderate		good	
	Lyoplant®	Lyodura®	Lyoplant®	Lyodura®	Lyoplant®	Lyodura®
Cutting properties	–	4%	–	49%	100%	47%
Modelling propert.	–	44%	–	49%	100%	7%
Suturing propert.	–	2%	5%	54%	95%	44%
Thickness	–	5%	8%	58%	92%	37%
Stiffness	–	10%	2%	74%	98%	16%

Intra-operative complications

There were no intra-operative complications with 96% of the patients. In 4% of the interventions there were complications such as haemorrhage, cerebral inflammation and dura laceration. These were operation-dependent complications (3 Lyoplant®, 2 Lyodura®) no association with the use of Lyoplant® or of Lyodura® was detectable in any of the cases.

Postoperative complications

There was healing primarily without infection or wound dehiscence after 97% of the interventions. Infections in the region of the operation only occurred in one case (Lyoplant®); disturbances of wound healing were not observed. This infection does not have to be attributed to the patch, this also applies to the remaining postoperative complications, such as postoperational haemorrhage, haemotoma and cerebrospinal fluid fistula, which are listed in Table 2. These are operationdependent complications and are known to occur at this frequency after neurosurgical intervention.

Of the total of three infections observed, 1 (Lyoplant®) was a specific neurological infection in the region of the operation - known for this type of intervention. In two cases (Lyodura®) there was a general infection (respiratory tract infection). All infections were amenable to treatment with antibiotics. It was possible to exclude a causal connection with the implant in every case.

Table 2: Postoperative complications

Type of complication	Number (n)		Proportion (%)	
	Lyoplant®	Lyodura®	Lyoplant®	Lyodura®
Wound dehiscence	–	–	–	–
Infection	1	2	12%	33%
Haematoma	3	1	38%	17%
Cerebral inflammation	–	–	–	–
Others	4	3	50%	50%
Total	**8**	**6**	**100 %**	**100 %**

List of other:

Lyoplant®: cerebrospinal fluid fistula, cerebral attacks, subcut. cerebrospinal fluid cushion

Lyodura®: cerebrospinal fluid fistula, A. c. media infarct

Postoperative examinations after 2, 6 and 12 months

During the period between discharge and postoperative examination after 2 months only one complication was found in the form of an infection originating in the osseous cap over the Lyoplant® implant. In the following investigation periods (2-6 months) 1 infection (Lyoplant®), 1 haematoma and 1 osteomyelitis in the Lyodura® group were observed. One Lyodura® patient died in the period of postoperational observation from 6-12 months as a result of a hypostatic pulmonary infection.None of the complications were related causally to the implants.

Re-intervention

A total of 19 re-interventions (Tables 3) was carried out, with 13 patients being re-operated once and 2 repeat operations in the case of 3 patients. Of these 10 re-interventions were carried out prior to discharge. There were 4 re-interventions before the first postoperative examiniation after 2 months, a further 3 reoperations were carried out before the next postoperative examination after 6 months and 2 re-operations were carried out between then and the examination 12 months after the first operation. None of these re-interventions was causally connected with the implant.

Table 3: re-operations

Reason for re-operation	1. Re-operation Number (n)		2. Re-operation Number (n)	
	Lyoplant®	Lyodura®	Lyoplant®	Lyodura®
Recurrence of tumour	4	3	–	–
Haematoma	3	2	–	–
Infection	1	-	1	–
Other	-	3	–	2
Total	8	8	1	2

Other: (1. Re-operation)

Lyodura®: cerebrospinal fluid fistula, removal of tumour, A. c. media infarct

Other: (2. Re-operation)

Lyodura®: cerebrospinal fluid fistula, removal of tumour, A. c. media infarct

Death

By the end of the postoperative investigation period the trial centre had reported the deaths of 16 patients. The causes of death of these patients are listed in Table 4. In no case was acausal relationship with the trial material established.

Table 4: Cause of death

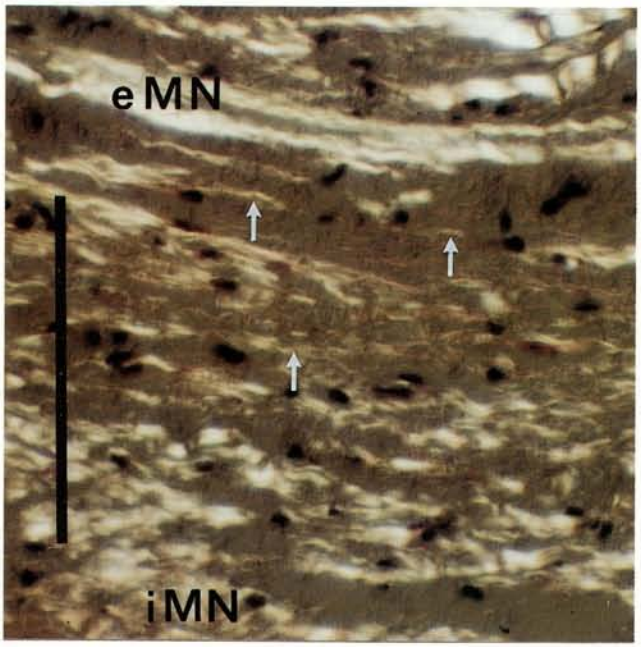
Cause of death	Number(n)		Proportion(%)	
	Lyoplant®	Lyodura®	Lyoplant®	Lyodura®
Progression of primary disorder	2	8	40%	73%
Pulmonary embolism	1	–	20 %	–
Cerebral/cardio-resp. collapse	1	1	20%	9%
Tumour of other manifestation	–	1	–	9%
Septic shock	1	–	20%	–
Other	–	1	–	9%
Total	5	11	100%	100%

Discussion

Numerous materials have been employed for the plastic replacement of dura since the first duraplasty carried out with exogenous tissue by Abbé in 1895. These include in addition to endogenous tissue, whose use should always be preferred, the use of lyophilised dura (homologous) or the use of xenogenous material. As the present study demonstrated Lyoplant® can be recommended without restriction for the replacement of dura; this is particularly the case in view of its handling properties. Product-specific complications were not observed in this investigation. Thus, specifically, there were neither early nor late infections in association with the product. The number of complications such as "inflammation" or "cerebrospinal fluid fistula" was conspicuously low, so that Lyoplant® can be recommended for all neurosurgery, except for application in already infected regions or when there is potential contamination of the region operated (open cerebrocranial trauma), where foreign material should, in general, be avoided, if possible. The results obtained in this clinical trial do not suggest any further contra-indications for the use of Lyoplant® as dura replacement material.

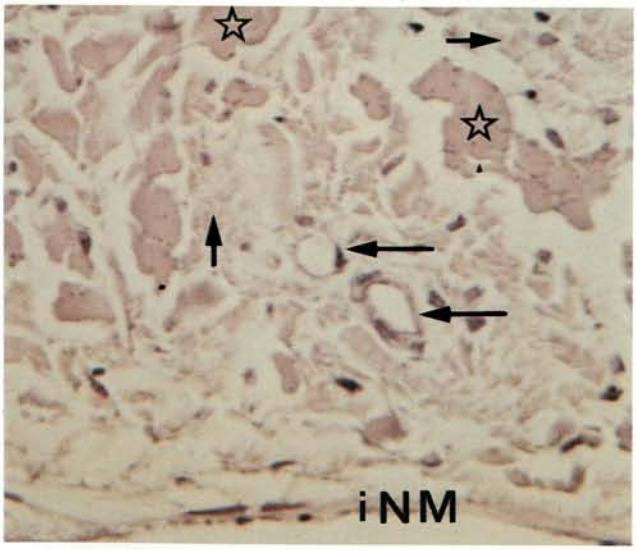
Histological findings

Fig. 7a:



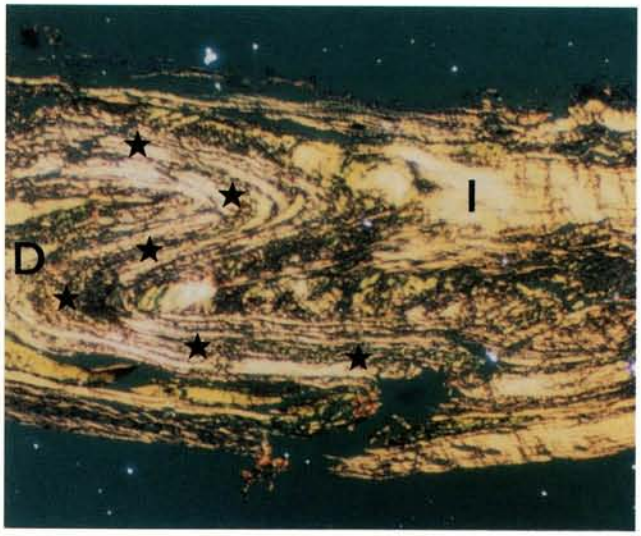
The Lyoplant® implant (bar) is fully vital and has grown together with the inner and outer neomembranes (iNM, eNM). Fine fibrils (arrows) indicate the neoformation of collagen. (IT 6 months, 135 x).

Fig. 7b:



Eight months after operation the fibroblasts that had immigrated have produced new collagen (short arrows) between the fibre bundles of the Lyoplant® (stars). Enzymatically degraded implant collagen can be replaced by the recipient's own tissue because of the maintenance of a loose structure and ingrowing capillaries (long arrows). The noemembranes are exceptionally thin. (IT 8 months, 220 x)

Fig. 7c:



After an implantation time of 10 months, the Lyoplant® (I) has been so remodelled that it can no longer be distiniguished from dura (D). (Stars mark the suture line). (Polarized light, IT months, 35 x).