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Fibrin Sealant in Operative Medicine

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Preface

Fibrin plays a prominent role in wound healing. It has a hemostatic effect, induces cellular response to wound damage, and, by forming strands to build a matrix, assists in neovascularization and fibroblast proliferation.

The concept of using clotting substances from human blood for wound management and to achieve hemostasis in bleeding parenchymatous organs can be traced to 1909, when Bergel [1] reported on the hemostatic effect of fibrin powder. In 1915, Grey [3] employed fibrin to control bleeding in neurosurgical operations of the brain. A year later, Harvey [4] used fibrin patches to stop bleeding from parenchymatous organs in general surgery.

It took more than two decades for this ingenious idea to be rediscovered. In 1940, Young and Medawar [8] reported on experimental nerve anastomosis by sealing. Similarly, Tarlov and Benjamin [7] reunited nerves with plasma clots in 1943. Tarlov improved the results obtained with clot anastomosing of nerves by avoiding tension at the nerve stumps. In 1944, Cronkite et al. [2] reported on an initial series of eight cases in which fibrinogen and thrombin had been used successfully for anchoring skin grafts.

Although these early attempts suggested the basic advantages of using a biomaterial for wound closure – such as complete absorption, improved wound healing, and excellent tissue tolerance – the failure rate was relatively high, mainly because the fibrinogen employed had poor adhesive strength and the sealing did not last. It was because of these unsatisfactory results that the technique was not further pursued in the decades to follow.

In 1972, the use of fibrin as a biologic adhesive was revived by Matras et al. [6], who successfully employed a fibrinogen cryoprecipitate for reuniting peripheral nerves in an animal model. Matras and Kuderna used autologous material in the first successful human application in 1975 [5]. It was not until a special cryoprecipitation process had been developed that it was possible to produce a highly concentrated fibrinogen solution with an enriched factor XIII content, as the basis of two-component fibrin sealant.

In the meantime, the controversial issue of virus transmission, including the transmission of HTLV-III, by the blood product Tisseel (Tissucol) has been resolved. In addition to subjecting Tisseel (Tissucol) to in-process virus inactivation, both the source material and final product are routinely screened for HTLV-III antibody.

Following the first international symposium on fibrin sealant in Vienna in 1985, which dealt with the use of the product in various surgical disciplines, this seven-

volume study attempts to present current knowledge relating to the method of fibrin sealing. The disciplines covered are: general and abdominal surgery; ophthalmology and neurosurgery; otorhinolaryngology; plastic, maxillofacial and dental surgery; thoracic and cardiovascular surgery; traumatology and orthopaedics; urology, gynaecology and obstetrics. Each volume is preceded by a general chapter on the principles of fibrin sealing, methods of application, aspects of quality control, and safety studies.

Today, fibrin sealing has become an accepted tool in many fields of surgery. In many areas, fibrin sealing has superseded conventional surgical techniques, increased postoperative safety, and even made new therapeutic approaches possible.

We would like to thank all authors for their excellent contributions and helpful photographs, which have made these seven volumes on fibrin sealing possible.

Vienna, Juni 1986

G. Schlag
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Table of Contents

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I. Principles of Fibrin Sealing

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The Importance of Fibrin in Wound Repair

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Key words: wound healing, fibrin, macrophages, granulocytes

Abstract

A review is given, beginning with the inflammatory phase of wound healing and explaining the role of macrophages, platelets, and granulocytes. Beside the cellular response the special importance of fibrin and factor XIII is demonstrated, particularly their function for fibroplasia. Special emphasis is put on the effect of highly concentrated fibrin – fibrin sealant. Its beneficial role in promoting the growth of fibroblasts is shown by a study on rats, in which a new model of granulation tissue formation was used. With this model it can be demonstrated that the application of fibrin sealant leads to significantly higher amounts of fibroblasts in newly formed granulation tissue. However, it is also demonstrated that fibrin sealant cannot overcome the inhibition of wound healing caused by, for example, adriamycin, though the beneficial effect of fibrin sealant in other cases of disturbed wound healing, e.g., ulcus cruris, has been demonstrated previously.

General Aspects

Three phases of wound healing are seen following trauma:
- Inflammatory phase
- Fibroplasia
- Protective maturation phase

Tissue trauma is immediately followed by coagulation and hemostasis. Coagulation eventually leads to conversion of fibrinogen into fibrin via the humoral pathway under the influence of thrombin and calcium (Fig. 1).

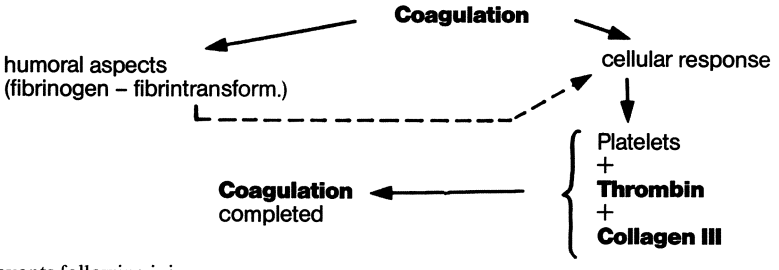

Fig. 1. Primary events following injury

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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During the coagulation process, a cellular response is seen. Together with thrombin and collagen III, the platelets complete coagulation. Adhesion of the platelets to collagen fibrils of type III [3, 20] leads to platelet aggregation, where the platelets change from a reversible into an irreversible form. 5-Hydroxy-tryptamine and epinephrine are released from the platelets, which undergo further aggregation. Other substances are released from platelets, like platelet factor III, which acts on the formation of thrombin. Platelets are also important for the fibrin network structure, since they make fibrin more resistant to mechanical shear forces and to fibrinolysis [13].

The coagulation activated via humoral as well as cellular pathways leads to the blood clot which acts as a sealant primarily because of its fibrin content. In this way, normal hemostatic mechanisms help to prevent contamination and loss of body fluids as well as providing a substrate material for cell growth [2].

Fibrin is essential since it causes chemotaxis [24] of PMNs (in vitro) in the presence of fibrin degradation products. Fibrin mainly leads to recruitment in the injured tissue and also activates the macrophages.

Immediately after trauma and the ensuing coagulation, the inflammatory phase (lag phase) starts and extends to the 4th or 5th day. This phase is a vital part of the wound repair process. The local neutrophils (PMNs) increase within several hours. The main task of PMNs is to degradate damaged tissue (debriding) and to phagocytose cell debris. The migration of PMNs is presumably caused by chemotactic substances released from aggregated platelets or from plasma components (proteases, fibrinopeptide A). During the first 48 h the PMNs increase markedly and are quickly subject to lysis. Only a few are engaged in phagocytosis [22]. Evidence from studies using antineutrophil serum suggests that the PMNs are not essential in normal wound healing.

After some days, the most important cellular components in the inflammatory phase, i.e., the "monocytes", migrate (Fig. 2), change into macrophages, and reach their maximum number in the wound between the 4th and 5th day. The macrophages serve many different functions. According to Gustafson [15], these include regulation of coagulation (macrophage-induced procoagulant activity, factors V, VII, IX, and X) and fibrinolysis; elimination of cells, tissue debris, and bacteria; and regulation of fibroblast activity (fibroblast growth factor). Their main tasks include

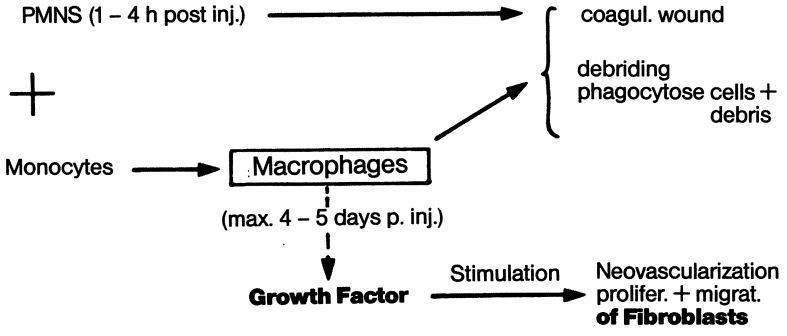

Fig. 2. Inflammatory phase of wound healing

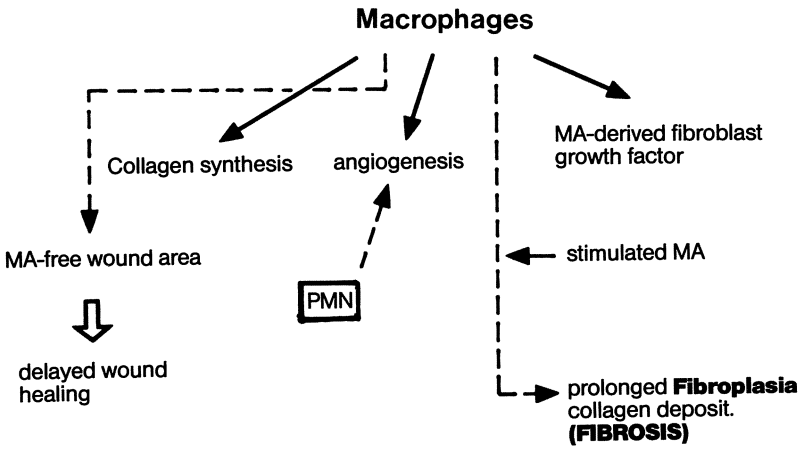

Fig. 3. The role of macrophages during the inflammatory phase of wound healing

phagocytosis of fibrin and the release of growth factors which stimulate fibroblast and endothelial cell proliferation in vitro [14, 28]. Induction of angiogenesis by wound macrophages has been confirmed [12, 21] (Fig. 3). Hunt et al [21] reported that this activity involved macrophages more than PMNs; however, a granulocyte component in the production of angiogenesis could not be excluded. Macrophages are responsible not only for neovascularization in the wound but also for stimulation of collagen synthesis. Collagen synthesis requires fibroplasia. Here, the "macrophage-derived fibroplast growth factor" apparently plays a vital part. If antimacrophage serum is administered, wound healing is severely delayed. On the other hand, prolonged activation of macrophages (endotoxin, bacterial products) may result in exaggerated fibroplasia and collagen deposition, which ends in fibrosis [21].

As to the cellular response in wound healing, the platelets in connection with fibrin play an important part [25]. Activated by thrombin, the platelets release a mitogen for fibroblasts and smooth muscle cells and stimulate collagen synthesis. This mitogen was isolated as "platelet-derived growth factor".

Fibroplasia and collagen synthesis start within 24 h following trauma. The platelets also activate neovascularization. Thus vital factors for wound healing are released by the platelets, which are largely responsible for the healing process (Fig. 4).

Wound healing is influenced by local oxygenation. Banda et al. [4] have shown that anoxia leads to stimulation and activation of the macrophages. This causes production of an angiogenesis factor and a macrophage-derived growth factor which stimulates the fibroblasts.

Knighton et al. [26] have demonstrated hypoxic stimulation of angiogenesis by macrophages in a corneal assay. Hyperoxia appears to suppress angiogenesis as shown in a second experiment with an ear chamber equipped with oxygen-perme-

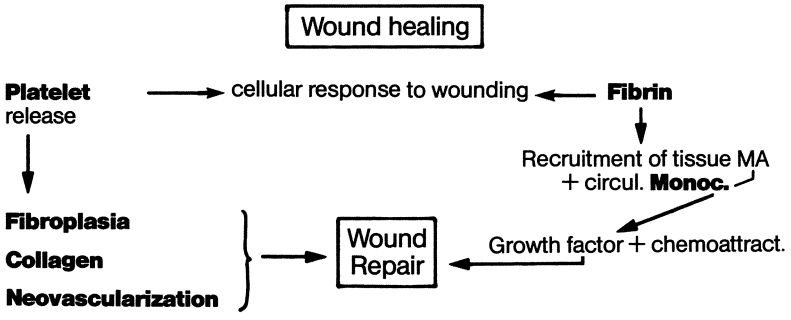

Fig. 4. The concert action of fibrin and platelets

able or -impermeable membranes. The demonstration that respiratory oxygen concentration affects the tensile strength of healing wounds and granulomas may reflect macrophage regulation of angiogenesis or fibroplasia [31, 32].

Granulation tissue plays a key role in the healing of all organs, except for those of epithelial origin. Granulation tissue largely consists of macrophages, endothelial cells, and fibroblasts [36]. The hallmark of granulation tissue is the proliferative response of fibroblasts. Proliferation is stimulated by a substance produced by macrophages (growth factor). It is thus very important that the cellular phase (inflammatory phase) is not influenced as to the quality and quantity of the cells. Macrophages are a crucial component of the initial inflammatory reaction which precedes fibroplasia. The administration of corticosteroids in experimental conditions results in significantly fewer monocytes and macrophages in the cellular infiltrate. The effect of fewer macrophages is that the accumulation of collagen – measured as hydroxyproline content – is decreased and neovascularization is inhibited [38].

Fibroblasts proliferate within the first 3 days after trauma. In connection with neovascularization, fibroblasts become the dominating cells in collagen and proteoglycans synthesis. Collagen is also lysed throughout wound repair, perhaps due to fibroblasts. Fibroblasts are responsible for the synthesis of glycosaminglycans, which surrounds the collagen network and absorbs the compressive load as a hydrated viscous gel [27, 30].

Specific Effects of Fibrin, Thrombin, and Factor XIII

Fibrin is vital in wound healing since the network formed in the wound acts both as a scaffold for migrating fibroblasts and as a hemostatic barrier [33]. This scaffold is formed by fibrin strands in connection with fibronectin. In large quantities, fibrin has an inhibitory effect on cell migration and may even delay wound healing. Fibroblasts are quickly followed by new capillaries. These are essential for the granulation tissue. The endothelial cells contain plasminogen activator, the subst-

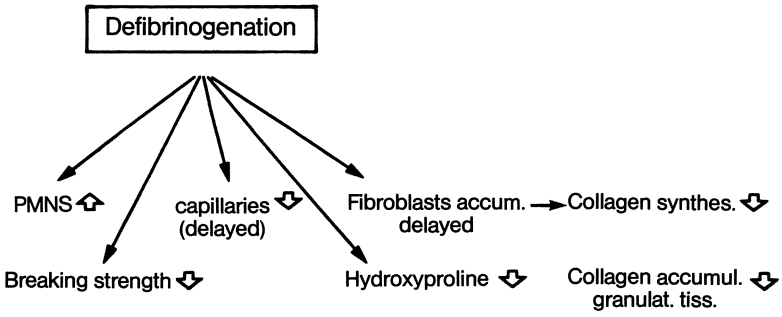

Fig. 5. Effect of defibrinogenation on the different aspects of wound healing

ance that initiates the process of fibrin removal (fibrinolysis). Banerjee and Glynn [5] have demonstrated that implanted fibrin clots are invaded by new capillaries and fibroblasts.

The importance of fibrin in wound repair was confirmed by Brändstedt et al. [7–11]. Defibrinogenation with! Arvin has been used in studies on the formation of granulation tissue (Fig. 5). Under these conditions the fibrin strands are irregular and disrupted, and the number of fibroblasts and collagen fibrils is reduced. As a result of this, a reduction of collagen accumulation in the granulation tissue has been observed. Controlled fibrin deposition appears necessary for granulation tissue formation and for normal healing.

Deposited fibrin apparently stimulates the formation of granulation tissue, including increased collagen precipitation [17]. Hydroxyproline directly reflects the collagen concentration and was significantly high in a fibrin-filled Teflon implanted cylinder [16]. Pohl et al. [34] confirmed the influence of fibrin on growing fibroblasts in vitro by showing that fibrin markedly enhances cellular growth as well as mitosis of the fibroblasts. After 10 days, the cell growth stops. The network of fibrin fibers promotes growth and multiplication of the fibroblasts. As long ago as 1960, Banerjee and Glynn [5] demonstrated that implanted fibrin clots are invaded by new capillaries and fibroblasts.

Thrombin has mitogenic characteristics in cell cultures, aside from its effects on platelet activation, such as long-lasting hormone-like influence on fibroblast proliferation [34], on transformation of factor XIII to XIIIa, on conversion of fibrinogen to fibrin, on prostaglandin production, and on activation of protein C [15]. The effect of thrombin in wound healing is manifold and is a vital part of wound repair.

Factor XIII is needed in the cross-linkage of fibrin in order to produce a stable fibrin network which provides the matrix for the ingrowing fibroblasts. The delay in wound healing in factor XIII-deficient patients may be due to lack of stimulation of fibroblast proliferation [23]. The attachment of fibroblasts is not only obtained by the fibrin matrix, but also (indeed, mainly), through the cross-linkage by activated factor XIII. Cross-linkage between fibrin fibers promotes the cellular response and thus subsequent migration and proliferation of fibroblasts. Factor XIII is also cross-linked with collagen, fibronectin, and α^2-antiplasmin [29].

Fibrin Sealant

For more than 10 years, fibrin sealant (Tissucol/Tisseel), a two-component sealant, has been widely used in surgical medicine and its disciplines.

Tissucol has a triple effect on wound healing. Due to its hemostatic effect, hematoma formation is avoided; consequently the lengthy process of absorption and possible organization of the hematoma does not take place and the rather negative influence of the hematoma on the quality of the granulation tissue is also avoided.

As far as the adhesive effect of Tissucol is concerned, critics have repeatedly pointed out its limited adhesive strength, which will not tolerate major stress exposure. It should, however, be remembered that the objective of using fibrin sealant is not confined to sealing severed tissue segments. Proper adaptation of dissociated surfaces is just as important because it ensures smooth wound healing unhampered by an artificial barrier such as is introduced with synthetic sealants.

The third effect of a fibrin sealant, at least as far as Tissucol/Tisseel is concerned, is on the physiological network structure [35]. This fibrin network is an excellent substrate for the ingrowth of fibroblasts, which will be demonstrated in the study below.

Materials and Methods

To determine the effect of Tissucol on the formation of granulation tissue we developed a spongiosa-based granulation tissue model. The model uses blocks of lyophilized Kieler spongiosa. They were decalcified with hydrochlorous acid and fixed with glutaraldehyde to cross-link the collagen structure. The blocks were then subcutaneously implanted into rats. The cavities of the spongiosa were either filled with a substance that influenced local wound healing, e.g., homologous fibrin sealant, or left empty for controls. The spongiosa blocks were removed at certain time intervals. The granulation tissue was biochemically examined after proteolytic removal from the spongiosa, e.g., to determine the DNA and hydroxyproline content. On the other hand, the granulation tissue was morphometrically evaluated following fixation and prepared for electron microscopy using standard techniques. The space filled by granulation tissue within a given time was precisely determined and the composition of the granulation tissue evaluated. With these methods, we determined the quantity of granulation tissue and the cellular (fibroblasts, capillaries) and biochemical (hydroxyproline, DNA) composition. This model seems very useful since no foreign body reaction was seen, in contrast to the reaction frequently observed after cellular sponge implantation according to Hølund [19].

A total of 72 male Wistar rats were distributed into four equally sized groups. The animals were given intramuscular anesthesia with Ketalar-Rompun, and some of them then received adriamycin (6 mg/kg body weight) before implantation of the spongiosa blocks. All animals underwent paravertebral implantation of two sterile spongiosa blocks with or without fibrin sealant under the dorsal skin. The four groups were thus as follows:

Groups O (F + A): Implantation of spongiosa blocks soaked with fibrin sealant with systemic application of adriamycin.

Group 1 (F): Implantation of spongiosa blocks soaked with fibrin sealant without systemic application of adriamycin.

Group 2 (A): Implantation of spongiosa blocks with systemic application of adriamycin, without fibrin sealant.

Group 3 (CO): Implantation of spongiosa blocks without further systemic or local treatment (control group).

The animals were killed on the 7th or 14th postoperative day.

Results and Discussion

We found a significant fibroblast-stimulating effect of the sealant (16% fibroblasts per volume granulation tissue in controls, 22% in the fibrin sealant group without adriamycin). As opposed to this, the inhibitory effect of the cytostatic agent adriamycin on the formation of granulation tissue was not improved by the sealant (11% without and 10% with sealant). As a cytotoxic chemotherapeutic drug, adriamycin inhibits wound repair. It causes inflammatory arrest, suppresses protein synthesis, and inhibits cell replication [6].

On the electron micrograph, immature (undifferentiated) cells were seen in the adriamycin group (Fig. 6). After 7 days, fibrin strands were markedly visible (Fig. 7), as against the pure fibrin sealant group, in which the fibrin was largely

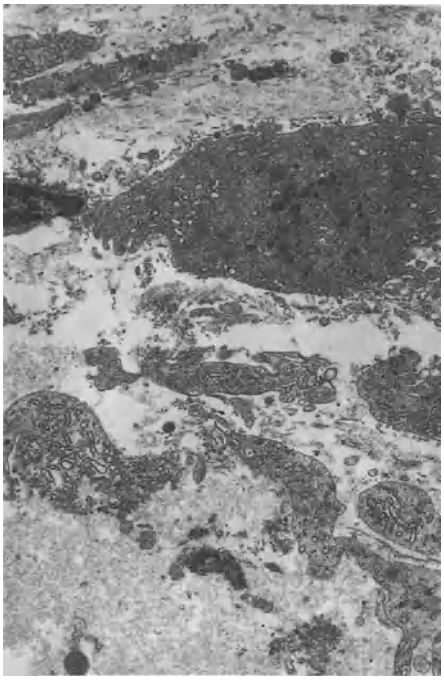

Fig. 6. Immature (undifferentiated) cells in granulation tissue of adriamycin-treated rats (7 days after implantation of spongiosa blocks). EM, x5 000

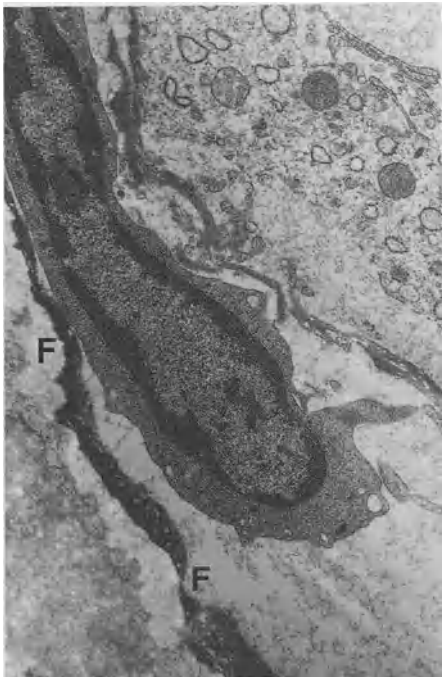

Fig. 7. Seven days after implantation – fibrin strands (*F*) of applied sealant are still visible in the adriamycin group

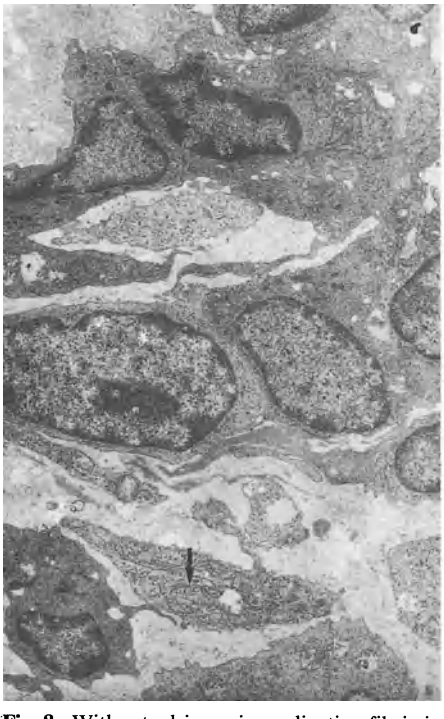

Fig. 8. Without adriamycin application fibrin is completely degraded after 7 days; mature cells are seen in which rough endoplasmic reticulum is already visible (*arrow*)

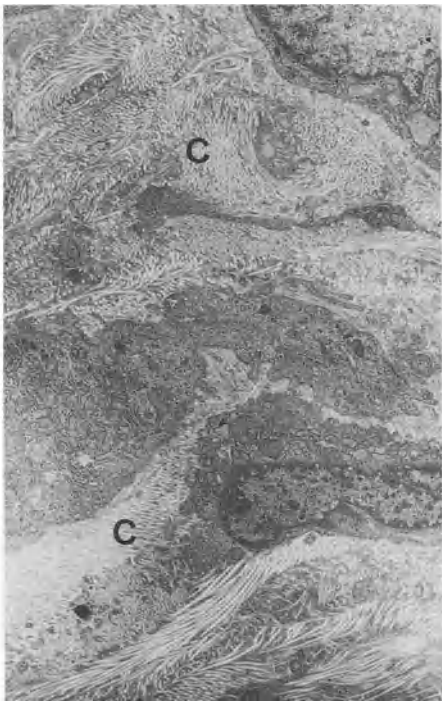

Fig. 9. Two weeks after implantation a marked collagen (*C*) structure is visible in the Tissucol group, which cannot be observed after adriamycin application

degraded (Fig. 8). After 2 weeks, a marked collagen structure was seen in the fibrin sealant group (Fig. 9); such a structure was not observed in the adriamycin-fibrin sealant group. In the latter group, many collagen-free zones were seen around the fibroblasts, as compared with a dense network of collagen fibers along the fibroblasts in the fibrin sealant group, which also showed abundant granular endoplasmic reticulum, corresponding to type B fibroblasts [1], as described in healing rat and human wounds [37].

It stands to reason that fibrin sealant cannot act on wound healing when cytotoxic drugs are applied simultaneously, since the fibroblasts are directly damaged. Nevertheless, in contrast to these findings, it has been shown that other forms of disturbed wound healing, such as ulcus cruris, can be cured by fibrin sealant in clinical settings, even when the ulcera have been unresponsive to other kinds of treatment [18].

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Fibrin Sealant and Its Modes of Application

H. REDL, and G. SCHLAG

Key words: antibiotics, collagen fleece, Duploject system, fibrin glue, hemostasis, spray, tissue adhesive, tissue sealing, wound healing

Abstract

After reconstitution, the two components of fibrin sealant – sealer protein/aprotinin and thrombin/$CaCl_2$ solution – can be applied in different ways. Besides sequential application or premixing of the reactant, application of the sealant components with the double-syringe applicator (Duploject) is advantageous in a number of ways, e.g., single-handed operation, thorough mixing, thin-layer application. Use of the Duploject is almost universally applicable. Thrombin concentration can be varied depending on the need for rapid or slow clotting of the sealants. The sealant can be delivered using needles, spray heads, or catheters, as indicated by the specific application. The spraying catheter can be easily used through the biopsy channel of an endoscope. Furthermore special micro-application techniques are possible. Fibrin sealant may also be used in connection with other biomaterials such as collagen (fleece), dura, and vascular grafts. Tests are reported on different collagen fleeces as well as on the addition of antibiotics. Finally visibility (including X-ray) and histological techniques are discussed.

The Material

Fibrin sealant is available under the trade names Tissucol, Tisseel, or Fibrin-kleber Human Immuno as a kit containing freeze-dried powder, freeze-dried thrombin, calcium chloride, and aprotinin solution. The substances mix to form two components: sealer and thrombin solution. To prepare the sealer, protein concentrate is dissolved in the accompanying stock solution of fibrinolysis inhibitor (aprotinin 3000 KIU/ml) or a dilution of it, where applicable. To simplify and speed up reconstitution (5–10) min of the highly concentrated sealer proteins, we developed a combined heating and stirring device – Fibrinotherm (Fig. 1). Thrombin is reconstituted in the accompanying 40 m*M* of calcium chloride solution, to yield concentrations of either 500 or 4 (NIH) units (NIH-U) of thrombin per milliliter depending on the chosen method of application. As the two components combine during application, fibrin sealant consolidates and adheres to the site of application, i.e., to the tissue.

The most important of the sealer proteins is fibrinogen, whose molecular weight is about 340 000 daltons. The molecule consists of six polypeptide chains of three different types – α, β, and γ. Through the action of thrombin, the fibrinopeptides A

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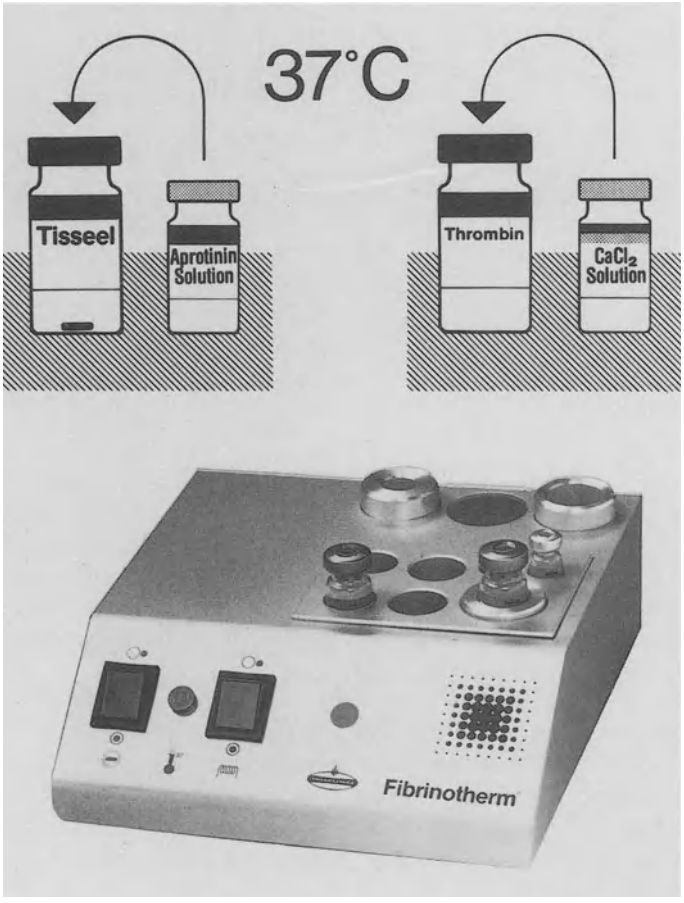


Fig. 1. Component preparation – Fibrinotherm

and B are split off from the resulting fibrin monomer. These fibrin monomers aggregate largely because of hydrogen bonding and thus produce the resulting fibrin clot. These reactions duplicate the last phase of the clotting cascade (Fig. 2). The time required for the onset of coagulation is dependent on the amount of thrombin used.

To achieve maximal tensile strength, cross-linking between fibrin α-chains is necessary. Fibrin seal itself contains sufficient factor XIII (which is activated by thrombin) to produce a high degree of cross-linking; the latter proceeds slowly, but the initial steepness of the α-cross-linkage curve results in sufficient tensile strength after about 3–5 min. In previous studies [1, 2], we were able to demonstrate the direct dependency of tensile strength on α-chain cross-linking. In other experiments [3, 4] we found that the intrinsic tensile strength of a clot formed with fibrin seal was about 1200 g/cm^2 (157 kPa) while that of a sealed rat skin was approximately 200 g/cm^2 (17 kPa) after 10 min cross-linking at 37°C, implying that adhesion of the sealant

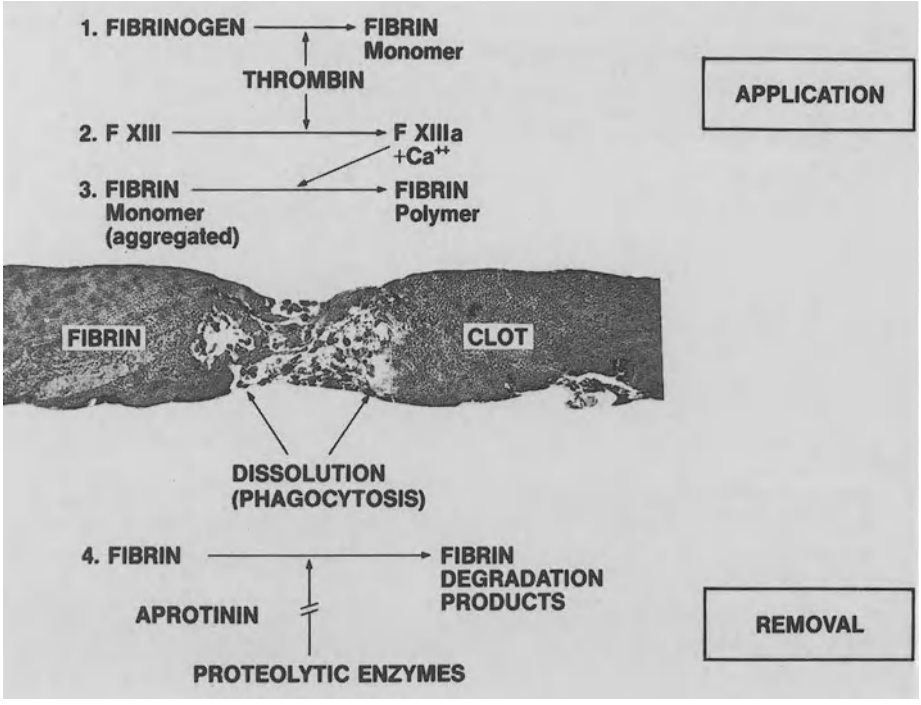


Fig. 2. Fibrin clot formation and removal

to the tissue is the decisive factor for gluing tissue. The adhesive qualities of consolidated fibrin sealant to the tissue might be explainable in terms of covalent bonding between fibrin and collagen [5] or fibrin, fibronectin, and collagen.

As far as the adhesive effect is concerned, critics have repeatedly pointed out its limited adhesive strength compared with synthetic acrylate adhesives. This is compensated for by the high elasticity of the material [6], which makes the material especially useful for nonstatic tissue, e.g., lung parenchyma. In addition, applications onto wet surfaces are equally possible, as is shown in Table 1. However, the applications of fibrin sealant are not limited to sealing severed tissue segments, as adequate hemostasis is also achieved.

Table 1. Tensile strength of sealed rabbit skin in relation to tissue moisture before application of Tisseel (method similar to that described by Redl et al.[26]

Dry (with pads)	Wet (with Ringer's solution)
× 48.2 g STD ± 10.7	53.3 g ± 12.8

To a variable extent, sealant persistence in vivo can be controlled by adding an antifibrinolytic agent [7]. Previous studies have demonstrated that aprotinin, a natural antiprotease, is superior to synthetic antifibrinolytic agents [8]; this has been confirmed by other reports [9]. Sealant degradation rate depends on

a) the fibrinolytic (or more generally the proteolytic) activity in the area of application,
b) the thickness of the sealant layer – which should be as thin as possible – and
c) the amount of aprotinin present.

Thus expected clot persistence can only be dealt with on an individualized basis. However, excessively long survival of the sealant may not be desirable [10].

Application of Fibrin Sealant

General

Historically the components were applied sequentially with relatively poor mixing owing to fast buildup of fibrin membranes between them. This prompted us to study mixing ratios, and alternative application techniques and their effects on the seal produced. Ever since the first applications of fibrin sealant the strength obtainable has been known to depend both on the fibrinogen concentration [11] and on the amount of cross-linkage [8]. Using a design for measuring intrinsic clot strength [3], we tried to find the optimum mixing ratio [12]. The mixture of one part sealant and one part thrombin solution gave the best results, although thorough mixing appears to be the decisive factor.

The gross and microscopic data obtained from experiments on rat skin revealed [12] that seals produced with premixed reactants (4 NIH-U thrombin/ml) or with the Duploject applicator (4 or 500 NIH-U/ml) had a superior tensile strength to those obtained with sequential application of reactants. There is no doubt that cavitation, as observed microscopically, is one factor involved. Another factor is insufficient availability of the reactants at the reaction site, since adequate cross-linkage requires a minimum concentration of Ca^{2+} [13], which may not be achieved locally if mixing is incomplete.

Duploject System with Needle

While we have repeatedly stressed the disadvantages associated with sequential application (poor mixing and cumbersome handling) [8, 12], the technique has not lost its role in selected cases, e.g., in combination with collagen fleece or vascular graft material so as to facilitate mixing.

In most cases, application of the sealant components with the double-syringe applicator (Duploject) is advantageous, e.g., single-handed operation, thorough mixing, and thin-layer applications. Use of the Duploject is almost universally applicable (Fig. 3).

Low thrombin concentrations (4 NIH-U/ml – slow clotting) are beneficial in all those applications where the parts to be sealed require subsequent adaptation, e.g.,

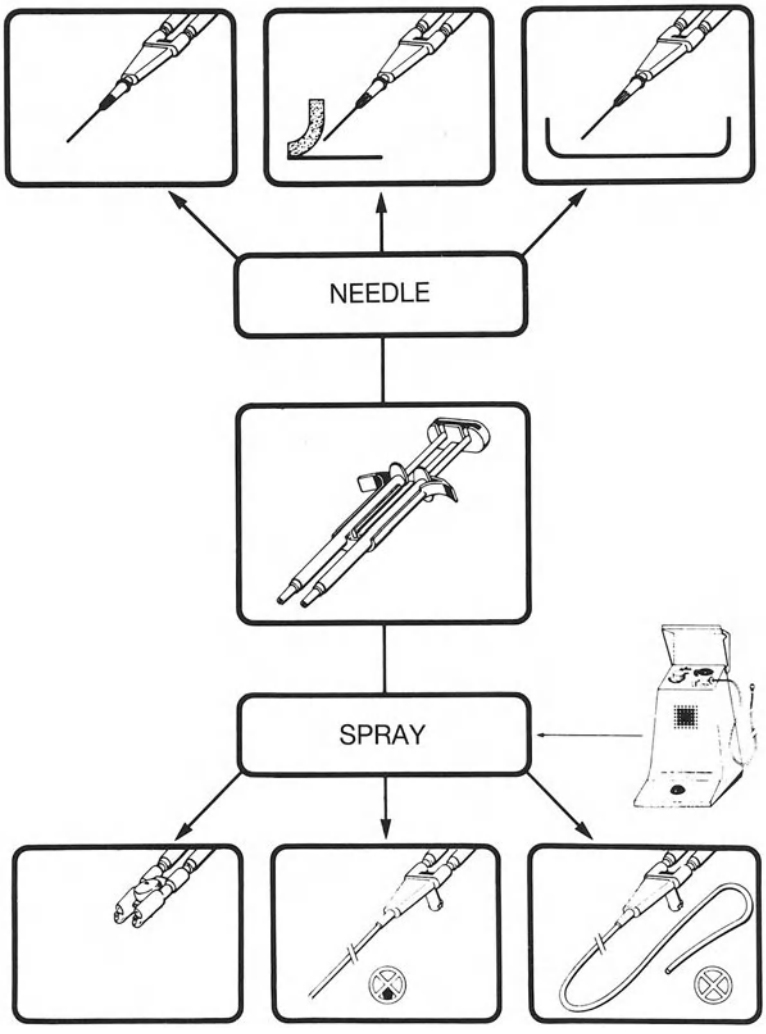
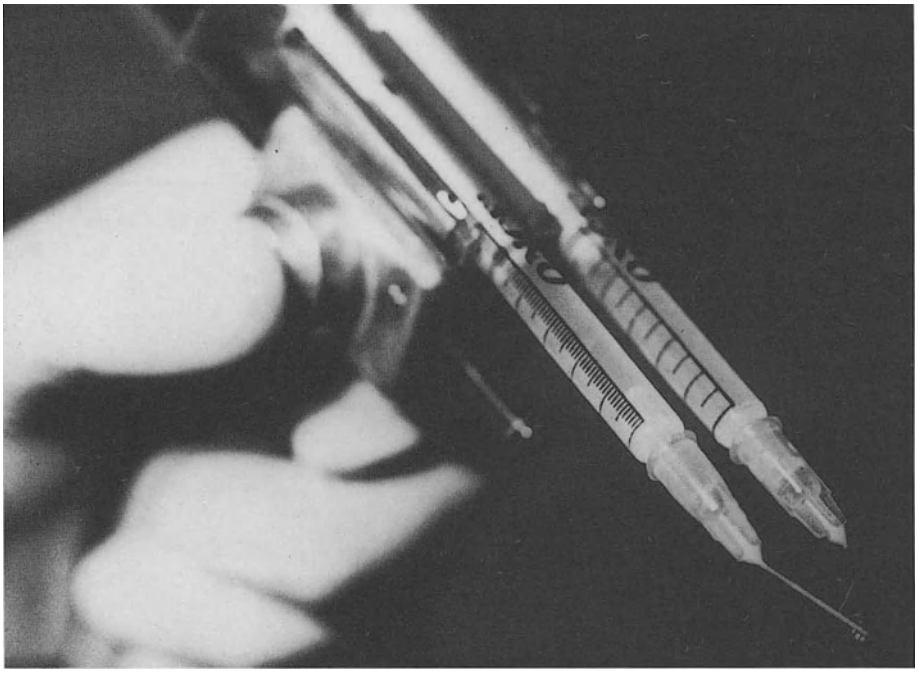


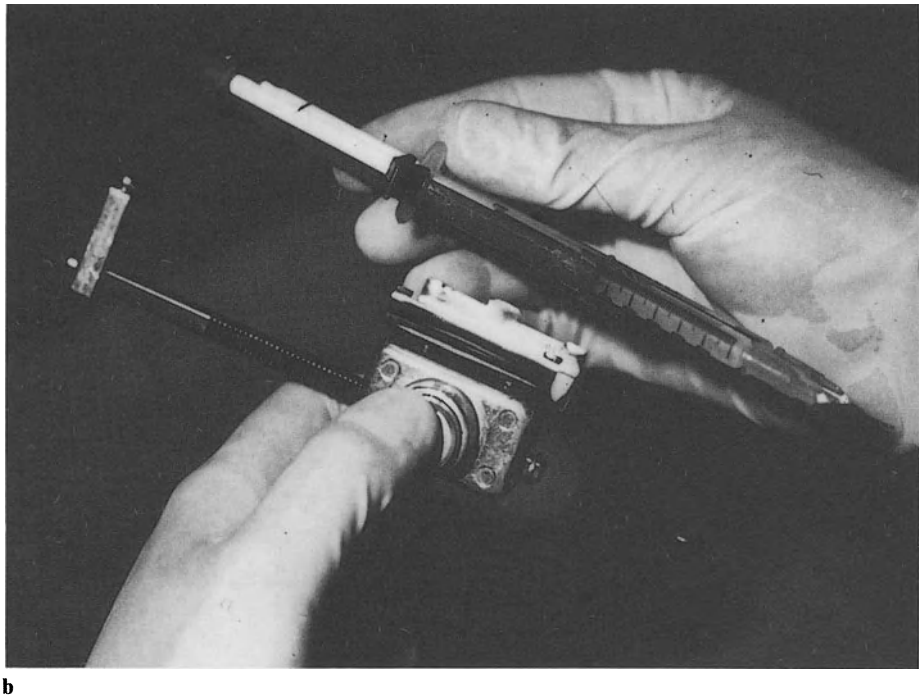
Fig. 3. Duploject system

in skin grafting and in some microsurgical operations. If, however, hemostasis is of primary interest, a high thrombin concentration, i.e., 500 NIH-U/ml, should be used as this ensures almost instantaneous clotting.

The double-syringe unit with mixing attachment – needle or catheter – is designed for simultaneous operation of the two barrels so that the two components are ejected at the same time but separately via the exchangeable mixing needle. As long as the sealant is being applied, there will be no clogging of the needle. Once application is interrupted, insertion of a new needle makes the applicator ready for use again.



a



b

Fig. 4 a, b. Microapplicator to be used with Duploject system

Certain operations require the use of a microapplicator (Fig. 4) which allows repeated application of the same small volume per ejection; this is especially useful when using 4 NIH-U thrombin per milliliter. A similar system was developed by Tange [14]. An alternative is to mix the two components on a piece of aluminum foil and apply the premixed sealant with a spatula [15]. To get an "ultramicro" dosing (but without mixing) the special device of Chüden [16] may be used.

Duploject System – Spray Applications

The spray head or spray catheter (lower part of Fig. 3) is connected to a conventional pressurized gas source. The gas pressure is reduced to 2 bar (head) or 4 bar (catheter) in order to obtain a gas flow of 5–10 liters/min, which is optimum for use with the Tissomat (Fig. 3). The two components are injected separately into the continuous gas jet. The optimal distance between the spray head and the wound surface is approximately 10 cm for the head and 1 cm for the catheter. As the droplets bombard each other in the air and on the wound surface, they mix, and at a high thrombin concentration instantly form a delicate fibrin film. A thin film so produced is optimum and is required for the sealant to promote wound healing [10]. Spray head application also allows coating of extensive surfaces with a small amount of sealant. Thus an area of about 100 cm^2 can be coated with the 1-ml kit.

The spray head is especially useful for covering large areas, e.g., resected surfaces of parenchymal organs [17], for fixation of skin grafts and coating the donor area [18, 19], and for hemostasis of diffuse epicardial bleeding [20].

In the four-lumen spray catheter (Fig. 5), two lumens are used for the components, the third one for the gas, and in the short version a malleable wire is contained within the fourth lumen. The "spray catheter" can also be used, without spraying gas, to mix the two components in an otherwise inaccessible area, e.g., an esophageal-bronchopleural fistula [21]. In the latter case, the third lumen may be used to apply X-ray contrast dye for catheter localization.

Catheter spray systems can be modified to seal otherwise inaccessible areas by either:

1. The use of endoscopy (with biopsy channels) and a 150-cm-catheter (Figs. 5, 7), or
2. The short catheter with a malleable wire which allows any specific catheter shape (Fig. 6).

These catheters may be used for pleurodesis in recurrent pneumothorax [22–24], to occlude bronchopleural [25], rectovaginal, and esophageal-bronchopleural fistulas [26], to arrest gastric [27] and esophageal bleeding to ensure tissue sealing of the larynx, to fix flaps in plastic surgery, and to achieve hemostasis in epistaxis and after prostatectomy. An additional advantage offered by spraying with the Duploject spray is that the gas jet can be operated separately and can be used to clean and dry the operating site. The sealant is thus applied to a "dry" surface, which facilitates hemostasis. In addition, no clogging occurs when the sealing procedure is interrupted.

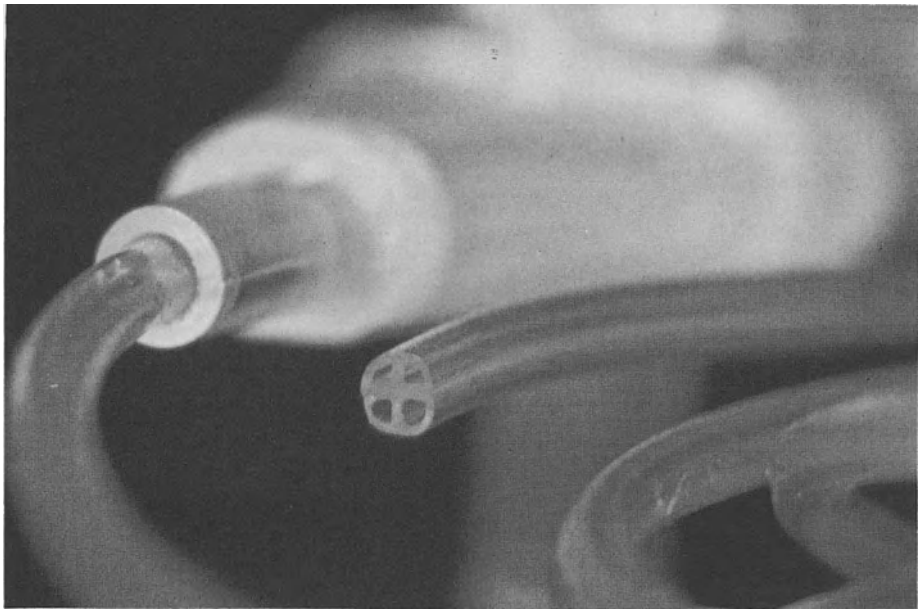


Fig. 5. Spray catheter with characteristic four-lumen design

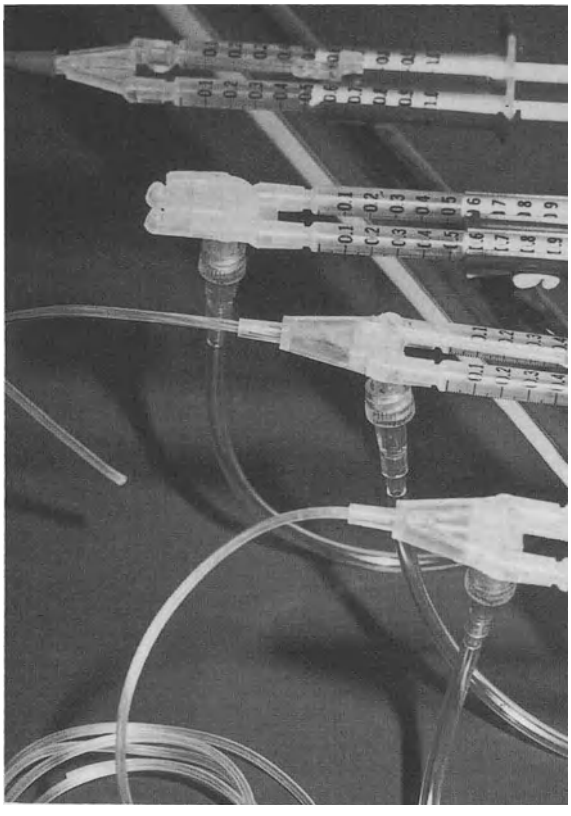


Fig. 6. Spray adaptors of the Duploject system

a

b

Fig. 7a u. b. Use of spray catheter through the biopsy channel of the endoscope. **a** Insertion into the channel. **b** Catheter in action, fibrin coming out of the biopsy channel at the tip of the bronchoscope

A cut Swan-Ganz catheter can also be used, as outlined by Linscheer [28]. This technique [15] has been successfully employed to treat patients with pneumothorax [29].

Combination of Fibrin Sealant with Matrices

For some applications the additional use of sealant support, e.g., Dacron patches, lyophilized dura, fascia, or collagen fleece, proved useful. However, not all of the commercially available fleeces are suitable for this purpose, and preliminary tests are therefore mandatory before clinical use. Some fleeces were tested by Stemberger [9] to assess their effects on platelet aggregation. We feel that pliable collagen fleeces are best suited for this purpose. Therefore we performed a preliminary study with some of the available fleeces. Test criteria were:

1. Uptake of liquid
2. Tensile strength in the wet state
3. Ease in handling
4. Tissue reactivity

Preliminary Results

1. To test the absorption of water, 1×1 cm pieces of collagen fleece of different thickness were used (for results, see Table 2). Absorption of H_2O was negligible with Collatamp and slow with Gelfix, all other fleeces absorbed H_2O immediately, which seems to be of essential importance in ensuring adequate soaking with sealant components. Some of the fleeces absorbed H_2O differently at the upper and the lower surfaces.
2. There were great differences in tensile strength in wet conditions (Table 2). Gelfix showed the highest tensile strength of all the fleeces tested. As expected, the Braun fleece had negligible tensile strength, whereas the Helitrex fleece of only 3 mm showed a remarkable tensile strength of 40–50 g.
3. Most of the collagen fleeces were easy to work with in wet conditions, with the exception of the Braun fleece, which broke into pieces and stuck to the gloves. (However, after our examinations had been completed, an improved fleece was developed.) The application of collagen fleece in dry conditions deserves special

Table 2. Test criteria and results of tests on different collagen fleeces in vitro

Company	Hydrophilic surface	Tensile strength	Handling	Tissue reaction
Braun	+	2 g	−	∅
Collatamp	−	10 g	+/−	+
Gelfix	−	150 g	−	+
Pentapharm	+	15 g	+/−	+/−
Helitrex	+	55 g	+	+/−
Savolon	+	50 g (inhomogeneous)	+	∅

mention, especially in regard to spray applications. The only fleeces suitable for this mode of application are Helitrex and Savolon 3 mm, whose properties with regard to ease of handling and H_2O absorption (in particular rapidity and volume of absorption of water) are excellent.

4. For histological examination, moistened pieces of fleece (size: 1×0.5 mm) were applied subcutaneously in rats according to a similar model of wound healing used by Rudas [30]. Blinding evaluation was performed after 14 days. The findings may be summarized as follows: In principle, every fleece tested was still detectable after 14 days; the larger pieces, however, were less disintegrated. The loosely textured Braun fleece and Savolon were absorbed relatively rapidly. The foreign body reaction seemed relatively limited with Braun, Savolon, Pentapharm and Helitrex, while Gelfix and Collatamp cause a more severe reaction. In view of our experience thus far, we recommend the use of Helitrex as a standard fleece for fibrin sealing. In addition to its properties outlined above, it has a further special property: if pressed in a dry condition it may be greatly compressed, yet when absorbing liquid, e.g., fibrin sealant, it expands to its original dimensions. This may result in interesting applications, e.g., endoscopy.

Combination of fibrin sealant with either decalcified bone (ongoing studies in this laboratory) or hydroxyapatite (see orthopedic section) is a further example of heterogenic combination. Fibrin sealant may also be used to fix bioprostheses, such as the middle ear bones [31].

Combination of Fibrin Seal with Antibiotics

The practice has been to apply fibrin seal only to areas unlikely to become infected. To overcome this limitation, the addition of antibiotics to the fibrin seal seemed desirable. As early as 1950 a patent was described in the USA in which the combined application of fibrin and antibiotics was used [32]. Fibrin seal has also been used in combination with antibiotics both experimentally and clinically [33, 34]. Therefore we studied the in vitro properties of mixtures of fibrin seal and antibiotics, particularly their effect on coagulation time, cross-linking, and drug release [3, 4].

For the practical application of fibrin seal, it is important to note that the clotting time can be regulated by the use of higher thrombin concentrations and the rate of fibrin-α-chain cross-linkage with additional factor XIII. Drug release from fibrin seal is probably by simple diffusion, and therefore to a large extent, dependent on the concentration gradient between the clot and its environment. This implies that although antibiotics incorporated into fibrin clots are retained for longer than when they are directly instilled into body cavities, drug retention is much lower than with bone cement-antibiotic mixtures and is insufficient to maintain adequate local drug concentrations for more than 3 days. This observation has also been confirmed in a recent in vivo study [35]. The limitations may be overcome by newer, less soluble antibiotics [36]. Nevertheless, infections may be controlled in the early stages after bone surgery using fibrin seal containing relatively high antibiotic concentrations. However, the total dose of drug should be less than the recommended maximal daily systemic dose.

Detection of Fibrin Seal in Tissues

Owing to the opaque white appearance of coagulated fibrin sealant, it is usually easy to detect fibrin in the sealing area. However, for special indications (e.g., in eye surgery) or with sequential application, in which one might wish to observe the delivery of the sealer protein solution, adding disulphine blue dye (ICI) (10 μl/ml sealer protein solution) is effective in rendering the fibrin seal visible.

For X-ray detection the addition of different contrast media was tested by Richling [37]. Metrizamide was found to be superior, but its general use cannot be recommended because of slight depression of fibrin-α-chain cross-linking.

Reviews on histological techniques for identifying fibrin sealant have been published by Dinges [30] and Heine [38]. With the phosphotungstic acid method of Mallory and the trichrome technique of Lendrum it is possible to visualize easily the fibrin sealant with light microscopy, but the fibrin sealant does not react as well as endogenous fibrin (perhaps due to the thicker network of fibrin strands). The histological differentiation between exogenous fibrin sealant and endogenous fibrin requires some experience if standard fibrin techniques are employed. If heterologous fibrin glue is used in animal experiments, its demonstration with the immunoperoxidase technique gives optimal results [30]. It is also easily seen with hematoxylin-eosin stain and shows up nicely on trichrome stain.

Conclusions

In summary, for the optimal use of fibrin sealant the application technique should meet the following requirements [12].

1. The sealant components should be fully dissolved and kept at a temperature of 37°C (which is easy with the Fibrino thermsystem — Fig. 7).
2. The wound surfaces should be as dry as possible (though application to wet surfaces is feasible).
3. The components should be thoroughly mixed on application.
4. The thrombin and aprotinin concentrations may be adjusted to the purpose of application.
5. The sealant should be applied as a thin film.
6. After clotting has occurred, further mechanical stresses should be avoided for about 3–5 min because of the time course of α-chain cross-linking.

Fibrin sealant is useful in controlling microvascular or capillary bleeding from ruptured or surgically dissected tissues. It is particularly beneficial in patients with increased bleeding tendencies undergoing surgery. It might also be used to seal tissue with different kinds of biomaterials. Thus fibrin sealant has a place in all surgical disciplines for the purposes of tissue sealing, hemostasis, and support of wound healing. There seem to be few drawbacks, not even such as the risk of viral transmittance [39, 40]; however, the benefits of combining fibrin sealing with modern-day surgery far outweigh any known risks.

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Properties of Different Tissue Sealants with Special Emphasis on Fibrinogen-Based Preparations

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Key words: fine clot, coarse clot, fibrin sealant, fibroblast proliferation, tissue adhesive, fibrinogen, wound healing, hemostasis

Abstract

Different tissue sealants are described with special emphasis on the performance of different fibrinogen-based sealants. Therefore the biochemical properties of four different fibrinogen-based tissue adhesives are compared in detail. The major difference is in clot structure – coarse versus fine. Related to this structural difference are additional dissimilar properties. The coarse type fibrin sealant proved to be superior in tensile strength, cell compatibility and fibroblastic proliferation.

Introduction

The use of tissue adhesives as an alternative method for repairing injured tissues, and more importantly, as a means for improving wound healing, may be based either on natural or synthetic materials. Therefore, it is necessary to compare various natural adhesives (e.g. fibrin sealant) to each other, as well as to synthetic preparations (e.g. cyanoacrylates), in order to asses their relative advantages and disadvantages in regards to clinical applicability (Table 1).

Table 1. Tissue Sealants

Synthetic	Natural
Acrylates	(Plasma)
Gelatine-Formaldehyde-Resorcin	(Cryoprecipitate)
	Fibrin Sealant

One obvious advantage of fibrinogen-based materials is their complete degradation and rapid removal from the body. Thus, local and systemic toxicity are avoided.

In the present study, we compare the biochemical properties of four different fibrinogen-based tissue adhesives; in addition, the similarities and differences of these natural adhesives, as compared to synthetic preparations, are discussed (Tables 2, 3).

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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Table 2. Advantages and Disadvantages of Fibrin Sealant Versus Acrylates

	Fibrin sealant	Acrylates
Application to wet area	Possible	Impossible
Adhesivity	Good	Better
Elasticity	Very good	None
Tissue compatibility	Excellent	Poor
Absorption or degradation	Complete	None
Hemostasis	Excellent	None
Supporting of wound healing	Obtainable	Unobtainable
Application in bone and cartilage	Possible	Impossible
Foreign granulation tissue	None	Invariably present
Risk of virus infection	None*	None

*according to current knowledge

Table 3. Clottable Material [mg/ml]

Cryoprecipitate	AF	Fibrin sealant
29	11	80

Special emphasis is put on two fibrin sealants, which differ mainly in their ionic composition. Ferry and Morrison [1] described the influence of ionic strength on clot structure in 1947. High ionic strength results in "fine" clots and physiological ionic strength in "coarse" clots.

Material and Methods

The four fibrinogen-based, natural adhesives utilized in the study were cryoprecipitate, autologous fibrin (AF)[2] and two fibrin sealants. One fibrin sealant contains a physiological salt concentration (PS) while the second has a high salt concentration (HS) to achieve fast reconstitution.

Protein concentration and composition, kinetics of fibrin alpha-chain crosslinking, factor XIII content, conductivity and osmolarity were measured as described in Redl et al [3]. Intrinsic strength of the formed fibrin clots was tested in an apparatus similar to the one described by Redl et al]4], but using a 0.2 ml butterfly shaped mould for the breaking strength test and a larger one, 0.8 ml with 1.5 cm usable length, for elasticity measurements. The velocity used for stretching the fibrin clots was 1 cm/min.

Human diploid embryonal lung fibroblasts MRC5 were cultivated and their viability tested as described by Redl et al ([3]. Fibroblast proliferation was evaluated according to Mosmann [5] either on cell layers or in cell suspension using the substrate (3-(4,5 Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) (Sigma, USA) (= MTT). The effect of the two fibrin sealants on fibroblasts was assessed in either a liquid or solidified state.

In order to assess the influence, if any, of the liquid sealants on the cells, the latter were seeded into the wells of TC Cluster 24 plates (Coster) and incubated at 37°C under 95% air + 5% CO_2 until an almost uniformly dense cell layer had formed. Following dilution of the sealants with equal volumes of isotonic NaCl solution, the cell cultures were covered with 0.5 ml of sealant solution for a maximum of 30 minutes. The effects of dilute sealants on the cells were observed using light microscopy and the supernatants removed at fixed intervals. The cells were then washed with isotonic NaCl solution and stained with Ziel-Neelsen Carbol Fuchsin (diluted 1:10 with water); micrographs were produced using a Polyvar microscope (Reichert).

The proliferation rate was determined according to Mosmann [5] and was used to obtain quantitative data. After incubation with liquid sealant as described above, 50 µl of MTT (5 mg/ml) was added and incubated further for 2^h at 37°C. Simultaneously 0.1 ml MRC5 cell suspension (5×10^5/ml) was added to 0.1 ml of each sealant solution, incubated for 30 min. and then incubated further at 37°C after the addition of 20µl MTT solution. The reaction was stopped with 0.4 N HCl in 2-propanol and the accumulated dye extracted. Photometric measurements were done after centrifugation of the supernatant fluid (diluted threefold with 0.4 N HCl/2-propanol) at 570 nm. This test has been shown to correlate well with the ^{3}H-thymidin uptake test [5].

In order to assess whether the solidified sealants differed in their influence on fibroblasts and to evaluate the fibrin structure, equal volumes of sealant solution were rapidly mixed at 37°C with thrombin-$CaCl_2$ solution (4 IU of thrombin/ml, 40 mmol of $CaCl_2$/l) and 0.5 ml of the mixture was poured into each TC Cluster 24 plate well (Costar) and incubated at 37°C and 100% rel. humidity for 1 hour. Plasma clots were produced similarly by mixing 0.9 ml of citrated human plasma with 0.1 ml of thrombin-$CaCl_2$ solution (4 IU thrombin/ml, 0.3 mol $CaCl_2$/l).

Some of the sealant clots were washed 4 times, each time with 0.2 ml of isotonic NaCl solution for 20 min at 37°C under continuous agitation; the washing efficiency was checked by washing clots of the same type with distilled water and determining the supernatant conductivity after each washing. The nonwashed clots and those washed with isotonic NaCl solution were each cut at a small angle (to obtain a rougher surface), covered with 0.2 ml MRC5 fibroblast suspension (5×10^5 cells/ml medium), and incubated for 24 hours at 37°C under 95% air + 5% CO_2. Direct examination of cells under the light microscope was possible only with the transparent HS fine clots, not with the milky white PS coarse clots. Therefore, the samples were prepared for histologic examination by fixing them in 3.5% formaldehyde solution followed by standard procedures of dehydration and paraffin embedding. For SEM examination, the samples were fixed with 1% glutaraldehyde (cacodylate buffer), refixed with 1% OsO_4, alcohol dehydrated, and critical point dried with CO_2. Dried samples were fractured in order to observe both surface and inner structures and gold sputtered (10 nm, Polaron Sputter) for scanning by a (Jeol-SM 35) SEM at 25 kV accelerating voltage.

Results

Cryoprecipitate and autologuos fibrin (AF) were found to have a low fibrinogen (clottable protein) concentration (Table 3), only moderate α-chain crosslinking (Table 4) and therefore only limited tensile strength (Table 5).

Both fibrin sealants require approximately the same reconstitution time (5–10 min) when PS dissolved at 37°C by using the combined warming and stirring unit described before [3] and HS at room temperature under manual shaking. Dissolution of HS at 37°C reduces the time required to 3–6 min.

PS and HS were found to be identical in their kinetics of fibrin crosslinking (Table 4) if FXIII is added to the latter.

Table 4. Crosslinking of Fibrin α-Chain (% of α-Polymer)

Incubation time (min)	Cryoprecipitate	AF	PS (coarse)	HS (fine)
120	35	36	80	80*

*(with additional factor XIII; see Table 6)

Intrinsic tensile strength was 4 to 5 times higher ($p < 0.001$, Student-t-test) in the PS coarse clots (Table 5). Due to the brittle nature of the HS fine clot, more than 50 % of the specimens broke during manipulation and were therefore excluded from the measurements. For the same reason, we were unable to obtain stress-strain results (length-tension relationships) of fine clots (Fig. 1). The decreased elasticity of the fine clots appeared to be unrelated to the fibrin sealant, as standard fibrin fine clots (with minimal lateral aggregation of protofibrils) were also irreversibly deformed, as compared to coarse type clots [6].

As was the case with fibrin structures, the different effects of solidified sealants on fibroblasts were best visualized on the cut surfaces of clots. On smooth PS clot

Table 5. Intrinsic Strength [g/cm^2] (kPa) (incubation temperature = 37°C)

Incubation time (min)	Cryo	AF	PS	HS
10	19845* (19kPa) n=7	237 (23kPa) n=2	616+101 (60kPa) n=5	
30		not investigated	899+155* (88kPa) n=8	192+41** (19kPa) n=8

* = signif. p. < 0.001 Student t-Test
** = 50% of the fine clot samples had to be eliminated during machine set up

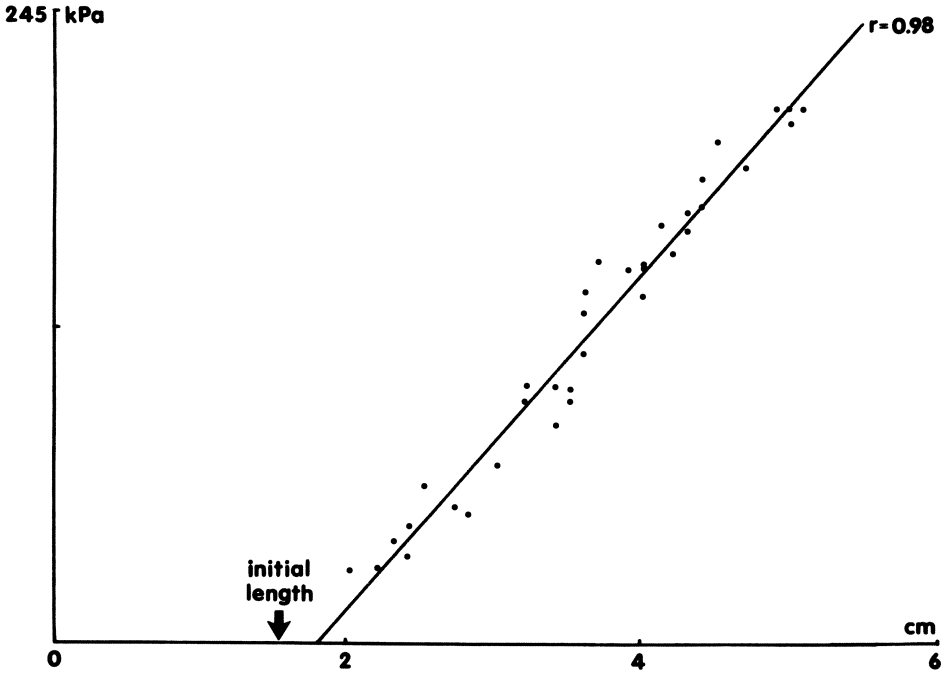

Fig. 1. Stress-strain diagram of PS coarse clot

surfaces, we observed a normal proliferation of fibroblasts. Mechanical disturbance of the clot surface greatly accelerated fibroblast proliferation, and the surface became completely covered with fibroblast growth (Fig. 2). HS clots treated in the same manner showed spheroidal deformation of cells, with no detectable proliferation (Fig. 3, Table 7).

The damage of cells on (nonwashed) HS clots was similar to the damage caused by the same sealant in liquid form, but the damaging effect occurred more slowly on the solid sealant.

Conductivity measurements on the supernatants of clot washings revealed the removal of more than 95% of salts contained in the clots after 4 washing cycles. Morphology and growth of fibroblasts were identical on washed and nonwashed PS clots, while the cytotoxicity of HS clots was reduced, but not completely eliminated, by extensive washing with isotonic NaCl solution (results not shown).

Table 6. Comparison of Fibrin Sealants

	PS	HS
Factor XIII (U/ml)	12.0	65.0
Conductivity (1:10 dilution with H_2O) (mS)	1.3	4.0
Osmolarity (mOsmol)	547.0	1 011.0

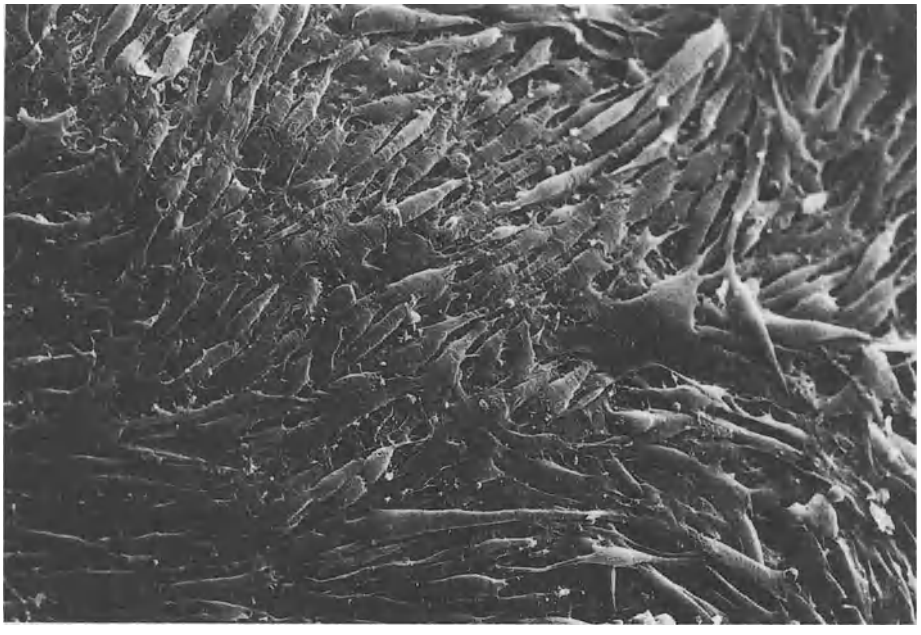

Fig. 2. Rich proliferation of fibroblasts on a cut PS clot. SEM, after critical point drying, x 1000

Fig. 3. Spheroidally deformed (damaged) fibroblasts on a HS fine clot after identical treatment as in Fig. 2

Table 7. Comparison of the Proliferation Rate of Fibroblasts (Cell Layer and Cell Suspension) when subjected to either PS or HS Sealer Protein Solution

	Photometric extinction at 570 nm		% Inhibition unphysiological – HS
	PS	HS	
Cell layer (mean of 3 diff. experiments ± SD)	.390±.130	.185±.077	53±3
cell suspension (mean of 3 diff. experiments ± SD)	.243±.190	.131±.112	67±7

Discussion

Because of the limited strength of Cryo and AF (Table 5), which results from a low clottable protein concentration (Table 3) and only ~ 35% α-chain crosslinking even after 2 hours (Table 4) no further experiments concerning histology and cell compatibility were carried out.

Fibrin sealant is a concentrated protein solution. Upon application, fibrinogen is coagulated by mixing with a thrombin-calcium chloride solution, following which the rigidity of the adhesives increases further as a result of fibrin crosslinking. The two preparations studied here produce clots with significantly different characteristics: PS clots are white (non-transparent) and of visco-elastic consistency, whereas HS clots are almost crystalclear and relatively brittle. Ferry and Morrisson [1] in 1947 described the formation of two different kinds of fibrin clots: white, non-transparent "coarse clots" formed at an ionic strength and pH value within the physiological range, and transparent "fine clots" produced at a higher ionic strength and/or pH value. Transition from one type to the other is smooth, with fibrinogen concentration, thrombin concentration, and reaction temperature as further influencing factors. In the present study, we determined electrical conductivity, osmolarity, and the kinetics of fibrin crosslinking of both sealants after adjustment of factor XIII content (Tables 4, 6). Micrographs of the fibrin clots produced were obtained under both light and scanning electron (SEM) microscopes and were compared with clots prepared from plasma and thrombin. Because HS differs from PS mainly by its high ionic strength outside the physiological range (causing the formation of almost amorphous clots), we examined the question of how the two sealants would differ in their influence on living cells. Considering the essential role of fibroblast proliferation in wound healing [7], we performed tests with human fibroblasts.

Our investigations were motivated by the striking differences in optical and mechanical properties between the two sealants after setting.

The essential difference between PS and HS is in ion content. PS conductivity is similar to that of isotonic saline solution, whereas HS conductivity is about three times greater.

Our results confirm the basic findings of Ferry and Morrison [1] that visco-elastic, nontransparent fibrin clots are formed at physiological ionic strength ("coarse" clots), whereas transparent, brittle "fine" clots are produced at a higher ionic

strength. Our results indicate that this influence of ionic strength persists over a wide range of fibrinogen concentrations.

Clots produced from PS or plasma show similar fibrin characteristics, consisting of relatively thick, branching strands (Fig. 4); HS clots appear almost amorphous under identical conditions (Fig. 5). The porosity of HS (with 4 IU/ml thrombin) seen in Fig. 5 might be even less when applied in vivo (with 400–500 IU/ml thrombin) as it was found by Blombäck et al. [8] that increasing thrombin concentration results in reduced porosity of fibrin clots.

Both sealants are very similar in terms of fibrin crosslinking kinetics. Ionic strength above the physiological range is known to inhibit fibrin crosslinking [9]; therefore this anticipated effect was compensated for by adding factor XIII.

The significantly higher tensile strength in the coarse clots (PS) is similar to previous shear modulus data from Kanykowski et al. [10]. The elastic rigidity measurements of fine clots (HS) revealed less than one-tenth the shear modulus found for coarse clots. It is possible that the rigidity of the latter clots is primarily due to steric immobilization as has been suggested by Nelb et al. [11].

Because mechanical union is just one aspect of successful surgery, wound healing and hemostatic properties of the sealants must also be simultaneously evaluated.

The formation of fibrin and its crosslinking by factor XIIIa are essential for wound healing. The fibrin network produced under physiological conditions serves as a

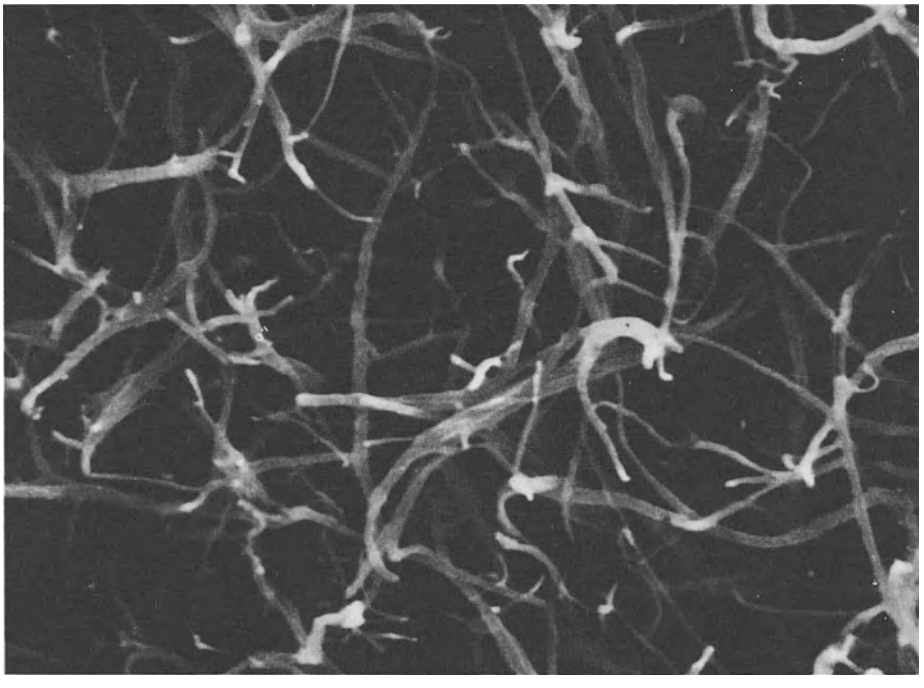

Fig. 4. Fibrin network in PS coarse clot very similar to plasma clot fibrin network. Scanning electron micrograph (SEM) after critical point drying.

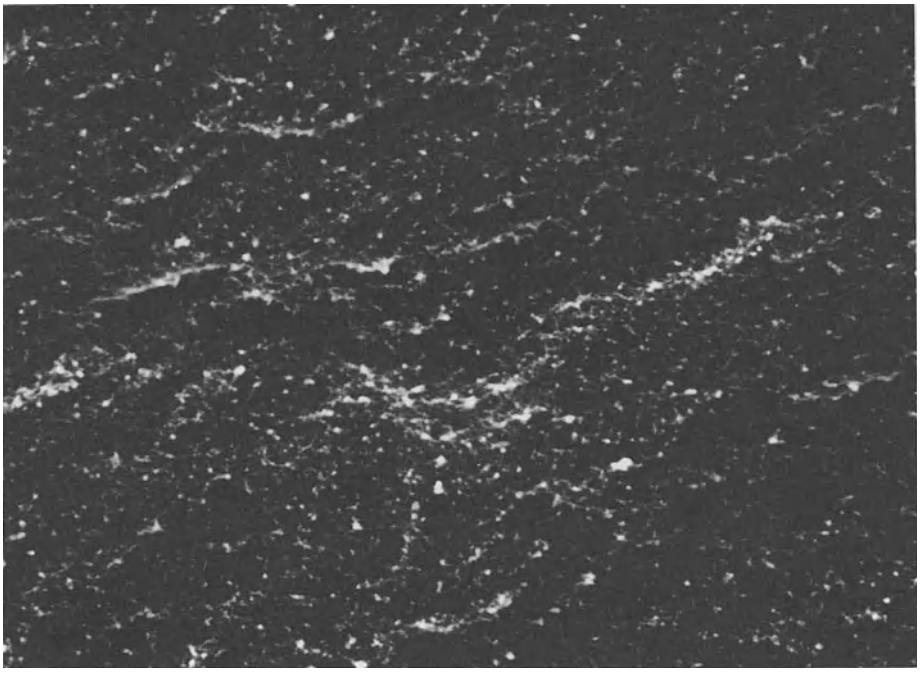

Fig. 5. Hardly detectable fibrin strands in a HS fine clot, conditions as in Fig. 4

matrix for the ingrowth of fibroblasts and the formation of collagen fibers [7, 12], thereby allowing for optimal wound healing. The formation of crosslinked fibrin is used not only for sealing tissues but for achieving hemostasis as well.

Up to now, many clinical and histologic reports [13, 14, 15, 16] describing satisfactory wound healing after fibrin sealant application have appeared. Thus the question arose whether the higher ion content of HS and the resulting altered fibrin structure of these clots influence fibroblast growth. Given the usual practice of mixing fibrin sealant with an equal volume of thrombin-$CaCl_2$ solution prior to application, we evaluated the influence of liquid sealants on fibroblasts after 1 + 1 dilution with isotonic NaCl solution. We found that human fibroblasts were severely damaged within minutes by contact with liquid HS, whereas liquid PS does not cause any detectable damage, even after prolonged incubation (Figs. 6, 7). The cytotoxic effect of liquid HS, which is also demonstrated by its 50–60% inhibition of cell proliferation (Table 7), is most easily explained by its high ionic strength and osmolarity. Both HS clots (nonwashed) and liquid HS cause similar damage to cells, but cytotoxicity develops more slowly with the clots. This is understandable if we assume cytotoxicity to arise from soluble additives; the solution trapped in the clot and the cell medium applied take a certain time to equilibrate, by when the damaging additives are further diluted.

In order to distinguish whether the cytotoxity of HS clots is due to soluble substances trapped in the clot or to the altered fibrin structure, we washed PS and

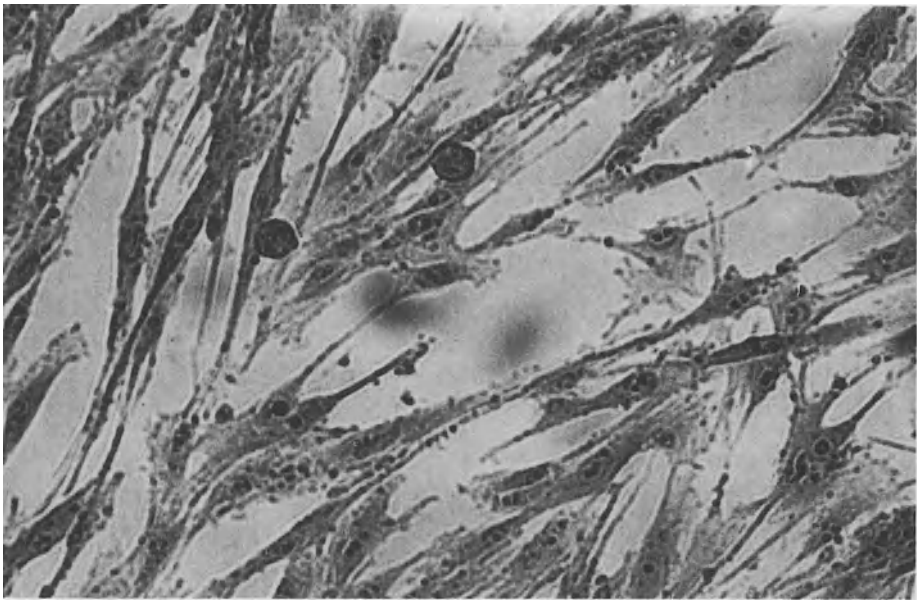

Fig. 6. Layer of fibroblasts 30 minutes after covering with PS, diluted 1 + 1. No detectable differences to controls. LM, carbol fuchsin staining, x 125

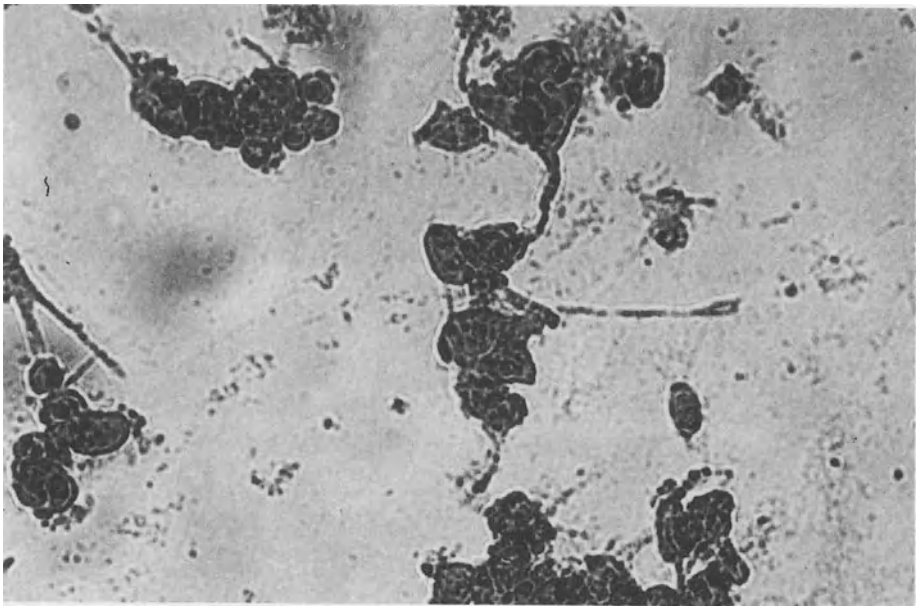

Fig. 7. Layer of fibroblasts 4 (!) minutes after covering with high salt concentration diluted 1 + 1. Damage to cell structure is clearly visible. Staining and enlargement as in Fig. 6

HS clots with isotonic NaCl solution. Conductivity measurements revealed that this procedure removed more than 95% of the conductive substances originally present in HS clots. Cells proliferated well on washed PS clots; washing reduced but did not eliminate cytotoxicity of HS clots [3].

Thus, the nearly absent structure of transparent fine clots appears to have a certain cytotoxic effect on fibroblasts in vitro. We consider this finding to have important implications in vivo. Although it may be assumed that the soluble components will diffuse out of a clot slowly, the typical "fine clot" structure will persist.

The importance of a stabilized fibrin network on fibroblast growth deserves special mention. The latter grow faster on cut PS clots than on the very smooth surfaces formed at the liquid-air interface of noncut clots. On the other hand, the same treatment on the cut surface did not improve fibroblast growth of washed HS concentration clots.

Beck et al. [17] in 1962 found that factor XIII is essential for normal fibroblast proliferation, they attributed the wound healing complications associated with factor XIII deficiency to a disturbance of fibroblast growth. These findings were later confirmed and extended by other investigators [18,19, 20]. According to Bruhn et al. [21], fibroblast proliferation is stimulated by the presence of factor XIII, whereas according to Kasai et al. [22], crosslinked fibrin rather than factor XIII is essential for the adherence of fibroblasts to the substrate and for well oriented cell growth. It was shown further that factor XIII itself may have an inhibitory effect on epidermal cell proliferation [23].

Our results indicate that crosslinked fibrin promotes attachment and growth of (human) fibroblasts only if present as PS coarse clots, whereas HS fine clots do not stimulate fibroblast proliferation and actually damage them, even at a comparable degree of crosslinking and after the additives that caused the formation of the fine clot structure have been removed.

Cryoprecipitate and glues from whole blood [2], carry other problems, such as poor standardization, lack of quality control, no virus inactivation, or little strength (e.g. AF, Table 5); the last point was corroborated by Hamm and Beer [24]. Other materials like COHN-fraction [24], though having good tensile strength, have very high viscosity as a major drawback.

It is obvious when comparing their different properties that synthetic sealants like acrylates [25] or gelatine-resorcin-formaldehyde [26, 27] have very limited applications.

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Lysis and Absorption of Fibrin Sealant (Tissucol/Tisseel)

(In Vitro and In Vivo Experiments)

H. PFLÜGER

Key words: Fibrin, wound repair, fibrinolysis, ^{125}I-elimination

Abstract

In order to determine the optimal fibrin thrombin adhesive system (FTAS) composition for resistance to fibrinolysis, in vivo lysis was tested by adding increasing amounts of the fibrinolysis inhibitor aprotinin to ^{125}I-FS; urokinase and plasminogen were administered in vitro while measuring protein and iodine^{125} release. The correlation between protein and iodine^{125} release clearly reflects the interdependence of these parameters; disjunction of radioactivity from the protein molecule was ruled out. In vivo, fibrinolysis is inhibited to a nearly unlimited extent by aprotinin. In vivo, aprotinin improves fibrinolysis inhibition only up to a maximum of 1500 KIU/ml clot, thereby significantly altering the maximum elimination of ^{125}iodine and FS half-life as well. Higher doses of aprotinin applied in vivo remain without effect upon FS stability. In human surgery, the addition of aprotinin to FS is recommended for strictly hemostatic application only, not for tissue synthesis such as nerve and microvessel anastomoses in plastic reconstructive surgery.

The aim of the second study was to investigate the degradation of fibrinogen thrombin adhesive system (FTAS) and the process of wound healing after partial kidney resection in rats using FTAS for induction of local hemostasis. In 28 rats partial kidney resection was performed bilaterally. Hemostasis was achieved with FTAS. Four experimental groups were formed. Group F (n = 3): hemostasis with unlabeled FTAS, subcutaneous injection of 0.1 ml = 60 μCi Na ^{125}I. Group G (n = 3): hemostasis with unlabeled FTAS, subcutaneous injection of 0.1 ml = 60 μCi ^{125}I-labeled FTAS, Group H (n = 6): hemostasis with ^{125}I-labeled FTAS. Group I (n = 16): treated like group H. In groups F–H ^{125}I elimination in 24-h urine samples was determined with a gamma-scintillation counter. Pairs of animals in group I were killed after 2, 6, 12, and 24 h and 3, 7, 14, and 21 days.

Kidneys were examined under the light and electron microscope and by autoradiography. In animals of groups G and H two peaks of ^{125}I excretion were observed: one peak within the first 48 h postoperatively which corresponded to the amount of free iodine injected with FTAS (FTAS contains 15% free iodine) and a second peak after 120 h which was most probably due to the degradation of FTAS. Fibrinolysis was not observed. FTAS was resorbed mainly by macrophages. The time course of wound healing paralleled that of physiological fibrinogen concentration. Renal parenchymal damage was not observed.

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Introduction

Fibrin plays a central role in the physiological process of wound healing. According to examinations by Key [8] fibrin induces the chemotaxis of polymorphonuclear granulocytes and introduces the initial inflammatory phase of the healing process. There is no doubt that the concentration of fibrin and the platelet content of the thrombus as well as a variety of other factors are in direct interaction and influence the duration of the healing process.

Use of Tissucol, a sealing method that has been employed for years, imitates the physiological process, applying unphysiologically high concentrations of fibrinogen. The influence of the artificial clot on chemotaxis and the resulting induction of macrophages and fibroblasts and of collagen fiber formation is unknown. Other unanswered questions are

a) the importance of the local potential of the sealed tissue for lysis and degradation of the fibrin clot and
b) the necessity of adding fibrinolysis inhibitors to the film clots and their appropiate concentrations. It was the objective of the experiments described below to test the fibrinolysis of a Tissucol clot in vitro with and without proteinase inhibitors, and to obtain further results on cellular fibrin degradation in in vivo experiments.

First Study

Materials and Methods

In Vitro Experiment

0.1 ml ^{125}I-FS Human Immuno (60 µCi/0.1 ml) was clotted by adding 0.1 ml thrombin (4 NIH-U/ml) and $CaCl_2$ (0.04 *M*/Liter) and incubated for 30 min at 37°C. Aprotinin (5000 KIU/ml clot) was added to series A. There was no aprotinin in series B.

In vitro lysis of FTAS was performed by layers of 1 ml urokinase (5.25 Plough-U/ml) and 1 ml plasminogen solution (0.2 CTU/ml) at 37°C permanent incubation. The supernatant was exchanged every 12 h. Protein content was established photometrically at an extinction of 280 nm, and the content of ^{125}I was measured by a gamma-scintillation counter.

In Vivo Experiment

Twenty-one albino rats (Wistar) with an average weight of 320 g were used as test animals. The animals were kept in single metabolite cages and fed with Tagger whole food and water ad libitum. In order to avoid any intermediary retention of ^{125}I in the thyroid gland, the animals were given 25 drops of Lugol's solution (ÖAB 9, solutio jodi aquosi) in 40 ml drinking water 3 days before the tests were started. In Ketalar (60 mg/kg body weight) and Rompun (8 mg/kg body weight) general anesthesia, two skin pockets of 1.5×0.5 were formed on the back of the animals and 0.2 ml FTAS was injected into these pockets.

Fibrin sealant:
0.1 ml ^{125}I-FS human Immuno (60 μCi/0.1 ml)
0.1 ml thrombin (4 NIH-U/ml)
$CaCl_2$ 0.04 *M*/liter

Group C ($n = 7$) was treated without aprotinin, while in group D ($n = 7$) 1500 KIU/ml clot and in group E $n = 7$) 5000 KIU/ml clot were added to the FTAS. In animals of group C–E ^{125}I elimination was counted by gamma-scintillation counter in urine collected over 24 h until the 7th day after surgery.

All the results were indicated as mean value with standard deviation.

Results

In Vitro Experiment

The correlation coefficient of protein concentration (extinction at 280 nm) and radioactivity counted was $r = 0.97$ for both series A and series B. Regression line $y = 0.02 \times + 0.04$ (Fig. 1).

The samples with aprotinin (series A) showed slow fibrinolysis. A maximum of 5% of the total activity was absorbed per 12 h and the stability of the clot lasted for more than a week. In the samples without aprotinin (series B) the maximum degradation was found after 36 h, 40% of the total activity being released (Fig. 2). After 60 h the whole FS clot was dissolved. All the animals survived the surgical intervention and the observation period of 7 days.

The maximum ^{125}I excretion in animals of group C was found after 1.75 ± 0.5 days, in group D (1500 KIU/ml clot) after 3.2 ± 0.45 days, and in group E after 3.5 ± 1.29 days.

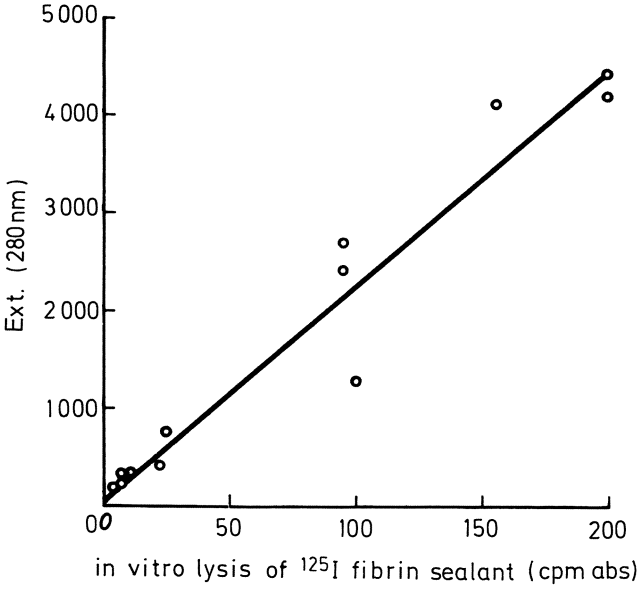

Fig. 1. Correlation of protein concentration and counted radioactivity. Measurement every 12 hours after lysis of a ^{125}I. Fibrin sealant clot with urokinase – plasminogen

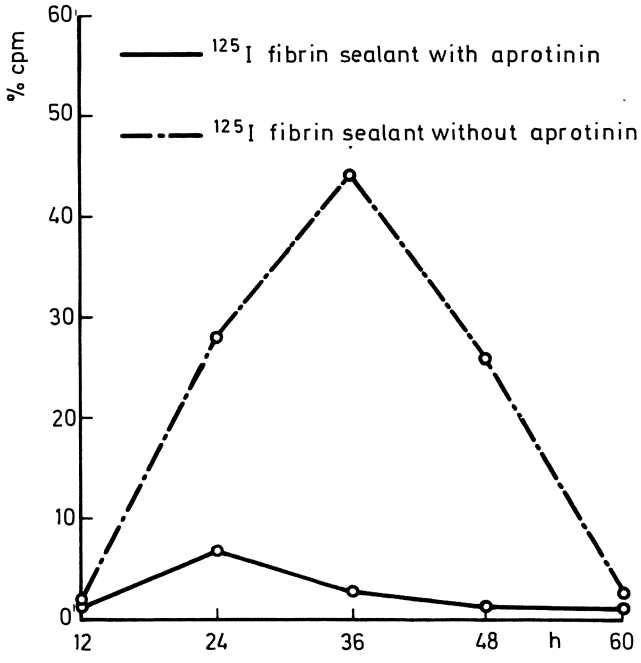

Fig. 2. Fibrinolysis expressed in radioactivity counted in 24[h] urin samples of rats

Statistical evaluation of the results by means of the hour *t*-test showed a significant time difference in the elimination maximum ($p<0.01$) between animals of groups C and D, and C and E. Comparison of the groups D (1500 KIU/ml) and E (5000 KIU/ml) showed no significant time difference in the ^{125}I excretion maximum. (Fig. 3) shows mean values of ^{125}I elimination as a percentage of the total dose applied in animals of groups C and D during an observation period of 7 days. In animals of

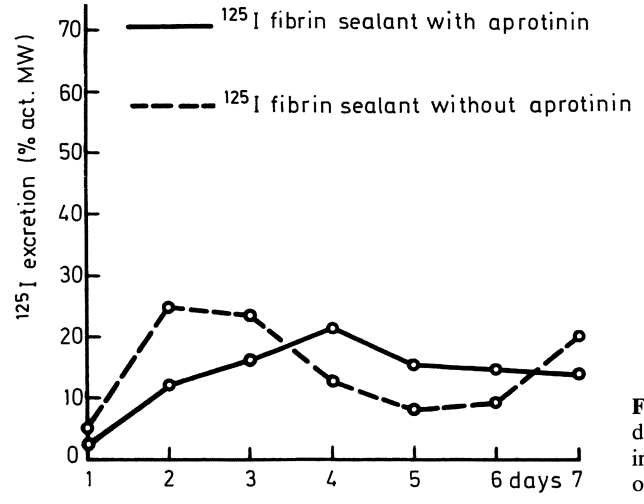

Fig. 3. Mean values and standard deviation of ^{125}I.-excretion in 24[h] total urin, indicated in % of total excretion per 10 days

group C we found a two-stage course of the graph: A first elimination peak occurred after 2 days ($\bar{x}$~24%); ^{125}I excretion then reached a minimum on the 5th day after surgery ($\bar{x}$~8%) but a further increase was observed on the 7th day after surgery ($\bar{x}$~20%). In animals of group D this two-stage course of the graph could not be observed. They showed a slow increase in ^{125}I excretion, the peak being on the 4th day after surgery ($\bar{x}$~21%), as well as a slow decrease in ^{125}I excretion. On the 7th day after surgery 14% of the total dose applied was eliminated. ^{125}I elimination in animals of group E was almost identical to that in animals of group D.

FS Half-life

The Half-life (period of time after which half of the iodine dose applied has been eliminated) was 2.16 ± 0.13 days in animals of group C, 2.82 ± 0.31 days in group D, and 2.92 ± 0.25 days in group E. There was a statistically significant difference ($P < 0.01$) between animals of groups C and D, and groups C and E. There was no statistically important difference between groups D and E.

Second Study

Materials and Methods

In the second study we examined the degradation of fibrinogen thrombin adhesive system (FTAS) during healing after partial kidney resection in rats, using FTAS for production of local hemostasis. We followed the fate of the autologous fibrin clot histologically and by monitoring the redistribution of ^{125} iodinated fibrin fragments.

FTAS was applied on a supporting collagen fleece (Disperger, Vienna), placed on the resection wound [12], and lightly pressed digitally on to the resection area for 60 s. Twenty-eight male albino rats were used (Wistar SPF breed, average weight 350 g). The animals were kept in single cages and fed with Tagger complete food and water ad libitum. In order to achieve complete blockage of iodine absorption into the thyroid gland, all animals were given 25 drops of Lugol's solution (ÖAB 9, solutio jodi aquosi) in 40 ml drinking water 5 days before starting the experiment. Under diethyl ether anesthesia the kidneys were exposed through lumbar incisions, bilateral lower partial kidney resections were performed, and hemostasis of the parenchymatous wound was achieved with FTAS as described above. Twenty percent of the renal parenchyma was removed. The 28 animals were divided into four experimental groups:

Group F (n = 3): Bilateral partial kidney resection, hemostasis with unlabeled FTAS, subcutaneous injection of 0.1 ml = 60 μCi Na ^{125}I (Amersham, IMS, 1 P ^{125}I sodium thiosulfate).

Group G (n = 3): Bilateral partial resection, hemostasis with unlabeled FTAS, subcutaneous injection of 0.1 ml = 60 μCi ^{125}I-FTAS.

Group H (n = 6): Bilateral partial kidney resection, hemostasis with ^{125}I-FTAS.

Group I (n = 16): Bilateral partial kidney resection, hemostasis with ^{125}I-FTAS.

The ^{125}I-elimination in a 24-h urine sample from animals in groups F–H was measured by a gamma-scintillation counter daily up to the 10th postoperative day.

For morphological studies pairs of animals of group I underwent laparotomy 2, 6, 12, and 24 h and 3, 7, 14, and 21 days after surgery. The kidneys which had been partially resected were perfused with Hanks' solution to remove all intrarenal blood and then perfused for 10 min with 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.4) [9]. The tissue samples were embedded in Epon 812 and 1-μ m sections were stained with 1% toluidine blue. For autoradiography Kodak Nuclear Track-Emulsion was applied to the sections, the exposure time being 28 days at 4°C. Ultrathin sections were examined in an EM9S electron microscope.

Serum creatinine and BUN were determined photometrically on the 3rd and 10th postoperative days.

Results

General

No animal died immediately after operation or within the period of observation. Three animals developed a unilateral wedge-shaped, ischemic renal infarction. Parenchymatous destruction to a maximum depth of 3–20 tubular lumina could be found in all other kidneys. In two cases a stone was found in the renal pelvis. Diffractometric X-ray analysis showed the stone composition to be calcium oxalate monohydrate. No animal developed uremia.

Dynamics of ^{125}Iodinated FTAS and ^{125}I Sodium Thiosulfate

Mean values and standard deviations of the ^{125}I excretion in 24-h urine samples indicated as a percentage of the ^{125}I total excretion during the 10-day observation period for animals of groups F–H are shown in Fig. 4.

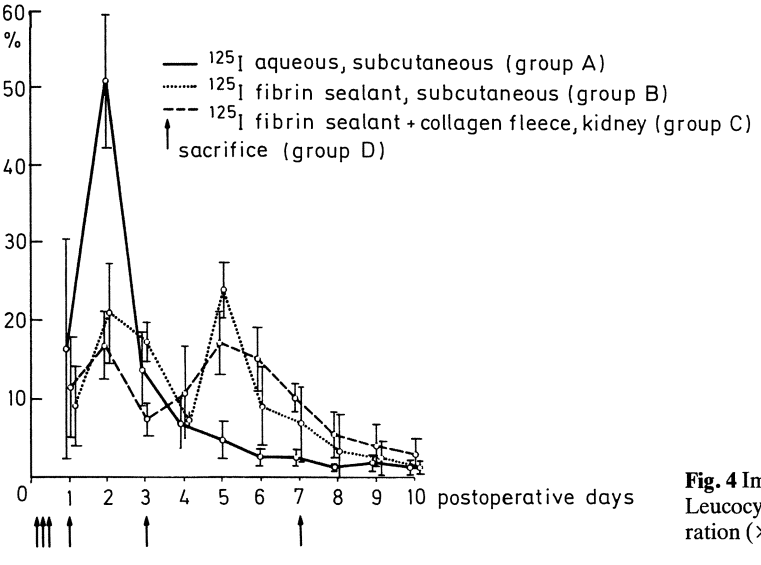

Fig. 4 Imigration of Leucocytes 24^{h} after operation (× 4.500)

Group F (Subcutaneous Injection of 60 μCi Na ^{125}I)

^{125}I excretion was maximal on the 2nd postoperative day (50.5 ± 8.4%) and an exponential decrease of ^{125}I elimination occurred after this time. By the 3rd postoperative day 80% of the measured total dose had been eliminated. ^{125}I elimination on the 10th postoperative day was 1.5 ± 0.75%.

Group G [Subcutaneous Injection of 0.1 ml (Containing Approximately 75 mg Protein) = 60 μCi FTAS]

Maximal ^{125}I excretion occurred on the 2nd (20.6 ± 6.2%) and 5th postoperative days (23.6% ± 3.5%). The least ^{125}I elimination occurred during the 4th postoperative day (7% ± 1.3%). A slow decrease in ^{125}I elimination occurred from the 5th postoperative day onwards. ^{125}I excretion on the 10th postoperative day was 1.1% ± 0.6% of the measured total dose.

Group H ^{125}I-FTAS for Hemostasis of Kidney Wounds

Maximal ^{125}I elimination occurred on the 2nd (16.5% ± 4.2%) and 5th postoperative days (16.5% ± 3.8%). The lowest excretion rate occurred on the 3rd postoperative day (7.3% ± 2%). A slow decrease in ^{125}I elimination occurred after the 5th postoperative day. ^{125}I elimination on the 10th postoperative day was 3% ± 2% of the measured total dose excreted.

Radioisotope excretion did not depend on the daily volume of urine.

Histological, Electron Microscopic, and Autoradiographic Findings in Animals of Group I

2, 6, and 12 Hours After Operation. No reaction of connective tissue was observed under the light or on the electron microscope. Collagen fleece was inhibited with erythrocytes and partly lifted off the parenchymatous area by small hematomas.

24 Hours After Operation. Light and electron microscope studies showed emigration of neutrophilic granulocytes and macrophages into the intersticium (Fig. 5). Autoradiography showed larger amounts of labeled fibrin at the area of adhesion.

3 Days After Operation. Cell-rich granulation tissue and infiltration of granulocytes was seen under the light microscope (Fig. 6). Marked resorption of fibrin clots by phagocytosing macrophages (Figs. 7, 8), as well as capillary outgrowth, was seen under the electron microscope. A high concentration of radioactively labeled FTAS was still present.

7 Days After Operation. Collagen fiber appeared and isolated remnants of radioactively labeled fibrin were seen in the granulation tissue with numerous macrophages.

14 and 21 Days After Operation. Collagen-rich granulation tissue with a markedly decreased number of infiltrating cells was found. Until the 14th postoperative day,

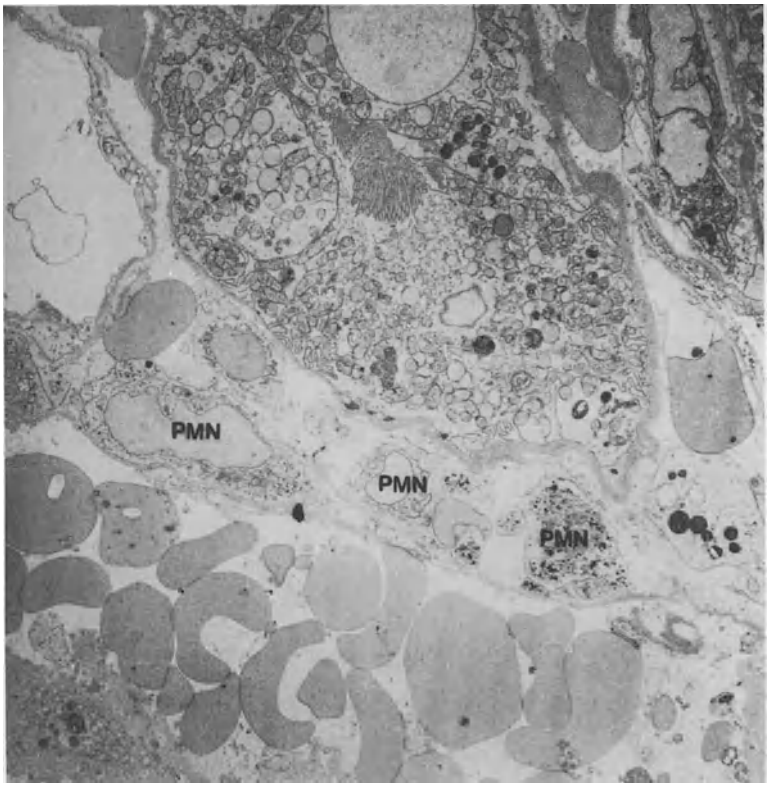

Fig. 5. Cell rich granulation tissue and Leucocytes 3 days after Operation (× 32)

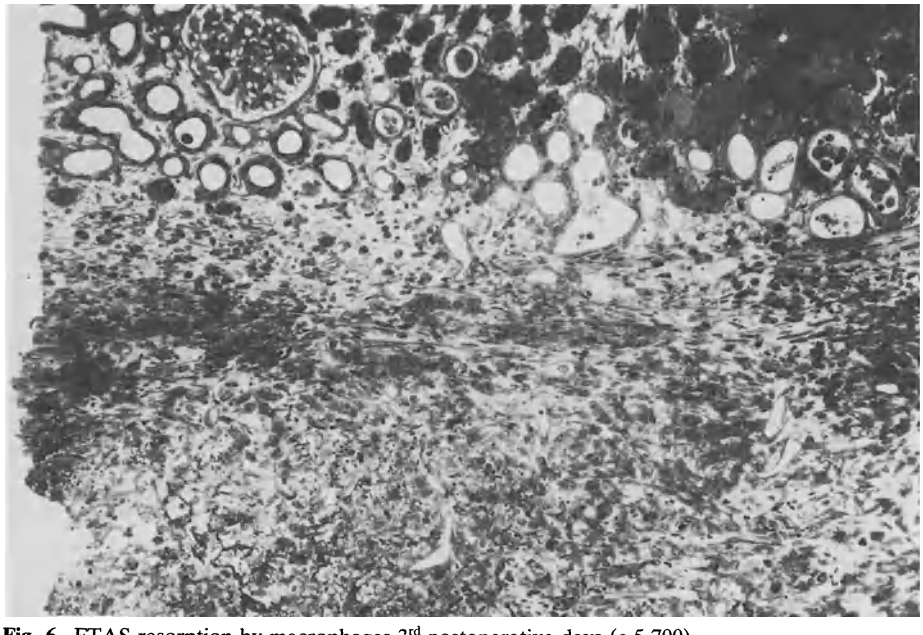

Fig. 6. FTAS-resorption by macrophages 3rd postoperative days (c 5.700)

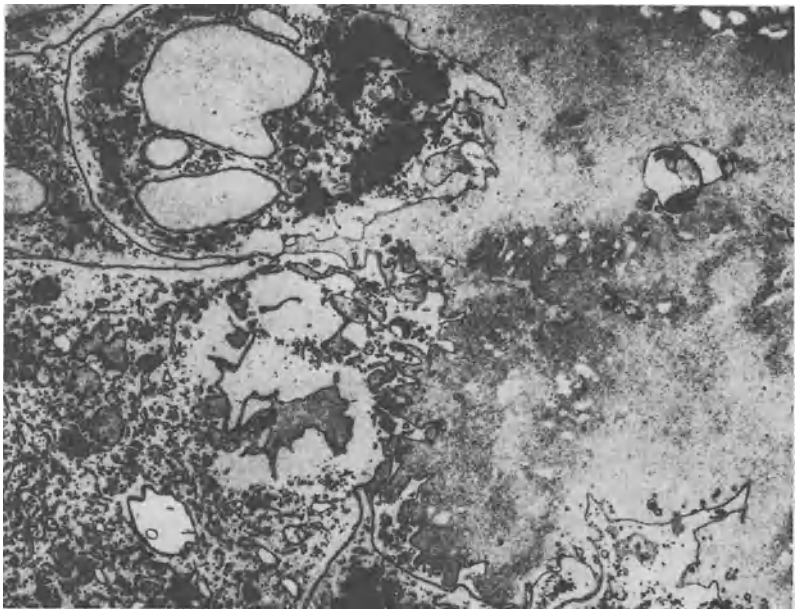

Fig. 7. Partial enlargement of Fig 6 (× 27.000)

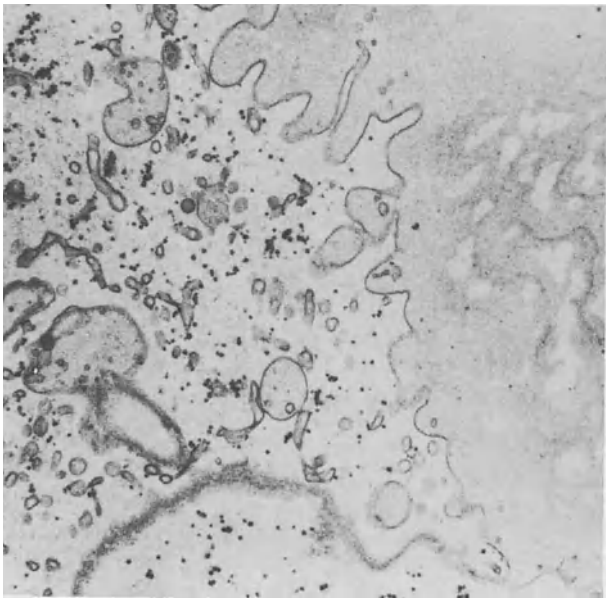

Fig. 8. Macrophages with stored labeled FTAS-degradation products 14^{rh} post-operative day (×320)

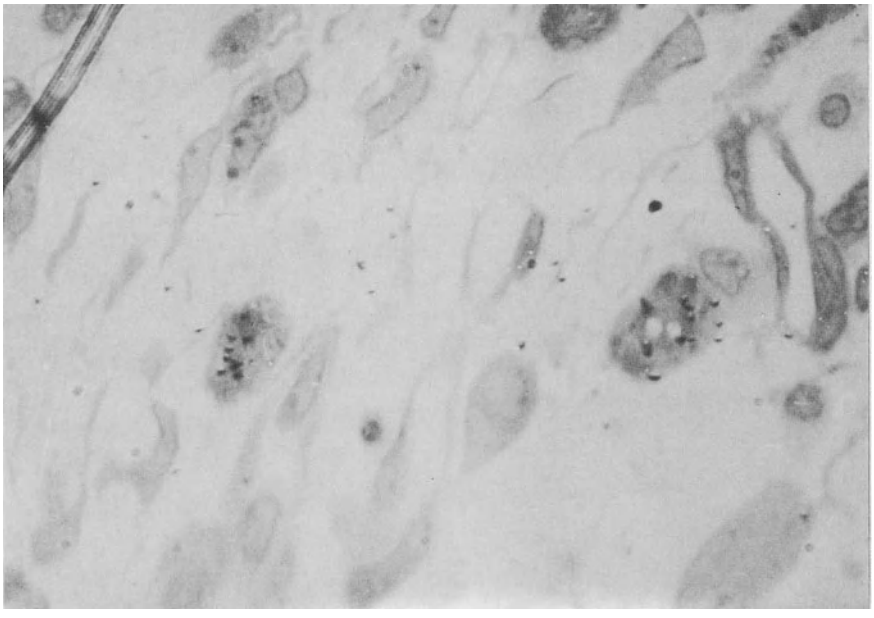

Fig. 9. Macrophages with incorporated degradation products of ^{125}I-labeled fibrin sealant

radioactively labeled fibrin was found in macrophages (Fig. 9). There was decreasing infiltration of round cells.

Discussion

Urokinase plasminogen-induced in vitro lysis of ^{125}I-labeled fibrin sealant shows an excellent correlation of both measuring parameters after measuring protein content and released radioactivity. This seems to prove that in vitro ^{125}I is not balanced out and washed off by protein molecules. It has to be presumed that measurements of ^{125}I excretion in 24-h urine in vivo are directly proportional to clot degradation.

In vitro the resistance of FS to urokinase plasminogen-induced lysis may be increased most efficiently and for as long as is wanted by the addition of aprotinin [6,11]. Measurement of ^{125}I activity released in series A and B supports these findings. As expected, the in vivo experiment showed that FTAS with aprotinin (1500 KIU/ml clot) is more resistant to fibrinolysis than FTAS without aprotinin. Increasing the aprotinin concentration to 5000 KIU/ml clot does not cause a delay in the ^{125}I elimination maximum nor any prolongation of the biological half-life. This seems to prove that aprotinin (1500 KIU/ml clot) is sufficient for stopping local fibrinolytic activity, and that the physiological degradation of FTAS by phagocytosis cannot be influenced by aprotinin.

^{125}I is mostly excreted in the urine after absorption of labeled iodine into the thyroid gland has been prevented by prior oral administration of an overdose of stable iodine. Analogous to the in vivo examinations by Alkjaersig [1] and Dudock

[4, 5] the determination of ^{125}I elimination in urine provides information about the degradation of labeled fibrin clots.

After subcutaneous injection, ^{125}I sodium thiosulfate was eliminated in the urine maximally on the 2nd postoperative day (50.5% ± 8.4% of total elimination per 10 days) in a single peak, reflecting the elimination pattern of free ^{125}I sodium thiosulfate. By contrast, after subcutaneous injection of ^{125}I-FTAS (Group G) and also after application of ^{125}I-FTAS in a collagen fleece directly to the renal parenchyma (group H) there were two peaks of ^{125}I excretion, one on the 2nd and one on the 5th postoperative day. The first peak after 2 days corresponded with the maximal excretion of unbound iodine in FTAS, which consisted of about 15% of the total applied radioactivity. (The TCA precipitable radioactivity of labeled charges of FTAS amounted to an average of 85%.) The operation itself may have delayed the maximum excretion of free, non-protein-bound iodine to the 2nd postoperative day.

The second peak of ^{125}I excretion between the 3rd and 5th postoperative days in animals of groups G and H coincided with the resorption of the fibrin clot by macrophages (group I) (Fig. 5) and may thus be derived from small iodinated fibrin fragments or from iodine freed in the process of clot organization. These data suggest that the fibrin clot was not dissolved until the 3rd day and could therefore provide hemostasis during this critical time. The protracted secretion of radioactivity after the 5th postoperative day in animals of group H may be caused by slow release of fibrinolytic fragments from macrophages (Fig. 6). We excluded the possibility that the collagen fleece interfered with the resorption of FTAS by finding that urinary iodine excretion was identical in groups G and H.

Wound healing after clot formation is initiated by emigration of granulocytes, macrophages and by capillary sprouting. (The Importance of Fibrin in Wound Repair, see G. Schlag et al.). Bösch [2] claimed that FTAS on a porous carrier accelerated wound healing in bone when compared with controls in which FTAS had not been used. Since proper controls for our kidney resections could not be obtained – because the untreated kidney wound would cause recurrent severe hemorrhage [3] and because mechanical damage of the kidney tissue may also cause conditions different from those caused by surgical treatment – no conclusions concerning the speed of wound healing in our experimental system could be drawn. In addition cyanoacrylate tissue adhesive cannot be used as a control because of its cytotoxic activity. The results of wound healing in rats after partial kidney resection and application of FTAS are similar to studies of wound healing in the rabbit's ear with physiological fibrin concentrations [7]. The use of homologous fibrinogen cryoprecipitate excluded any possible influence of foreign protein on hemostasis. Eosinophilic infiltration as a sign of allergic reaction [10] was not observed.

The wedge-shaped ischemic necroses (3/32) were due to the division at operation of a functional end-artery.

Comparing the results of the in vivo experiments with the data on the physiological wound healing process contained in the chapter "The Importance of Fibrin in Wound Repair" by G. Schlag et al., we find absolute agreement between the physiological process and the application of Tissucol as regards the time of wound healing, the inflammatory phase, and fibroplasia. The highly concentrated fibrin clot with addition of proteinase inhibitors in no way impedes the influx of polymorphonuclear granulocytes and macrophages and thus cellular fibrin degradation. Connec-

tive tissue proliferation and formation of granulation tissue are not influenced either. The excessive increase of proteinase inhibitor concentrations in the clot prevents the urokinase plasminogen-induced lysis of the fibrin clot in the in vitro test, although only a short-term delay in cellular degradation by proteinase inhibitors up to a maximum concentration of 1500 KIU aprotinin is possible. Taking into consideration that even short-term prolongation of fibrin stability induces increased influx of macrophages and thus fibroblasts and collagen fibers, it should be a clinical consequence of this examination to vary the addition of fibrinolysis inhibitors according to the clinical field of application of the fibrin sealant.

If atraumatic tissue synthesis has priority, such as in microvascular anastomoses and nerve anastomoses, no aprotinin should be added, thus avoiding unnecessary connective tissue proliferation, collagen fiber formation, and shrinking cicatrization. Sealing of parenchymatous organs such as the kidney, liver, and spleen certainly requires safe long-term hemostasis, justifying the application of aprotinin (1500 KIU/ml clot) in the fibrin clot.

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Preliminary Results of a Randomized Controlled Study on the Risk of Hepatitis Transmission of a Two-Component Fibrin Sealant (Tissucol/Tisseel)

G. EDER, M. NEUMANN, R. CERWENKA, and K. BAUMGARTEN

Key words: two-component fibrin sealant, hepatitis, ALT, gamma-GT, cerclage, conization

Abstract

A hundred patients who were to undergo cerclage or conization were entered into the study, being assigned to either group A or B on a random basis, irrespective of the type of surgery planned. Group A received conventional surgery plus two-component fibrin sealant, group B received conventional surgery alone.

The objectives of the study were to demonstrate the efficacy of fibrin sealant as a sealing adjunct in cerclage and as an aid to wound healing in conization. A further objective was to evaluate the risk of hepatitis B and hepatitis non-A/non-B transmission through fibrin sealant. Efficacy results are published elsewhere; here data are presented only on the risk of viral hepatitis transmission.

Of the 100 patients who had entered the study, 69 had a sufficient number of blood samples taken to qualify for evaluation of the hepatitis risk (group A: $n = 31$; group B: $n = 38$). None of the patients in either group contracted hepatitis B or non-A/non-B.

Introduction

Tisseel or Tissucol is a biological two-component fibrin sealant which is used to achieve hemostasis, to seal leakages, to glue tissue, or to support sutures. Tisseel has been found also to enhance wound healing [1]. The freeze-dried product is manufactured from pooled plasma of selected donors. Donors of this plasma are tested at every donation for HBs antigenemia using radioimmunoassay. To reduce the risk of nonA/non-B hepatitis transmission [2, 3, 4], only plasma of alanine aminotransferase (ALT) levels below 25 U/liter (reaction temperature 25°C, optimized method; [5] are used for manufacturing fibrin sealant. Thus far, two prospective, nonrandomized studies have been published investigating the risk of hepatitis transmission associated with the use of fibrin sealant. One such study was conducted in general surgery [6], the other in ENT surgery [7]. In neither of the two studies has a case of hepatitis B been seen that might have been attributable to the use of fibrin sealant. In a substudy to the ENT study, two groups of ten patients each were also tested for transaminase at biweekly intervals for a total period of 8 months. In none of these patients could an increase in ALT beyond 50 U/liter be seen.

The study which is described below was a prospective, randomized, controlled study investigating the efficacy of fibrin sealant as an adjunct to conventional surgical techniques employed for cerclage and conization in obstetrics and gynecology, respectively. In its context patients were monitored for potential virus hepatitis associated with its use.

Material and Methods

Patient Group Assignment Procedure

Patients were assigned to group A or B by computer random numbers, irrespective of whether they were to undergo cerclage or conization. Patients in group A received two-component fibrin sealant in addition to conventional surgical methods; patients in group B served as a control and received conventional surgical treatment only. Cerclage was performed around the 16th week of pregnancy.

When patients were entered, they received envelopes bearing consecutive numbers which assigned them to either group A or B. This made it impossible for the surgeon to give preference to one of the two methods (conventional surgery alone or conventional surgery plus fibrin sealant). Each patient consented to entering the study in writing.

Dosage

All patients in group A were treated with 1 ml of fibrin sealant, which corresponds to approx. 100 mg fibrinogen. One lot of product was used.

Laboratory Tests for Viral Hepatitis

Blood samples were taken immediately before surgery, on the 3rd, 7th, and 14th days postsurgery, and 4, 6, 8, 10, 12 and 24 weeks postsurgery. Shorter intervals, it was felt, would have led to poor patient compliance. From each sample of whole blood, serum was obtained by routine hospital methods. Two milliliters of each serum sample was deep-frozen to provide documentation samples and the rest was tested for ALT, γ-GT, HBsAg, and HBsAb. If a sample proved positive for HBsAg or HBsAb, further tests were done to clarify the patient status, including tests for HBcAb, HBeAg, and HBeAb.

ALT was determined using commercially available reagent kits (Boehringer Mannheim; GPT optimized) at a reaction temperature of 25°C (recommendation of the German Society for Clinical Chemistry) [5]. For internal quality control three commercially available control sera were used (Monitrol I and II, Merz and Dade; and Precinorm U, Boehringer Mannheim) along with an in-house serum. The controls were tested at the beginning and end of each test series. Kinetics were measured using a Beckman spectral photometer (Model 25) and printer. Samples were measured manually and extinctions were printed at 60s. intervals. Extinction

differences per minute (△ U/min) were converted into U/liter using an extinction coefficient of 1756. Measuring time: 3 min; wavelength: Hg 365 nm. The manufacturer defines the normal range for women to be ≤ 17 U/liter [8, 9].

γ-GT was determined using commercially available kits by Boehringer Mannheim (Monotest Gamma-GT new) [10]. Reaction temperature: 25°C; measuring time: 3 min; print out of extinctions every 60 s.; wavelength: Hg 405 nm. Extinction differences (△ U/min) were converted into U/liter using an extinction coefficient of 1158. The manufacturer defines the normal range for women as between 4 and 18 U/liter [11]. The internal quality control of γ-GT was performed in analogy to ALT determination.

HBsAg and HBsAb were determined using RIA-QUICK (Immuno AG, Vienna), AUSRIA, and AUSAB (Abbott). Determination of HBcAb, HBeAg and HBeAb was performed using CORZYME and HBe-EIA (ELISA Method, Abbott). Four in-house quality control sera which were calibrated against international standards were used to determine HBsAg and HBsAb. For HBsAg determination the internal quality control sera were calibrated against the HBs Reference Antigen (subtypes *ad* and *ay*) of the Paul-Ehrlich-Institute, Frankfurt/Main (concentration 50 000 U/ml), and the British Reference Preparation of Hepatitis B Surface Antigen (1st British Reference Preparation established 1982 – concentration 100 Units by definition) [12]. The limit of detection for HBsAg was also tested using the standard of the Paul-Ehrlich-Institute and was found to be 0.5 ng of HBsAg/ml. For quality control of HBsAb determination the HBV-Referenzserum (IgG) of the Paul-Ehrlich-Institute Frankfurt/Main was used in a concentration of 25 IU/vial and the WHO Anti-Hepatitis B Immunoglobulin Standard, 1st Reference Preparation 1977, lot 26.1.77, in a concentration of 50 IU anti-hepatitis B immune globulin [13, 16]. HBsAg-positive results were confirmed using the inhibition test in the radioimmunoassay. HBsAb-positive findings having a concentration of ≤ 10 mU/ml were considered negative.

Results

Altogether 100 patients (group A, $n = 50$; group B, $n = 50$) were recruited into the study; 72 underwent cerclage, 28 conization (Table 1).

An evaluation of the efficacy of fibrin sealant in the treatment of cerclage and conization has been published elsewhere. In the following only the hepatitis risk associated with fibrin sealant is discussed. To evaluate this risk, only patients were

Table 1. Patient sample and group assignment

Group	Treatment	*n*
A (with Fibrin Sealant)	Cerclage	37
A (with Fibrin Sealant)	Conization	13
B (without Fibrin Sealant)	Cerclage	35
B (without Fibrin Sealant)	Conization	15
	Total	100

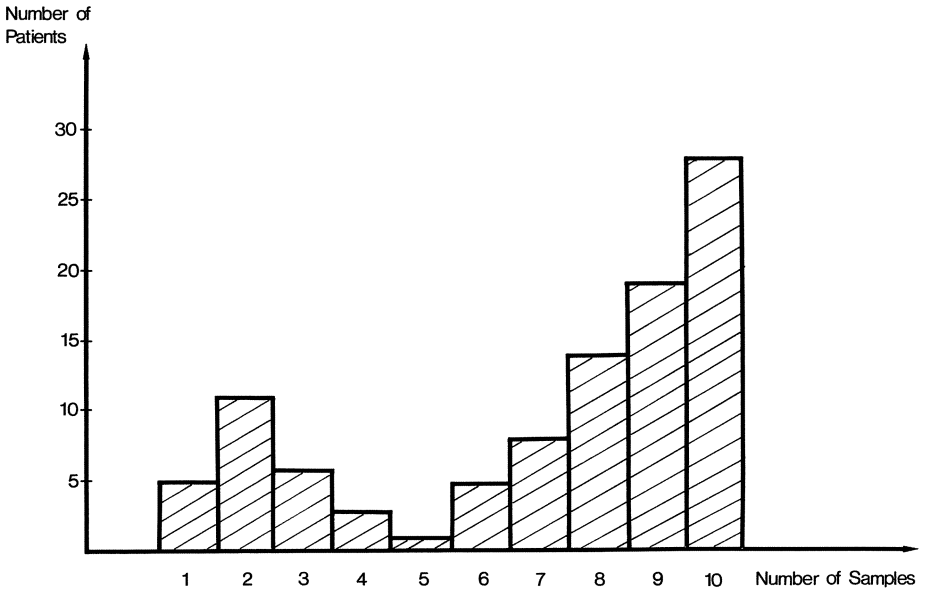

Fig. 1. Frequency distribution of patients and blood samples

used from whom at least seven consecutive blood samples (over a period of at least 10 weeks postsurgery) were available. Of the 100 patients in the study only 69 presented for blood sampling seven times or more (Fig. 1). Table 2 gives the proportion of patients who had at least seven blood samples taken and qualified for evaluation of the hepatitis risk and those who did not. As can be seen, 31 patients (23 cerclage and 8 conization) qualified in group A and 38 patients (29 cerclage and 9 conization) in group B. The percentage of patients undergoing conization in groups A and B was about the same (62% and 60%, resp.). In group B the percentage of patients undergoing cerclage (83%) was higher than in group A (62%).

Table 2. Proportions of patients qualifying and not qualifying for the evaluation of hepatitis transmission

	Nonevaluable patients	Evaluable patients	Sum (100%)
A/cerclage	14 (38%)	23 (62%)	37
A/conization	5 (38%)	8 (62%)	13
B/cerclage	6 (17%)	29 (83%)	35
B/conization	6 (40%)	9 (60%)	15
Total	31	69	100

Hepatitis B Markers

From among the 69 patients who had at least seven consecutive blood samples taken, one had to be excluded from evaluation because of receiving hepatitis B vaccination in the 4th, 8th and 12th week post fibrin sealant application.

Of the remaining 68 patients, two tested positive for HBsAb of all blood samples taken, including the preoperative one. These patients must be considered immune to hepatitis B. In three more patients HBsAb was detected on the 2nd and 7th postoperative days in concentrations below 15 mU/ml. Two of these three patients tested negative for HBsAb on all other blood samples. In one patient in group B who underwent cerclage, HBsAb findings were positive, in a concentration of as low as 13 mU/ml 6 months postsurgery. Two of the patients tested positive for HBsAg on all samples, including the preoperative sample.

Hepatitis Non-A/Non-B Markers

By definition, non-A/non-B hepatitis can only be suspected if ALT is increased postoperatively to 2.5 times the upper limit of normal. In all, 12 ALT increases (ALT > 20 U/liter) were detected, eight in group A and four in group B. Two patients (both in group B) only had slightly increased values initially (in one case 39 U/liter preoperatively, followed by normal findings on all postoperative samples; in another case a pathological 49 U/liter on the preoperative sample, which normalized in the course of the subsequent 2 weeks). Two more patients in group B had slightly increased ALT values without hepatitis B markers 6 months postoperatively (41 and 46 U/liter). One patient in each group had increased ALT values in postoperative weeks 6 and 8 (46 and 40 U/liter, resp.) without hepatitis B markers. None of the patients had clinical symptoms of non-A/non-B hepatitis.

In addition to ALT, γ-GT was monitored as an indicator of the possible presence of non-A/non-B hepatitis. Nine increased γ-GT values were found in all (six in group A, three in group B). The increased γ-GT results were often borderline. One patient in each group had preoperatively increased γ-GT (114 and 41 U/liter, resp.) which returned to normal in the course of the observation period in one patient and dropped to half the initial value in the other. One more patient in group A had a one-time increase in γ-GT of 46 U/liter 10 weeks postoperatively.

Discussion

The main ingredient of two-component fibrin sealant is fibrinogen, which is present in a concentration ranging from 70 to 110 mg/ml. Products made from human plasma are known to have the potential of transmitting viral hepatitis, unless special donor screening methods are used and/or products are subjected to a virus inactivation procedure. To exclude hepatitis B, donations have been tested for HBsAg before such plasma is used for processing into plasma derivatives ever since third generation test methods became available (radioimmunoassy and/or ELISA). This measure dramatically reduced hepatitis B transmission. Nevertheless, a high percen-

tage (up to 98%) of hemophiliacs have been shown to have hepatitis B markers [14]. In addition, chimpanzee trials have shown that infectivity of some plasmas persists despite negative HBsAg findings in the radioimmunoassay, rendering hepatitis B transmission possible [15].

The difference between the infection titer and HBsAg titer in the radioimmunoassay may be as large as two or three orders of magnitude. This means that plasmas testing negative for HBsAg can still transmit hepatitis B.

Probably, there is a relation between dosage and infectivity. As Tabor [16] has demonstrated, hepatitis B virus infectivity which might still be present in such plasmas or plasma derivatives may be neutralized by hepatitis B immunoglobulin. Therefore, addition of anti-HBs either during or after the manufacture of such products is a practical approach to prevent hepatitis B [17]. A similar immunologic neutralization of non-A/non-B hepatitis agent is not possible because neither the agent nor the protective antibody has been identified so far.

To reduce the risk of non-A/non-B hepatitis transmission, the manufacturer of fibrin sealant tests all donations of plasma for ALT levels. As early as 4 years ago serum alanine aminotransferase in donors could be shown to have a correlation with the risk of non-A/non-B hepatitis transmission [2, 3, 4]. However, experience has shown that rigorous quality control criteria – every donation with ALT levels ≥ 25 U/liter (25°C reaction temperature) is discarded and excluded from processing – reduce non-A/non-B hepatitis transmission, but do not eliminate it completely. The mechanism involved in the transmission of non-A/non-B hepatitis by fibrinogen or fibrin sealant was investigated in 1980 [18]. At that time, one lot of fibrinogen triggered non-A/non-B hepatitis in two patients and one patient developed chronically persisting hepatitis 2 years after the onset of the acute phase of the disease. The same lot of fibrinogen was injected intravenously into a chimpanzee in a concentration of 200 mg and produced typical non-A/non-B hepatitis with ultrastructural changes of the hepatocytes [19]. The chimpanzee developed an ALT level of 55 Karmen U/ml (five times the baseline 11 weeks after the intravenous administration of that fibrinogen lot). Two milliliters of pooled serum from samples drawn from that chimpanzee in weeks 4–10 postinoculation were given to another chimpanzee by the intravenous route. A typical non-A/non-B hepatitis developed in that chimpanzee 8 weeks postinoculation with that serum pool, manifesting itself in ALT increases of 4–5 times the baseline. In another study [20] a young chimpanzee was inoculated with ~100 mg of fibrinogen intravenously. The chimpanzee developed an ALT increase to 227 U/liter after a 16-week incubation period, with light microscopic and ultrastructural changes typical of non-A/non-B hepatitis. The two studies have shown that concentrations ranging from 100 to 200 mg may trigger non-A/non-B hepatitis if given intravenously. It must be borne in mind, however, that fibrinogen is not given intravenously when fibrin sealant is applied, but that clottable protein is transferred into a viscid solution which solidifies rapidly into a rubberlike mass after the addition of aprotinin, thrombin, and calcium chloride. The course of this solidification bears analogy with the physiological process of coagulation. For that reason, it is not likely that fibrinogen enters the circulation. It was the aim of this study to show that fibrinogen given in concentrations which produce non-A/non-B hepatitis if given intravenously, do not transmit non-A/non-B hepatitis if applied in the routine product combination.

Evaluation of the Risk of Hepatitis B Transmission

A prospective study on the viral transmission of hepatitis B carried out from 1979 to 1981 in the same department has shown 23 (or 0.52%) of 4400 pregnant women who were examined consecutively for the presence of hepatitis B markers to be antigen carriers [21]. The prevalence of HBsAg-positive pregnant women is determined by the ethnic composition of a patient population, particularly in countries with a low incidence of HBsAg (22). Sixty-five percent of the antigen carriers identified in the above study came from Southern Europe, Turkey, and the Philippines. The frequency of HBsAg carriers in the 3 year study varied widely. The small patient sample in the fibrin sealant study (68 evaluable patients out of 100) explains the nonrepresentative frequency of HBsAg and HBsAb in this group of women. Since in all samples (including the one taken preoperatively) HBsAb could be identified in only two patients, the percentage of patients considered to be immune to hepatitis B is too low, while the percentage of HBsAg carriers (2 of 68) is too high. One of the HBsAg carriers was a 29-year-old woman with an incompetence of the cervical canal who had a cerclage performed in the 17th week of gestation. HBs antigenemia in patients with normal liver function have been known for well over 4 years. The second case was a 33-year-old woman who had the same problem and the same intervention performed in the 15th week of gestation and gave birth in the 41st week. This patient had no history of HBs antigenemia and none of her relatives had hepatitis B.

The HBsAb which was detected in three patients on the 2nd and 7th postoperative days in concentrations of $\leq$ 15 mU/ml could not be clearly confirmed to have been HBsAb by inhibition. The concentrations were too low. HBcAb could not be detected. This suggests the HBsAb involved to probably have been a nonspecific one [23, 24].

In only one patient (group B) could HBsAb be detected 6 months after cerclage had been performed, in a concentration of 13 mU/ml. Since HBcAb was absent, this could not be considered a seroconversion. The results, therefore, suggest that fibrin sealant does not transmit hepatitis B, since none of the patients underwent hepatitis B infection serologically or clinically within the 6-month observation period.

Evaluation of the Risk of Non-A/Non-B Hepatitis Transmission

Since at the present time no serological test methods are available for the detection of non-A/non-B hepatitis virus(es) [25], the non-A/non-B hepatitis risk can only be assessed based on biochemical tests such as ALT or to a certain extent γ-GT. Some time ago determination of reverse transcriptase was described as an indicator of non-A/non-B hepatitis[26]. The sera available to us cannot be used for this determination, since this requires the plasma or serum samples to be deep-frozen at $-70°C$ immediately after they are taken, which was not done with the documentation samples collected. If typical clinical and biochemical findings were present, non-A/non-B hepatitis could only be diagnosed by exclusion of other forms of hepatitis, including cytomegaly and Epstein-Barr. Liver biopsies could not be taken for ethical reasons. Therefore, the risk of non-A/non-B hepatitis transmission was assessed

based on elevated ALT and γ-GT levels. Numerous chimpanzee studies have shown that increases in ALT or γ-GT values beyond 2.5 times the baseline or normal upper limit are indicators of non-A/non-B hepatitis.

For well over a decade, statistically significant correlations have been known to exist among age, weight, sex, and enzyme activities. A correlation between weights and ALT levels is more markedly present in men than women. In women, on the other hand, age plays a more important role in younger women (below 30). The normal range of ALT values does not exceed 10 U/liter (0.95 quantile). In women between 30 and 40 years of age, the normal range lies between 5 and 21 U/liter with a median of 9 U/liter [9]. Studies on the normal range of ALT during pregnancy (where higher enzyme activities must in principle be expected) have not been done. The small patient sample did not allow stratification by age, body weight, or weeks of gestation if cerclage was involved. For all of the above reasons, the upper limit of the normal range was defined to be 20 U/liter and the limit beyond which non-A/non-B hepatitis was present was defined to be 50 U/liter (2.5 times the upper limit of normal). γ-GT levels were interpreted analogously. However, little is known about the correlation between γ-GT, age, weight, and sex.

Since γ-GT levels are also expected to be slightly higher during pregnancy, the upper limit of normal was again taken to be 20 U/liter and the limit for non-A/non-B hepatitis 50 U/liter (2.5 times the upper limit of normal). The slightly increased ALT levels of 41 and 46 U/liter 6 months postoperatively in the two patients in group B cannot be correlated with non-A/non-B hepatitis. In one patient ALT levels were increased 5 days before delivery of twins. In the other the increased enzyme activity showed 2.5 months postpartum.

The increased ALT levels of 46 and 40 U/liter in two patients, one in group A, one in group B, are below the defined limit for non-A/non-B hepatitis. The increased γ-GT value of 46 U/liter 10 weeks postoperatively in one patient (group A) cannot be interpreted as indicative of non-A/non-B hepatitis either.

Conclusions

Two-component fibrin sealant does not transmit hepatitis B or non-A/non-B hepatitis. Of 69 patients who qualified for evaluation of viral hepatitis transmission out of 100 entered into a randomized controlled study, none had hepatitis B, seroconversion, or clinically or biochemically manifest non-A/non-B hepatitis.

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II. Ophthalmology

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Fibrin Sealant in the Treatment of Perforating Injuries of the Anterior and Posterior Lens Capsule

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Key words: Perforating injuries, traumatic cataract, lens restoration

Abstract

A microsurgical method has been developed, experimentally tested and clinically applied in perforating injuries of the lens. Removal of the damaged lens matter and closure of the capsule wounds with a human fibrinogen concentrate resulted in a high percentage of the patients in maintenance or restoration of the clear lens with a circumscribed scar in the lesion area. Even extended subcapsular traumatic rosettes disappeared in the long term, if the capsule wounds were successfully sealed. The surgical technique in the anterior lens capsule and in through-and-through perforation is described, and an analysis of the results in the first consecutive series of 31 patients treated is given with special reference to the time interval between injury and fibrinogen application, age of patients, extent of capsule lesions and technique of fibrinogen application.

Introduction

Until now, the surgeon's role in perforating injuries of the lens capsule has been just to wait, to hope for the rare favourable course of spontaneous healing and later to remove the traumatic cataract in most patients. Spontaneous healing of the capsule wound with restoration of a useful visual acuity was observed by us in 11 out of 131 perforating injuries with lens lesions (= 7.6%). An analysis of these spontaneously favourable courses gave a hint as to the role of iris trauma and spontaneous protein exsudation. Extended experimental research proved that fibrinogen application could markedly support the healing of lens capsule wounds. Several microsurgical application techniques were developed. Clinical application was started in early 1982.

Many ophthalmic surgeons do not believe that wounds of the lens capsule may heal. Again and again they have seen that even small capsule lesions usually result in progressive traumatic cataract. The posterior capsule even has no epithelium at all. That is the background to the widespread feeling that this is a hopeless situation. However, spontaneous healing has been observed and reported by a number of authors during the past 100 years, and a detailed evaluation was given by Duke-Elder [6].

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Patients, Indications and Application Techniques

This is an analysis of the first consecutive series of 31 patients with perforating injuries of the lens capsule, treated in 1982–1984. Microsurgical fibrinogen-sealing of capsule wounds was routinely applied in all patients who where admitted to our hospital and had an anterior lens capsule perforation or a through-and-through lens perforation (anterior and posterior capsule, e.g. intraocular foreign bodies). The method was not applied if symptoms of sufficient spontaneous healing were found or diffuse damage of the lens (apart from the lesion site) was already visible at slit-lamp examination. Some patients had a spontaneous scar formation initially, but later on this proved insufficient and we decided to apply fibrinogen despite the delay.

Application Technique

The Duploject device is not suitable for our purposes. We need 0.01–0.02 ml fibrinogen. A reliable 1:1 mixture of fibrinogen and thrombin cannot be achieved with the Duploject in such small quantities. Therefore, we had to apply both fluids successively. It is nearly impossible to feel the necessary small syringe piston movement, and it is nearly impossible to see the unclotted, clear fibrinogen within the aqueous humour. Stained samples of the fibrinogen glue are now on test, but were not available in the mentioned period. Thus we took care that the syringe was filled only with the desired amount, but had to make sure that the needle volume was regarded. Usually some degenerated lens matter already protrudes through the capsule wound. This is removed using suction with a blunt needle. Then, thrombin S is injected with a blunt needle, the tip located far from the capsule lesion. Subsequently the fibrinogen is directly applied to the lesion site. Unclotted fibrinogen must reach and overlap all exposed lens cortex and capsule edges. The fibrinogen should not reach the chamber angle or the corneal endothelium, and the margin of the pupil should remain fibrinogen-free for at least half of its circumference. This can be achieved by the mentioned limitation of the fibrinogen amount.

In through-and-through perforations of the lens, initially we treated the anterior capsule wound only, because we did not have a technique for the posterior capsule defect and wanted to learn about the effectivity of vitreous tamponade. Later on, we approached the posterior defect via basal iridectomy, introducing a blunt, bent needle beside the lens equator. This technique did not enable us to remove all swollen lens matter from the posterior lesion area. Finally, we approached the posterior wound by injection of the fibrinogen through the lens perforation channel with the blunt needle placed in the cortex level at the anterior lesion. In this way, the degenerated lens matter at the posterior capsule perforation is pressed into the posterior chamber together with a small amount of the fibrinogen. It forms a clot between the lens and vitreous and this becomes absorbed within a few days.

Results

The intervals between the accident and fibrinogen application were as follows: In the group of patients treated within 24 h, 40% ended up with a visual acuity of 1.2 to 0.3. In patients treated on the 2nd or 3rd day after trauma, the percentage of successful courses was the same. Two patients were treated even later, on the 8th and 142nd days, respectively, after trauma, and have so far achieved a visual acuity of 1.2 and 0.8, respectively. However, both needed (on the 80th and 176th days, respectively) a second fibrinogen application before finally a sufficient capsule scar was achieved. No influence of the *age of the patients* was found in this series. The mean age of all patients with a successful course was 28.4 years, and in the patients with an unsuccessful course (progressive cataract) 30.9 years. The eldest patient treated was 59 years old; the capsule wound healed promptly and the preexisting cataracta coronaria incipiens was not progressive in the postoperative period.

The extent of the capsule and lens lesion was of prime importance. The prognosis was better in the 12 patients in whom only the anterior lens capsule was ruptured. Eight patients showed a successful postoperative course.

In through-and-through perforations our treatment was primarily restricted to sealing of the anterior lens capsule wound (11 patients). In this way we succeeded in maintaining a clear lens with a circumscribed scar in only one case; the other ten patients developed progressive traumatic cataract. Thus, the subsequent four patients with through-and-through perforation of the lens were treated with additional fibrinogen application, using a blunt needle in the posterior chamber, introduced via a basal iridectomy. We had one success and three patients developed cataract.

Another subsequent four patients with through-and-through perforations of the lens were therefore treated with a third technique application of the fibrinogen sealant via the lens perforation channel from the anterior lesion site through the lens to the posterior capsule defect. All four patients had a successful postoperative course.

Discussion

Anterior lens capsule lesions can be treated successfully with this technique, but all swollen, degenerated lens matter has to be removed by suction with a blunt needle up to (and even somewhat behind) the capsule level. The cortex defect has to be filled with the fibrinogen. Postoperative cycloplegia is mandatory for 6 months (1% atropine once per day). The capsule scar cannot withstand the accommodation stress before and ruptured in two patients in whom the atropine treatment was stopped earlier.

In through-and-through perforations, the posterior capsule defect was sufficiently protected by formed vitreous in only one case (13-year-old boy). A more central location of the lesion (within Wieger's ligament) is important. Posterior capsule defects in which these favourable conditions are not given need to be closed by fibrinogen. Fibrinogen application with a blunt needle in the posterior chamber introduced via basal iridectomy was not too successful – it proved impossible to

remove the degenerated lens matter in this way from the posterior capsule lesion. This was better achieved in through-and-through perforations by application of the fibrinogen from the anterior capsule wound (blunt needle placed at cortex level). In this way the degenerated lens matter is pushed into the posterior chamber by fibrinogen application through the lens perforation channel. Regarding the results achieved hitherto, this technique should be preferred in through-and-through perforations.

Posterior rosettes and equatorial vacuoles usually disappear when the capsule wounds were closed sufficiently. This may need weeks, months, or even more than 1 year.

Fibrinogen appears to be a well-trained microsurgeon – it adapts the capsule margins perfectly, even in rolled-up capsule flaps. Fibrin contraction may produce capsule folds, which may smooth out in the long run.

Some surplus fibrinogen is usually present despite the small total amount used. Iris and anterior lens capsule are partially glued together, and at the pupillary margin broad posterior synechiae may be present at the end of the operation. Fortunately, this is unimportant (as long as parts of the pupillary margin and the chamber angle remained free for the passage of the aqueous). This fibrin is absorbed within a few days, and synechiae disappear completely except where traumatized iris is in contact with injured lens capsule. Such contacts, however, are rare, because we dilate the pupil. Elevated intraocular pressure was found postoperatively only in a few early cases, when a somewhat larger amount of fibrinogen was applied, resulting in seclusio pupillae. A basal iridectomy could be performed as a prophylactic measure, but proved unnecessary if adequate fibrinogen amounts were used. Applanation tonometry should nevertheless be performed repeatedly in the postoperative 48 h. We succeeded in controlling the intraocular pressure in the above mentioned cases by application of acetazolamide (Diamox) and beta-blockers. It is worth mentioning that this treatment, as a side effect, delays the absorption of the surplus fibrin.

In contrast to all the fibrin beside the lesion area, no absorption of the white scar formed at the lesion site was noted. This is difficult to understand. We suppose that the fibrinogen which came into direct contact with lens cortex protein undergoes an alteration which makes it more resistant against degradation and absorption.

Repeated applications of fibrinogen sealant in the same eye of a patient (maximum: three times) produced no local or systemic side effects. One patient (magnet extraction of an intraocular steel foreign body, one fibrin application for treatment of the through-and-through perforation of the lens) developed a partial optic nerve atrophy of yet unknown origin.

All patients received in the postoperative course, in addition to the above mentioned atropin application, Phakolen.

In conclusion, we achieved a successful course (clear lens, circumscribed scar, visual acuity 1.2–0.3) in 11 out of 16 patients, if we exclude the through-and-through perforations treated with the less-effective techniques and only consider the group of anterior capsule lesions and the through-and-through perforations treated with the further developed technique recommended now. This would be a 60.9% success rate, but the absolute figures are too small to give a reliable percentage. Neverthe-

less, it is quite clear that many lenses with perforating capsule injuries can be saved by application of these techniques.

Colour prints demonstrating the progress of lens wound healing and regression of posttraumatic subcapsular rosettes have been published in our earlier reports [1–4].

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   For further literature see Buschmann et al. [4]

Fibrogen Concentrate and Topical Antifibrinolytic Treatment for Conjunctival Wounds and Fistulas

W. BUSCHMANN

Key words: Conjunctival fistulas, fibrinogen sealant, fibrinolysis prevention

Abstract

A new microsurgical technique was developed and clinically applied in conjunctival fistulas. It is based on a fibrinogen sealant (Tissucol). Postoperatively, the remarkable fibrinolytic activity of the tear fluid was blocked by topical aprotinin application. In this way, the fibrin clot can be maintained long enough to allow conjunctival wound healing. Subconjunctival filtering blebs were not obliterated.

Introduction

In aged patients or after repeated surgical approaches in glaucoma cases, the conjunctiva is often very weak, and wound healing proves insufficient, with resulting external fistulas. It can be very difficult to close these fistulas with conjunctival flaps if larger areas of the conjunctiva are already immobile due to scars. Closure of such fistulas with a fibrinogen sealant could therefore be very helpful, but initial attempts by ourselves and others resulted in rapid disappearance of the fibrin clot within 1 or 2 days. Wound healing was still incomplete at that time and usually the fistulas recurred. In contrast to that experience, fibrinogen application at the lens capsule (see our other report) was followed by *slow* absorption of the fibrin clot and the capsule wound had time enough to heal without postoperative antifibrinolytic treatment.

Material and Method

Tear fluid and aqueous humour were examined by Blümel and Stemberger at the Institute of Experimental Surgery at Munich, to whom we sent frozen samples. The reason for the different postoperative courses was clarified. The fibrinolytic and proteolytic activity of the tear fluid was markedly higher than that of the aqueous humour [Buschmann, Stemberger, Blümel and Leydhecker, 1984]. Subsequently we studied the time for which eye drops with different methylcellulose (methocel) concentrations remained present in the conjunctival sac. Then, for postoperative topical prevention of fibrinolysis we decided to use aprotinin (Trasylol) eye drops with the following prescription: Methocel, 5.0; Trasylol, 10.0; and NaCl 0.9%–5.0 in 5-ml bottles (sterile filtration).

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

Surgical Technique

The area of fibrinogen application should be dry. Therefore, some general dehydration [acetazolamide (Diamox), e.g.] is used preoperatively. Slight compression of the globe (finger pressure) is applied at the beginning of the operation partially to remove the aqueous from the eye and the filtering bleb (if still present). A superficial abrasion of the conjunctival (and corneal) epithelium is necessary to obtain firm fibrin adhesion. Then, the fibrinogen/aprotinin solution and the thrombin S/$CaCl_2$ solution are applied. If necessary, several layers are formed subsequently to achieve a thick clot overlapping by 3–5 mm the conjunctiva/cornea surrounding the fistula. Sutures from former surgery should be removed before hand.

We wait until the fibrin clot changes to a white colour. Then, a fluorescin test is performed to make sure that watertight closure has been achieved. Trasylol eye drops are applied at the end of the operation and every 2 h subsequently. Intervals of 4 h may be used at night.

Results

Tear fluid was found to have, on the tests described, proteolytic and fibrinolytic activity, whilst primary aqueous humour proved negative in these studies [1].

This method was applied clinically in two patients with recurrent external conjunctiva fistulas following glaucoma surgery and in one patient with a dehiscent corneoscleral cataract wound and external fistulation. In all cases it was possible to close the fistulas, without obliteration of the subconjunctival filtering bleb. No disintegration or absorption of the fibrin clot was observed as long as the postoperative topical aprotinin eye drop application was continued. Wound healing is sufficient after 6–8 days; if one then stops the aprotinin application, the fibrin clot disappears within 1–2 days. Another possibility is to continue aprotinin application until the clot separates spontaneously en bloc from the wound area after reformation of the epithelium, just like a scurf on the skin after completion of wound healing.

Discussion

Healing of conjunctival or corneal wounds or fistulas by means of a fibrinogen sealant can be very effective and helpful especially in cases where stitching has become difficult or impossible. However, fibrin clots exposed to tear fluid are disintegrated too rapidly. Sufficient wound healing can be achieved if Trasylol-Methocel eye drops are used postoperatively, by which the fibrin clot can be maintained as long as necessary.

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Fibrin Sealing in Surgery of Extraocular Muscles: Experiments in Rabbits

H. AICHMAIR, F. LINTNER, and M. AICHMAIR

Key words: Fibrin sealant, extraocular muscles, strabismus surgery, recession, resection, muscular neurotization

Abstract

In 15 rabbits strabismus surgery (recession, resection, or muscular neurotization) was performed; instead of sutures, fibrin sealant was partly or exclusively used. The rabbit's eyes were enucleated 10–67 days thereafter and the fibrin-glued extraocular muscles examined histologically. A solid junction of muscles and/or muscle and sclera, formed by granulation tissue, was found in all specimens, whereas no significant scar formation or atrophy could be detected. The procedure was simple and less traumatizing than suturing; the conjunctiva showed no tendency to conglutinate.

Introduction

The first application of fibrinous glue in human patients was made on 19 December 1973, at the 2nd Surgical Department of the University of Vienna during cardiac surgery, in order to seal a vessel prosthesis. After this event, sealing with fibrin also found its place in many other surgical disciplines. In ophthalmology, however, experiments were started rather hesitatingly; on the gluing of extraocular muscles with fibrin, for example, up to now, as far as we know, only the reports of Fava et al. [3, 4] have been published. They used cyanoacrylate and fibrinous glue for strabismus surgery and for the *"Fadenoperation"* according to Cüppers and/or Bagolini and considered their results satisfactory.

Publications about animal experiments on the applicability of fibrin sealant for recession, resection and muscular neurotization of extraocular muscles [1, 2] have not been seen by us up to now, so we decided to perform a series of experiments in rabbits.

Material and Method

Fifteen rabbits (bastard animals with a mean body weight of 3 kg) were operated on both eyes, after anesthesia with urethane thiopentone sodium.

We performed the following operations, each variation on three eyes, respectively:

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

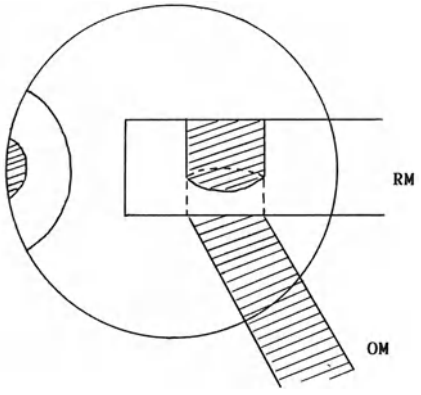

Fig. 1. Diagram of transposition method. *RM*, lateral rectus muscle; *OM*, inferior oblique muscle

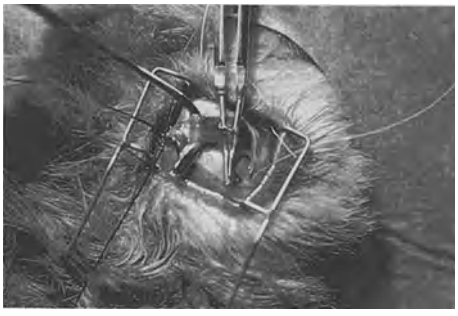

Fig. 2. Transposition operation in a rabbit: The healthy cut-off muscle is drawn by a pair of forceps through the slit in the paralyzed host muscle to its front side to be glued onto it at this point

1. *Recession:* a) Two 7.0 lateral Vicryl sutures, and gluing in the middle of the muscle
   b) One 7.0 middle Vicryl suture, and lateral gluing of the muscle
   c) Gluing only, no sutures
2. *Resection:* a), b) and c) as in recession
3. *Muscular neurotization:* The muscle to be transposed was
   a) Glued transversely across the host muscle, or
   b) Drawn from behind through a slit in the middle of the host muscle to its front and there glued onto it (Figs. 1, 2)

We used rabbit fibrin sealant of Immuno AG, consisting of fibrinogen, a solution of aprotinine-calcium chloride and lyophilized thrombin, 500 IE. Before the application of the sealant, the sclera and the surrounding tissue should be kept as dry as possible; during the sealing the site has to be free of tension. After 5 min of waiting the conjunctiva is closed again over the exposed muscle.

During the first postoperative days an antibiotic eye ointment was applied daily; no animals suffered from a postoperative infection. Most rabbits started to eat again the day after surgery and showed no change in their behavior in comparison to the time before surgery. Only four animals transiently had to have parenteral alimentation; therefore, two of these four were enucleated as soon as 10 days after the operation.

Enucleation was performed 10–67 days after surgery; then the eyes were fixed in 8% formalin for histological examination.

Results

Recession and Resection

The muscles at the new point of insertion and/or sealing were vital and showed a broad junction with the sclera, formed by a narrow strip of granulation tissue; the newly inserted muscle was drawn out cuspidally towards the limbus and presented, in its apical area as well as in some parts towards the granulation tissue, a modest fibrous lamination in an adjacent narrow strip. The conjunctiva adjacent to the muscle showed a continuous, unified course and a slightly raised fibrous content.

Fourteen days after *recession* there were formations of nuclear rods to be seen in the muscle fibers, and sporadically round cells and deposits of hemosiderin. After more than 2 months there were neither round cell infiltrates nor nuclear or hemosiderin deposits to be found.

In cases of *resection* a minimal atrophy of striated muscle fibers was demonstrated in the narrow border zone towards the conjunctiva, but in general the muscles were vital and showed no necroses or marked signs of degeneration. Only in some isolated parts of the fibrously laminated apical area did a sparse, partly also nodular round cell infiltration occur.

So at the point of sealing in recession as well as resection a broad, solid junction of muscles and sclera developed, without significant scar formation or fibrous lamination or atrophy of the muscles (Fig. 3).

Muscular Neurotization

At a distance of about 2 mm from the insertion of the host muscle a muscle could be seen running transversely (Fig. 4, upper left part), solidly adherent to the muscle running longitudinally by means of a very narrow strip of granulation tissue and/or fibrous connective tissue. The transversely running muscle showed primarily in its border areas a modest fibrous lamination with some solitary, mainly perivascular round cells.

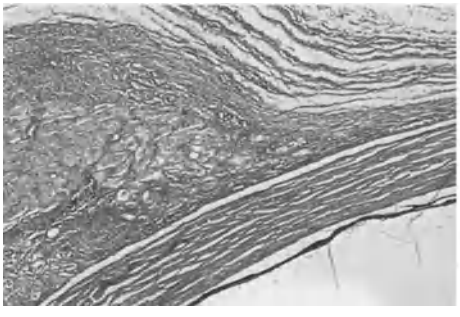

Fig. 3. Histological section of fibrin gluing (resection operation)

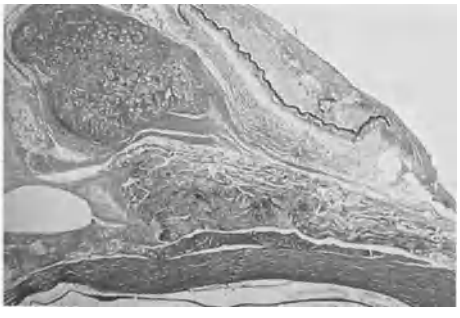

Fig. 4. Histological section of transposed muscle (*upper left*) and host muscle, connected by means of fibrin sealant instead of sutures

A junction of the muscles by a narrow formation of granulation tissue could be demonstrated, as well as the absence of a significant loss of muscle fibers due to cicatrization or atrophy.

Discussion

For strabismus surgery, the possibility to create a durable junction of muscles is of great importance, for recession and resection as well as for the newer method of muscular neurotization, where the transposition of an innervated muscle onto a paralyzed one leads to a growth of medullated nerve fibers and motor end plates into the host muscle and, slowly, to its reinnervation [1, 2].

Thanks to the more sophisticated suture materials today the surgeon causes considerably less traumatization of extraocular muscles than in former decades. However, with such methods as the *"Fadenoperation"* the possibility of a muscle lesion still exists, as well as of complications such as perforation of the globe or intraocular hemorrhage. Therefore, for a long time alternative methods have been looked for, and hence the gluing of muscles had seemed to be an ideal solution. There was the difficulty, though, that only synthetic glues were available which led to a rapid formation of new connective tissue and hence a solid junction, but on the other hand encouraged exaggerated formation of new connective tissue. Consequently, in 1981 Flick [5] reported rather sceptically on this method and pointed out that the sealing was sometimes effected so rapidly that a correction during the operation was no longer possible.

Contrary to this, sealing with fibrinous glue is effective only after some minutes. The conjunctiva near the area of surgery has almost no tendency to conglutinate and so can be closed without difficulty. With this method, no adhesions of wide areas and no bulging in the surgical zone because of reactive inflammatory processes and corresponding formation of new connective tissue were to be seen, so that the detrimental effects of synthetic glues could be avoided.

The rabbit experiments proved that in all three operation techniques (recession, resection, muscular neurotization) the muscles could be connected solidly with the sclera and/or each other, even without sutures, so that we started to test the fibrin sealant in human patients, too. The results have been satisfactory, but up to now we do not have long-time follow-up results.

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Intraocular Effects of Rabbit Fibrin Sealant Used in Experimental Retinal Holes and Detachments

I. NASADUKE, and G. A. PEYMAN

Key words: sealant (autologous, biodegradable), retinal holes, rabbit, vitreoretinal surgery, retinal detachment

Abstract

Contemporary vitreoretinal surgical techniques may prove ineffective in closing a posterior retinal break if traction on the tear cannot be relieved. In such cases a biodegradable nontoxic sealant may prove beneficial by plugging the retinal break temporarily.

We studied in adult pigmented rabbits the intraocular effects of a freshly mixed sealant containing pooled rabbit plasma/protein, fibrinolysis-inhibitor solution with bovine aprotonin, calcium chloride, and bovine thrombin. The fibrin sealant proved nontoxic to the retina by electroretinography and appeared to cause no inflammatory reaction. When the fibrin sealant was injected into and under retinal holes in experimentally induced detachments in eyes that had or had not undergone lensectomy and posterior vitrectomy, no clinical difference was found in retinal status between sealant-treated and control eyes during 3–7 weeks of observation. Histologic findings were similar in the two groups, indicating that the sealant does not stimulate periretinal or vitreal proliferation.

The rabbit fibrin – bovine thrombin sealant produced no adverse intraocular effects. A faster setting sealant with a higher thrombin concentration than that which was used might prove more effective for plugging retinal breaks in future experiments. A primate experimental model might yield results more closely applicable to human cases.

Introduction

Some retinal detachments display both rhegmatogenous and tractional components. In proliferative retinopathies [1–4] or after severe eye injuries, a resultant vitreoretinal band may tear a retinal hole at its base. Furthermore, retinal tears may be created at the time of vitrectomy by traction on membranes at the time of lysis [5, 6] or, inadvertently, by direct trauma with intraocular instruments. If traction on posterior retinal tears cannot be surgically relieved, a retinal detachment may ensue. The addition to the current vitreoretinal surgical armamentarium of a temporary plug for such retinal holes might be beneficial.

In our search for a biodegradable, nontoxic glue, we developed a technique to plug experimental posterior retinal holes in rabbits with fibrin mixed from an autologous plasma concentrate and exogenous bovine thrombin.

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

Material and Methods

To test for possible noxious effects of intraocular fibrin sealant on the vitreous or retina, rabbits were divided into three groups. In group A, fibrin was injected into the vitreous cavity to rule out noncontact retinal toxicity or vitreous proliferation by ERG, ophthalmoscopy, and histologic sectioning. In group B, a small retinal hole and detachment were created in eyes with an intact lens and vitreous. An attempt was made to plug the retinal hole to study fluid dynamics of the injected sealant at the retina/vitreous gel interface, as well as to detect possible vitreoretinal proliferation. Group C rabbits underwent lensectomy and vitrectomy with creation of a small retinal hole and large bullous retinal detachment. The distribution of sealant injected through the retinal hole in an aqueous medium and possible periretinal proliferative consequences were investigated.

Group A

Five adult pigmented male rabbits weighing 3–5 kg each were anesthetized with intramuscular ketamine hydrochloride containing acepromazine after their pupils had been dilated with 10% phenylephrine and 1% cyclopentolate eyedrops. Unipolar Burian-Allen contact lens electrodes were applied to the eyes with 2.5% hydroxypropyl methyl cellulose solution. Reference and ground needle leads were placed subcutaneously in the scalp. Electroretinographic (ERG) photopic responses from the retina to ganzfeld S8W intensities from a Grass PS-22 photostimulator were displayed on a Tektronix oscilloscope and recorded on Polaroid film. Scotopic retinal responses were similarly obtained after 20 min of dark adaptation.

Rabbit fibrin sealant was prepared. A total of 0.5 cc of a fibrinolysis solution (containing 3 000 KIU/ml bovine aprotonin, 4 mmol/liter $CaCl_2$ and 4 μg/ml bovine thrombin) was drawn up in a 1 cc syringe. Then 0.5 cc of frozen pooled rabbit sealer protein concentrate in a 1 cc syringe was thawed at 37°C. The two syringes were attached to a Y-shaped Duploject adaptor. The solutions were mixed in the barrel of the 22-gauge needle. The resultant 0.1 cc of rabbit fibrin sealant was injected intravitreally in the right eye (Fig. 1). In the left eye 0.1 cc of vitrectomy solution was

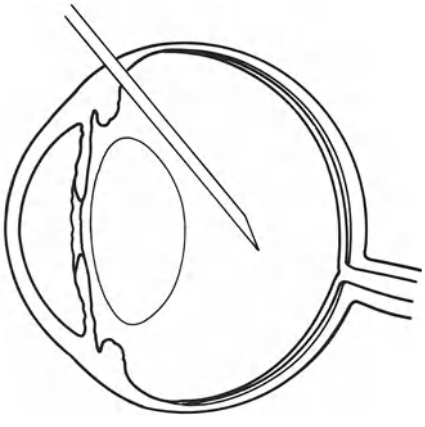


Fig. 1. Rabbit fibrin sealant or vitrectomy solution injected into vitreous cavity via sharp 22-gauge needle

injected intravitreally. An anterior chamber paracentesis was performed in each eye.

All eyes were followed up by indirect ophthalmoscopy. ERG testing was repeated in 1 month. Two months after the injections, the rabbits were killed. Their enucleated eyes were fixed in 1:1 formaldehyde/gluteraldehyde solution in a pH 7.4 phosphate buffer, grossly sectioned, dehydrated in ethanol, embedded in paraffin, sectioned on a microtome, and stained with hematoxylin and eosin.

Group B

Five adult pigmented male rabbits were prepared for surgery as in group A, with the addition of atropine 1% eyedrops. A stab incision was made in the ciliary body. A 30-gauge angulated blunt-tipped cannula was introduced into the eye. A retinal hole about ½ disc diameter (DD) in size was made 1.5 DD below the optic disc (Fig. 2). Vitrectomy solution was injected under the retina, causing a localized retinal detachment averaging about 3 DD in size. A limbal paracentesis was performed.

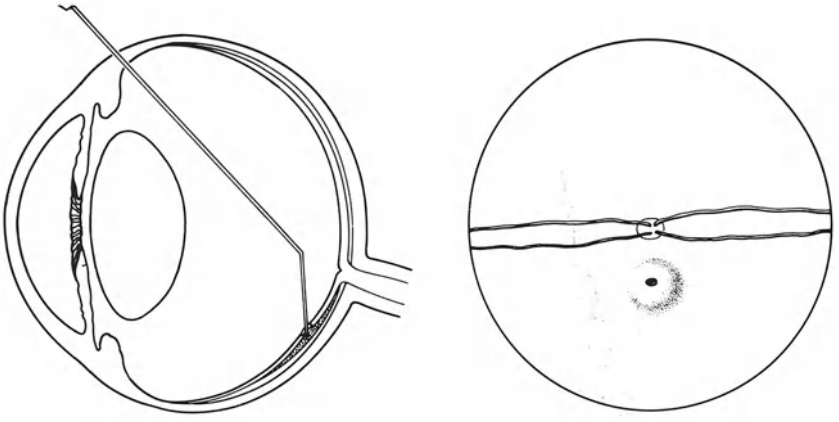


Fig. 2. Retinal held made below optic disc. Small amount of vitrectomy solution injected under retina using a 30-gauge blunt-tipped angled cannula to create a small retinal detachment

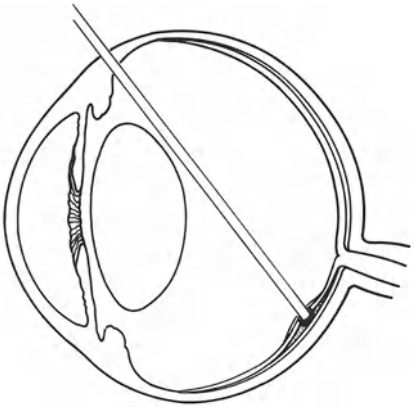


Fig. 3. Rabbit fibrin sealant injected into retinal hole via 22-gauge blunt needle

About 0.1 cc rabbit fibrin sealant mixture was injected in the right eye through the retinal hole (Fig. 3). In three eyes, the sealant ended up partly in the retinal hole and partly in the vitreous cavity over it. In two rabbits, the sealant would not stay in the hole, and rested in the vitreous gel. The left eye of each rabbit, which underwent retinal hole and localized retinal detachment formation but received no sealant, served as a control.

The rabbits were killed after 2 months of periodic ocular examination. The eyes were prepared as above.

Group C

Adult pigmented male rabbits were similarly prepared for surgery as in group B. The lens was removed from each eye (by superior limbal extracapsular extraction in 20 eyes, and using a Shock phacofragmentor in 6 eyes). Each eye underwent a posterior vitrectomy via a Peyman wide-angle vitrophage through a ciliary body sclerotomy, using vitrectomy solution for infusion. A retinal hole was made 1.5 DD below the optic disc, as in group B. An inferior retinal detachment from three-quarters to more than one quadrant in size was produced by injecting vitrectomy solution through the hole and thus ballooning up the retina (Fig. 4).

Rethawed frozen rabbit fibrin sealant was prepared as in group A. The sealant was injected into one eye in each of three rabbits; the other eye served as a control

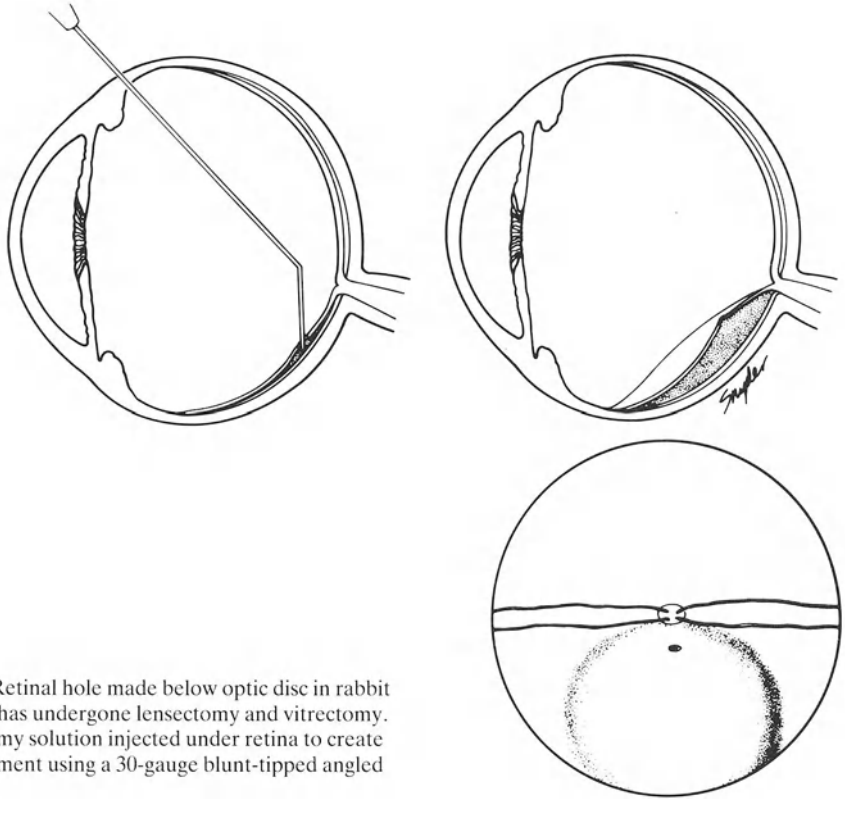


Fig. 4. Retinal hole made below optic disc in rabbit eye that has undergone lensectomy and vitrectomy. Vitrectomy solution injected under retina to create a detachment using a 30-gauge blunt-tipped angled cannula

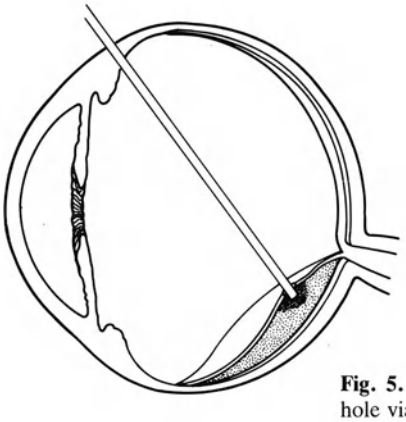


Fig. 5. Rabbit fibrin sealant injected into and under retinal hole via a 22-gauge blunt needle

after undergoing lens removal and vitrectomy. At that point, the rabbit fibrin manufacturer changed the preparation technique to that already in use for human fibrin sealant. Therefore, the remaining rabbits were treated with a reconstituted freeze-dried preparation of the same concentration as the rethawed frozen sealant used above. Freeze-dried rabbit fibrin was reconstituted with fibrinolysis inhibitor solution and placed in a 1 cc syringe, while a $CaCl_2$-thrombin solution was drawn into another 1 cc syringe. The resultant mixture, with similar ingredients to those used above, was injected through and under the retinal hole in one eye each of ten rabbits (Fig. 5).

By placing a minute quantity of sterile 10% intravenous fluorescein solution in one syringe and methylene blue in the other, the sealant appeared green as it emerged from the 22-gauge blunt-tipped needle. This permitted accurate visualization of the final location of the sealant.

In four of the ten rabbits, the other eye served as a control, having also undergone lensectomy, vitrectomy, and retinal hole and detachment formation. For technical reasons, the contralateral eye of six rabbits in this series could not be used as a control. Therefore, six eyes from three additional rabbits underwent similar surgical procedures to become controls.

All rabbits were killed after being followed up for 3–7 weeks. Their eyes were processed as above.

Results

Group A

The five rabbits that received intravitreal rethawed sealant or vitrectomy solution injections had unchanged ERG findings. On ophthalmoscopy, the retina appeared benign in all cases (Table 1). The intravitreal sealant absorbed, leaving only one tiny floater in one eye.

Table 1. Retinal status following experimental surgery[a]

Procedure	Number of eyes			
	Retina		ERG	
	Attached	Detached	Normal	Abnormal
Group A: Intravitreal injection of:				
Rabbit fibrin sealant	5	0	5	0
Vitrectomy solution (controls)	5	0	5	0
Group B: Retinal hole formation and intra-hole injection of:				
Rabbit fibrin sealant	5	0		
Vitrectomy solution (controls)	5	0		

[a] Determined by ophthalmoscopic examination prior to sacrifice, gross sectioning of enucleated eyes, and microscopic evaluation of histologic slides

One month after injection, the vitreous gel of one sealant-treated eye resembled synchysis scintillans. Two months after injection, the vitreous cavity in four of five sealant-treated eyes had the appearance of synchysis scintillans; two eyes showed a slight and two eyes a more pronounced multiple tiny reflective opacity effect.

On histologic examination, the retina appeared normal in all sealant-treated and control eyes. No vitreous debris was seen.

Group B

All five rabbits that received rethawed sealant or vitrectomy solution injection in the retinal hole had attached retinas (Table 1). One sealant-treated eye and one control eye had a retinal hole with slightly curled edges. The vitreous was clear in all but two sealant-treated eyes, in which a slight synchysis scintillans appearance was noted.

Histologic slides demonstrated no difference between sealant-treated and control eyes. The retina was attached and the vitreous cavity was clear in all cases. In general, the retina appeared normal. Only a small posterior area of scarring and atrophy was observed on a few sections at the site of previous retinal hole and detachment formation.

Group C

Two of the rabbits that underwent lens removal, posterior vitrectomy, and retinal hole and detachment formation died of pneumonia on postoperative days 18 and 20, respectively. The second of these two rabbits was excluded from the study results because of inadequate fundus visualization.

No difference was found clinically between the sealant-treated and control eyes. The rate of retinal reattachment was similar in both groups. Three to seven weeks after surgery, the retina was well attached in 6 of 12 (50%) eyes and was attached

Table 2. Retinal status following experimental surgery[a]

Procedure	Retinal state (no of eyes)				
Group C: *Lensectomy, vitrectomy, retinal hole detachment formation, with intra-hole injection of:*	Attached	Attached with retinal fold	Detached partially	Detached totally	Total
Rabbit fibrin sealant	6 (50%)	1 (8.3)	2 (16.7%)	3 (25%)	12
Vitrectomy solution (controls)	6 (50%)	1 (8.3)	2 (16.7%)	3 (25%)	12

[a] Determined by ophthalmoscopic examination prior to sacrifice, gross sectioning of enucleated eyes, and microscopic evaluation of histologic slides

with a retinal fold in one eye (8.3%) from both the sealant-treated and control groups. A partial retinal detachment was found in two (16.7%) eyes, while a total retinal detachment developed in three eyes (25%) from both groups (Table 2). Retinal detachments were associated with vitreous membranes.

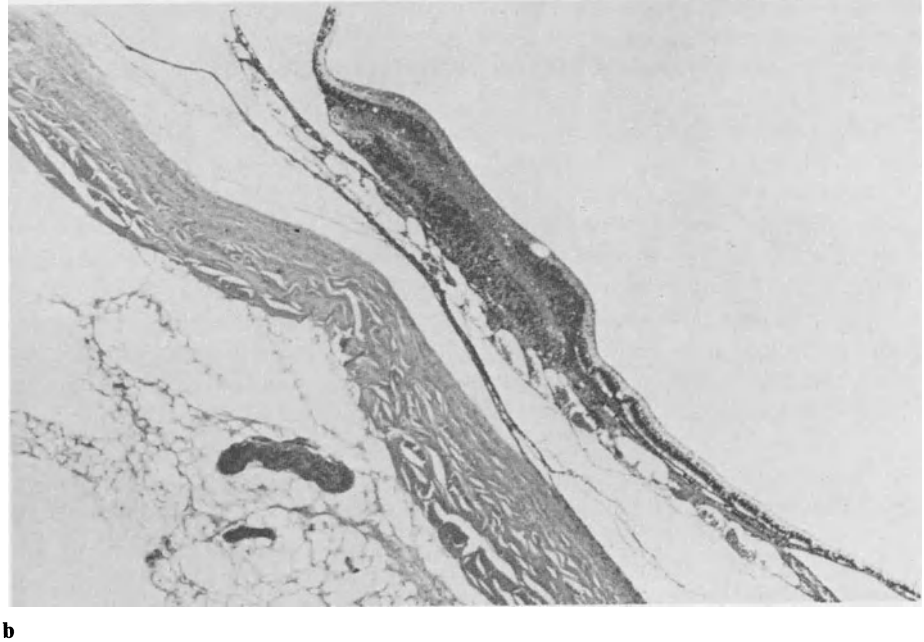
Histologic evaluation was carried out. Of the eyes that clinically and in gross section appeared to have attached retinas, four of six sealant-treated eyes and five of six control eyes had, in addition to normal retinal tissues, small retinal folds, usually one per eye and commonly located posteriorly near a healed previous retina hole but also occurring more peripherally. Small spots of retinal disorganization or scarring (Fig. 6) were located probably near a closed (former) retinal hole. Histologic findings confirmed clinical observations in eyes with retinal detachments. An increase in periretinal membrane proliferation in sealant-treated retinas as compared with control eyes was not observed.

Discussion

In 1920, Gonin [7] introduced the concept of closing retinal breaks using a thermopuncture technique for successful retinal detachment repair. In more recent years, iatrogenic retinal tears have resulted from vitrectomy and membrane dissection procedures. Between 10% and 13% of eyes [5, 6, 8] undergoing vitrectomy for proliferative diabetic retinopathy or epiretinal membrane dissection developed posterior retinal tears, half of which could not be repaired [6, 8]. If vitreoretinal traction on the posterior retinal break cannot be relieved, intraocular gas tamponade [9, 10], argon endolaser [11], or xenon arc photocoagulation [12] may prove ineffective in closing the hole. A silicone exoplant may be difficult to place and may risk damaging the optic nerve or macula [9].

In the 1940s, interest in a natural fibrin coagulum for skin grafting [13] and wound closure [14] was spurred by wartime shortage of sutures. A plasma-thrombin sealant was first used by Brown and Nantz [15] on rabbit corneal wounds. Subsequently, an autogenous fibrin sealant was used to reinforce [16] or close cataract wounds without sutures [17], to enable successful keratoplasty procedures [18], and to aid in retinal detachment repairs through transscleral subretinal injection [16].

a



b

Fig. 6a, b. Focal retinal scarring near previously made retinal hole in **a** sealant-treated and **b** control group C rabbit eyes

In more recent years, a commercially available human fibrin sealant has been applied in Europe for hemostasis, sealing leaks, end-to-end anastomoses, and wound healing in many surgical specialties [19–33].

Experimental work has also been done with this fibrin sealant in the anterior segment of the eye, for example, in corneal surgery [34–36] and for anterior lens capsule perforations [37, 38]. Rosenthal et al [39] added platelets to fibrinogen and thrombin to obtain effective corneal adhesiveness.

To our knowledge, the commercial human fibrin sealant has not hitherto been used intraocularly in the posterior segment of the eye. Our study was intended to show whether a similar rabbit fibrin sealant would cause toxicity or inflammation when injected in the vitreous cavity, and on or under the retina.

Our experiments indicated that the rabbit fibrin sealant is not toxic to the retina. It does not appear to cause an intraocular inflammatory response. Some eyes with intact vitreous developed minute reflective opacities suggestive of synchysis scintillans. Such reflective particles were not seen in eyes that had undergone vitrectomy.

The fibrin sealant could not be applied as a blob to the inner (vitreal) surface of the retinal hole because it would not adhere to the retina in vitreous gel or in an aqueous vitrectomy medium. We injected the sealant into an artificially created retinal hole, where it pooled under the retinal hole and on the retina.

In a slower setting preparation which contains 4 μg thrombin per milliliter and coagulates in 1 min, this sealant showed no effect on retinal hole closure, retinal detachment subsidence, or tractional retinal detachment formation. The faster setting sealant provided by the manufacturer, which has 500 μg thrombin per milliliter, coagulated in seconds. Because it tended to clog the mixing needle before it could be injected smoothly into the eye, it was not used in this series. An intermediate strength thrombin mixture may prove more effective in sealing retinal holes.

Our results with autologous rabbit fibrin sealant derived from pooled rabbit plasma are similar to those we obtained using an autogenous plasma-bovine thrombin coagulum to plug artificially created posterior retinal breaks in rabbits [40].

The rabbit, however, appears to be a suboptimal experimental animal for the study of posterior retinal breaks leading to retinal detachments. The retina in a rabbit, unlike that in a human, does not usually detach from a posterior retinal break. Furthermore, an experimentally induced rhegmatogenous retinal detachment may resolve spontaneously in the rabbit unless vitreoretinal tractional membranes form. For greater correlation with the human situation, a primate experimental model might be preferable in the future.

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The Use of Fibrin Sealant in Lid Surgery

F. J. STEINKOGLER

Key words: Eyelid tumours, lid surgery, skin defects, free skin transplant, fibrin sealing

Abstract

In ophthalmology the fibrin-glueing technique has been used in corneal, scleral, conjunctival and lens surgery. Fibrin sealing is also very suitable for ophthalmic plastic surgery, namely lid surgery. After excision of lid tumours skin defects can easily be closed with free full-thickness skin transplants. Optimal fixation of these autologous skin transplants can be achieved by fibrin glueing. The method is described and the results are reported.

Introduction

There are several reports on experimental and clinical use of the fibrin-sealing technique in ophthalmic surgery [1, 2, 3, 8, 9]. Fibrin glueing is also very common in plastic and maxillofacial surgery [4, 7]. Also in lid surgery this technique is favourably indicated [10]. Especially in tumour surgery (Fig. 1) in the lid area, skin defects can be closed by free full-thickness transplants with fibrin sealing.

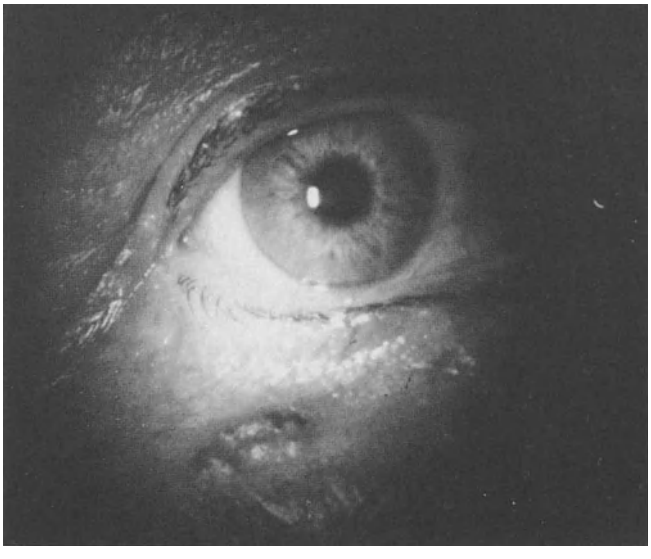

Fig. 1. Basal cell carcinoma of the right lower lid skin in a 66-year-old female patient

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

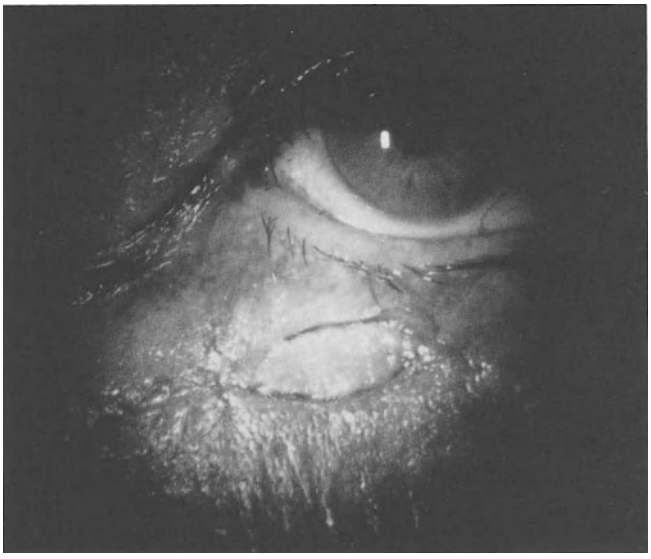

Fig. 2. Free full-thickness transplant in situ, fixed by fibrin sealant, after excision of a basal cell carcinoma of the right lower lid (immediately after the operation)

Material and Method

After tumour excision, defects of the anterior lamella (skin blade) often result. In 11 cases free full-thickness autologous skin transplants from the upper lid or from the retroauricular region were used for closing these defects. The excised skin was freed of the subcutanous tissue and prepared for grafting into the defect. For the fixation of the transplant fibrin sealant (*Tisseel*) was used. The components of the system (fibrin sealant, aprotinin solution, calcium chloride solution and 500 IU thrombin) were mixed and the skin defect was covered with a thin layer of fibrin sealant using the application set, provided by the producer. The transplant was brought to the fibrin-covered defect and was fixed on it under mild pressure for a short time. Primary coagulation takes place within several seconds (Fig. 2). No further pressure or sutures were necessary for final fixation. The wound was covered with a bandage for the first 24 h.

Results

With this fixation technique free full-thickness skin transplants can be fixed on skin defects without using any sutures. Former suggested pressure bandages on the skin transplant are no longer indicated [6]. In our 11 patients the free transplants were stable in situ as soon as the first postoperative day; their nutrition was sufficient. This method is suitable for upper and lower lid surgery, yielding good results.

Discussion

Before using the fibrin-sealing fixation technique of free full-thickness transplants either pressure bandages on the free transplant had to be used [6] or special sutures

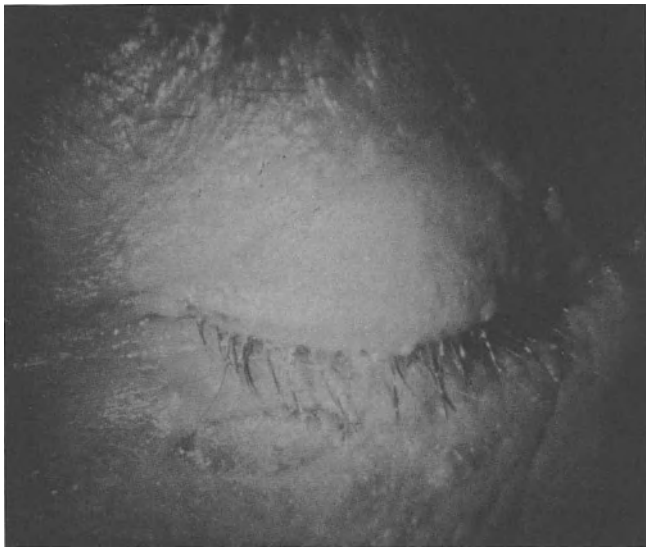

Fig. 3. Postoperative result of the same patient (7th day): primary healing of the donor area in the right upper lid; sufficient closure of the left lower lid skin defect

for keeping the transplant in the skin defect had to be made [5]. Fibrin sealing makes possible one-step procedure for the fixation of the transplant, which is easy to perform. Sutures often cause local irritation and tissue reaction. When using a fibrin-sealing technique no local reaction was seen; the fibrin sealant was always well tolerated. The primary healing of the transplant leads to an optimal cosmetic and functional result (Fig. 3).

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Experimental Experience with Fibrin-Glued Heterogenic Pericardium in Conjunctival Surgery

F.J. STEINKOGLER, and R. HADDAD

Key words: Rabbit study, heterologous pericardium, conjunctival defects, fibrin sealant, running sutures, microscopic findings

Abstract

A fibrin-glueing technique was used for the fixation of a new material for conjunctival reconstruction in a rabbit model. Thin glutaraldehyde-treated pericardium patches were glued by homologous rabbit fibrin tissue adhesive on conjunctival defects. The glueing technique was compared with conventional running suture fixation. Macroscopic findings are reported as well as the results of histological examination. Good results were achieved with thin, heterologous, fibrin-glued pericardium, yielding a matrix for reepithelization.

Introduction

Glutaraldehyde-preserved pericardium is a new material in ophthalmic plastic and reconstructive surgery [14]. Since glutaraldehyde has been used for preparing and storing xenogenic material, antigenicity was reduced [4] and this type of tissue was used instead of other transplants. Initial successful experimental and clinical studies using these xenografts [1] were carried out by cardiac and thoracic surgeons [5, 7, 8, 16].

These encouraging reports made us try this material for conjunctival reconstruction in a rabbit model and examine the use of a fibrin-sealing technique for fixation, which had not been done before. In cases in which conjunctiva from the contralateral eye [18, 19] cannot be used or local advancement flaps [3] are insufficient, homologous or heterologous transplants are necessary. Different tissues have been tried experimentally or clinically, e.g. buccal mucosa [2, 12, 17], peritoneum [6], vaginal mucosa [17], amniotic membranes [15] and lyophilized conjunctiva [9, 15]. These materials create a matrix for reepithelization [11].

The aim of our study was to compare conventional running suture-fixation technique with fibrin-glueing fixation of this new material.

Material and Methods

Equine glutaraldehyde-preserved pericardium (Xenomedica AG, Lucerne, Switzerland) patches were used for the repair of standardized conjunctival defects. In nine

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

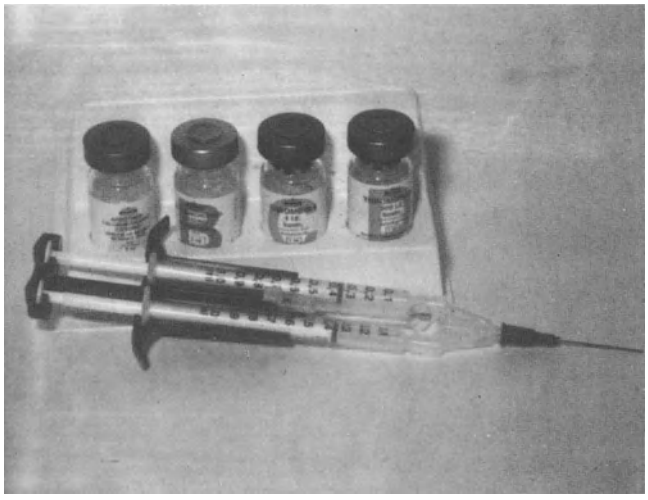

Fig. 1. Fibrin sealant application set

pigmented rabbits, anaesthetized with pentobarbital, 10-mm^2-sized pieces were excised in the temporal upper area of the conjunctiva. In nine eyes the patches were fixed by absorbable running sutures and in the other nine eyes a homologous rabbit fibrin sealant (Immuno AG, Vienna) was used [10]. The application set provided by the producer makes it easy to mix the components of the system (Fig. 1). We applied 2000 IU aprotinin and 4 IU thrombin. The defect was covered with a thin layer of fibrin sealant and the transplant was fixed on it under mild pressure for the first minutes. Postoperatively gentamicin ointment was applied for 1 week and the eyes were examined regularly. On the 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 20th and 50th days after operation the animals were killed and the eyes were enucleated and prepared for histological examination.

Results

Clinical observation showed a postoperative conjunctival irritation in all eyes, mainly in the vicinity of the patch. The sutured patches generally induced more conjunctival inflammation than the glued ones. Moderate conjunctival irritation was visible as long as the pericardium patch was in direct contact with the conjunctiva. An obvious difference between the two groups was found in the time of repulsion. Most of the sutured patches remained in situ for more than 14 days, but only one glued patch stayed in situ this long. None of the patches in any of the groups was lost before the 6th day. Some of the glued patches were slightly displaced anteriorly without causing severe irritation. Following repulsion of the patches, the conjunctival defects were obviously epithelized without marked scarring.

Histologically the beginning of epithelization could be watched on the second postoperative day. Complete epithelization under the pericardium patch was already seen on the 6th day. Smooth epithelization of the defect without marked scarring was especially found after early repulsion of the xenograft (6th–10th day)

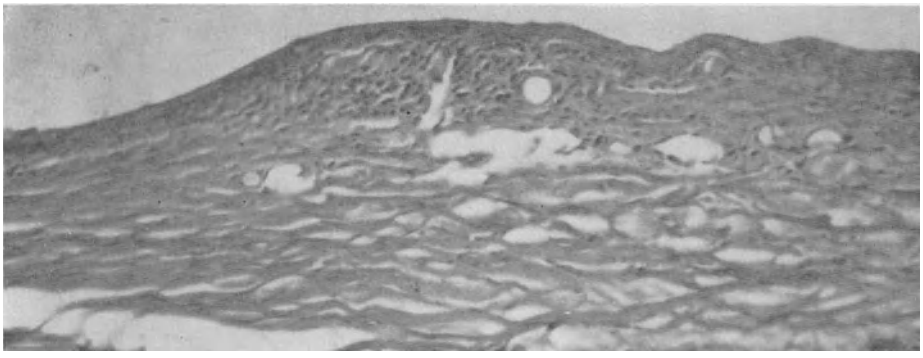

Fig. 2. Smooth epithelization of a conjunctival defect. H & E, X 50

(Fig. 2). The sutured patches elicited a more intensive stromal infiltration, occasionally preventing complete epithelization of the defect. The granulomatous inflammation of the conjunctival stroma was located especially around the sutures (polymorph nuclear cells, lymphocytes, epitheloid and giant cells). As long as the pericardium graft was in contact with the conjunctiva some stromal infiltration persisted. Around the implant and within its lamellar structures a certain amount of inflammatory reaction was sometimes present with little disintegration of the implant tissue. Severe pathological alteration of the ocular structures in the vicinity of the implant could not be found. There was only dilation of episcleral vessels with minimal cellular infiltration, but the sclera was always intact.

Discussion

The pericardium patches have served as a matrix for epithelization of conjunctival defects. Earlier experimental studies showed that this material maintains its structure, that it is easy to handle, and that it does not adhere to the surrounding structures, except at the suture line. Extremely thin equine pericardium grafts can be prepared and are suitable for conjunctival surgery. The consistency of the xenograft is sufficient to cover the defect without folding. It does not change its form or its structure. Below the pericardium, epithelization began on the 2nd day and was completed by the end of the 1st week. The glued patches were dislocated earlier and showed less inflammatory reaction of the stroma, which in turn made possible complete covering of the epithelial defect. Nevertheless a considerable inflammatory reaction around the fibrin-glued patches was found within the 1st week. Beneath the sutured implants the stromal inflammation with proliferation of connective tissue elements was significantly more pronounced. Sutured patches generally stay longer in situ, creating a chronic inflammation. At the end they are in contact with the conjunctiva mainly at the suture line, and as a result epithelization cannot be completed. The use of a thin layer of homologous fibrin sealant for the fixation of thin pericardium grafts yields the best results. After glueing, early repulsion of the patches can take place immediately after epithelization is finished. In this way

further conjunctival irritation is prevented. Fibrin glueing of pericardium heterografts will be useful in clinical conjunctival surgery.

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Fibrin Tissue Adhesive for the Repair of Lacerated Canaliculi Lacrimales

F. J. STEINKOGLER

Key words: Traumatic injuries of the canaliculi, microscopic canaliculus reconstruction, silicon ring intubation, fibrin sealing

Abstract

Eyelid traumas often affect the lower lid and the canaliculus lacrimalis. In order to prevent chronic epiphora the lower canaliculus has to be reconstructed. The best method is bicanalicular silicon ring intubation [7] combined with microsurgical reconstruction of the destroyed lid apparatus and canaliculus. Instead of atraumatic microsutures for the end-to-end anastomosis of the canaliculus, a fibrin-sealing technique was used on 14 patients. The method is described and its results are reported.

Introduction

Traumatic injuries of the lid apparatus often lead to destruction mainly of the lower canaliculus lacrimalis [3]. They are caused by various accidents, for example, traffic, work or sportsaccidents. Other reasons can be dog bites and fights [8]. The operative treatment has to be performed as soon as possible and must include the microscopic reconstruction of the eyelid and the canaliculi. Using bicanalicular silicon ring intubation [7], the end-to-end anastomosis of the torn canaliculi is usually performed by means of microsutures [2]. After using the fibrin-sealing technique in experimental and clinical ophthalmic [1, 4, 5, 9] and oculoplastic surgery, we had good results. These encouraged us to use this sealing technique in reconstructive canaliculus surgery.

Material and Method

This new technique was used on 14 patients (12 male, 2 female) with traumatic lower lid and canaliculus inferior injuries. After carefully cleaning the wound tissue differentiation and identification of the ends of the lacerated canaliculus has to be done under the microscope [2] (Fig. 1). Retrograde probing [6, 10] of the canaliculus system, including the proximal (torn) part of the canaliculus, and bicanalicular silicon ring intubation [7] are performed in the classic way. Instead of microsutures for readaptation of the canaliculus part fibrin sealing is used for the end-to-end anastomosis (Tissucol, Immuno, Austria).

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

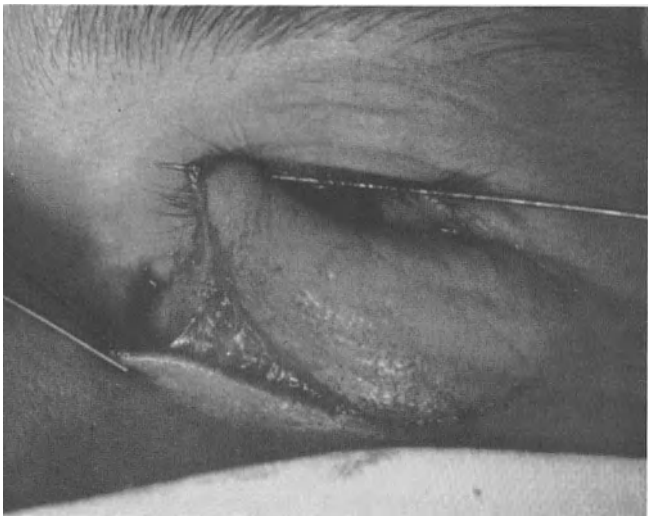

Fig. 1. Traumatic injury of the left lower lid and the lower canaliculus lacrimalis after a work accident in a 38-year-old male patient

To achieve quick primary agglutination the anastomosis area is instilled with the freshly prepared sealant, containing 500 IU thrombin. Finally the lid tendon and tarsus sutures are closed, fixing the glued canaliculus. The rest of the lid wound is closed layer by layer (in most cases the muscle is fixed by fibrin sealant and only the skin has to be sutured). The silicon tube has to remain in situ for 3–12 months.

Results

In all cases anatomical healing resulted. A good functional result was obtained in 11 patients. Only three patients complained of epiphora although irrigation was possible; all three patients are still wearing their silicon tubes. The follow-up time ranged from 3 weeks to 9 months. In six patients the silicon tubes have already been removed; these patients are free of epiphora. They have normal lid position and lid mobility and a normal position of the punctum lacrimale (Fig. 2). In these cases the lacrimal pump mechanism was restored anatomically and functionally. All of them were operated on within 5 h after the trauma.

Discussion

In cases of canaliculus inferior injuries a microscopic reconstruction with silicon intubation (for 3–12 months) has to be performed immediatly after the accident [8]. The end-to-end anastomosis of the canaliculus can be accomplished by means of fibrin sealant with good success. This success is also due to the exact reconstruction of the lid apparatus with refixation of the medial canthal tendon and the tarsus as well as an exact lid closure. Under these circumstances reepithelization of the

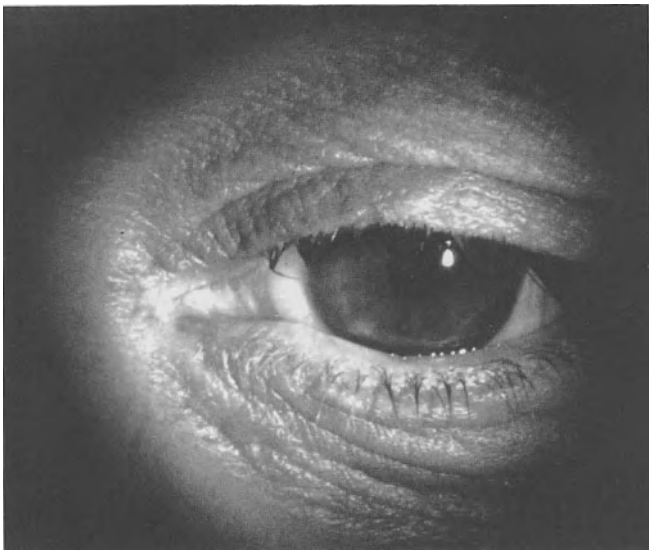

Fig. 2. Eight months after canaliculus reconstruction using the fibrin-sealing technique

proximal lacrimal system can be achieved in most cases. The main advantages of the fibrin-sealing technique are: simple and quick preparation of the glueing substance, fast anastomosing procedure without canaliculus microsutures, the possibility of orbicularis muscle glueing at the same time, marked hemostatic effect of the glueing substance, and good compatibility.

The results of canaliculus reconstruction with the fibrin-sealing technique are comparable to these after using the conventional suture technique.

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Fibrin Adhesive in Emergency Eye Surgery

K.-B. MELLIN

Key words: Conjunctival fistula, scleral fistula, scleral malacia, cyclodialysis

Abstract

It can be difficult to close a leakage in the conjunctiva or sclera. The closure of a scleral wound can be accompanied by diminution of visual function. In cases of a thin sclera or leakage into the sclera, adhesion with fibrin can be helpful. There are also new techniques to close leakages of conjunctiva with fibrin adhesive. These new techniques will be demonstrated.

Introduction

Fibrin has long been used in ophthalmology. To be effective, this adhesive substance must remain in the wound area for an extended period if it is to produce good scar formation. Adherent material does not stay fixed sufficiently on intact corneal and conjunctival epithelium. After only a few lid closures, the applied fibrin is usually dislodged. In addition, the fibrinolytic active substances present in tears and in the aqueous humour can lead to a reduction of the activity of the fibrin.

In eye microsurgery, the wound surfaces are thin and the wound edges have to be flat and in close approximation to achieve a good functional result and to produce a thin scar. These conditions cannot be achieved when treating the cornea using adhesive substances. Fibrin in this case actually enters the wound opening and results in wound widening.

Successful wound formation with suture in corneal, conjunctival and scleral surgery is still the rule today.

The support of corneal wounds with fibrin adhesive has not yet proved to be effective [5]. The application of fibrin adhesive for keratoplasty has also not been very successful [6]. The use of fibrin adhesive in the anterior chamber to treat circumscribed lens capsule trauma [1, 2] has been tried in animal experiments and also in clinical practice. The use of endogenous fibrin in the aqueous humour can result in a closure of a circular lens capsule defect and can delay lens clouding [9]. However, no clear decision has been reached in comparative studies on the use of fibrin adhesive or endogenous fibrin for the treatment of lens capsule closure.

Fibrin adhesive is not routinely used in eye microsurgery. However, the situation is very different when available suture techniques cannot be used. This is the case when tissue is not capable of healing or when a wide-faced wound is desired.

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

While fibrin adhesive adheres very poorly to intact corneal epithelium, conjunctival or scleral tendons can easily be fixed with fibrin adhesive.

Conjunctival Defect

Lypholized conjunctiva can be attached with fibrin to a plastic cover for closure of a conjunctival defect. The lypholized conjunctiva is, in this case, a stimulant for conjunctival epithelium growth. Conjunctival fistulas resulting after fistula operations for glaucoma usually present no great problems. In the area surrounding the fistula, the conjunctiva is usually avascular, atrophic and thinned; therefore, wound closure with sutures is not possible. Different techniques for fistula closure have been described using plastic conjunctiva. Scleral closure with minikeratoplasty has been reported [8]. The application of fibrin adhesive on the conjunctiva has already been performed [4]. In order to prevent fibrinolysis, it is recommended that an antifibrinolytic agent, such as aprotinin, be applied [3]. Fibrin adhesive, when used on the conjunctiva, surrounding a fistula opening, can produce fistula scarring. The quantity of applied adhesive as well as the retention time in the area of the fistula can result in conjunctival epithelial proliferation and scarring. These are of primary importance for the success of this method. A complication of this method may be the entry of fibrin adhesive into the anterior chamber. Within a few days, however, it is totally reabsorbed without permanent damage. One should carefully monitor the intraocular pressure (IOP) during this period. In the majority of cases numerous applications of fibrin lead to fistula closure.

When a wide avascular conjunctival fistula occurs after fistula glaucoma surgery, the injected fibrin may be degraded before conjunctival epithelization can take place. In these cases, the use of a conjunctival plastic patch may be effective. The technique involves opening and mobilizing the conjunctiva around the fistula. The conjunctival patch is then stretched over the fistula opening and abrased corneal epithelium is pulled down. The conjunctival patch is fixed with fibrin to the cornea and sclera and laterally with sutures. In the limbus area, a full conjunctival wound closure occurs within a few days. With this method, the intended scleral fistula remains intact.

Scleral Surgery

The other use of fibrin adhesive is in the treatment of complications of scleral surgery: scleral fistula and scleral malacia. Scleral fistulas usually appear as a result of inadequate wound treatment following perforating eye trauma. In addition, uveal tissue can become incarcerated within the scleral wound. It is advisable first to visualize the scleral fistula. Lypholized dura mater along with fibrin adhesive can then be applied to the fistula area. The entire area is then covered with conjunctiva.

Scleral malacia can develop after scleral buckling surgery for detached retina. Through the use of lypholized dura mater, the sclera can be repaired and the eye stabilized to such an extent that a new scleral buckling procedure can be performed. In this case, the applied lypholized dura mater becomes a good medium for scar formation.

Fibrin adhesive has no known adverse effects on uveal tissue and does not lead to any intraocular inflammation. Any accompanying conjunctival tissue reaction due to the fibrin adhesive is very minimal.

Rheumatoid scleral malacia can also be treated with lyophilized dura mater.

Intraocular surgery

Blunt trauma to the globe can result in ciliary body detachment. This condition can lead to global hypotonia which, if untreated, can lead to vision loss. In these cases, operative intervention to the ciliary body is usually very successful. After visualization of the cyclodialysis opening and attachment of the ciliary body to the scleral spur with 10/0 nylon suture [7], further fixation of the ciliary body can be achieved through the use of fibrin adhesive injected into the wound opening. A complication of this operation may be a rise in IOP which can interfere with further scleral wound closure. To avoid wound dehiscence, it is recommended that the sclera, in the area of the sutures, be further stabilized with lypholized dura mater.

Discussion

Based on the preceding discussion, it is obvious that fibrin adhesive plays an important role in eye surgery, especially in emergency cases. It is precisely in these emergency cases where surgical intervention can make a significant difference.

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Tissucol (Tisseel) in Surgery of the Ocular Anterior Segment

G. Falcinelli, P. Colliardo, V. Petitti, and C. Pinna

Key words: fibrin sealant, Tissucol, osteo-odontokeratoprosthesis, keratoprosthesis, corneal perforation, descemetoceles

Abstract

In surgery of the ocular anterior segment, the authors describe two conditions in which, in their opinion, fibrin sealant is indicated. These are:

a) in lamellar graft for the correction of spontaneous or traumatic perforation in highly vascularized and infiltrated corneas, and, in selected cases, in corneas with or without limited vascularization;
b) in surgery for keratoprosthesis, where fibrin sealant is used to improve the support, whether biological (osteo-odontokeratoprosthesis) or acrylic (polymethylmethacrylate), and its cohesion with the anterior segment.

Introduction

If one studies the rather meagre literature on the use of fibrin sealant (Tissucol/Tisseel) in ocular surgery, one is faced by negative, or at least doubtful conclusions about its value, arrived at by various authors [1–6]. But in our opinion there are two circumstances in surgery of the anterior chamber not yet resolved, in which fibrin sealant is of value, these being the treatment of loss of corneal substance, and during surgery for keratoprosthesis.

Regarding loss of corneal substance, it is well known that in ulcerative disease of the cornea, whether acute, subacute, or chronic, there are some forms which become complicated by spontaneous perforation. It is commonly agreed that the best treatment consists in emergency grafting, or, alternatively, conjunctival covering of the cornea [7].

In fact, perforating emergency graft, if performed in these circumstances on a cornea with fairly marked infiltration and vascularization, yields a low percentage of successful results, in spite of the prolonged and sometimes harmful use of corticosteroids and immunosuppressives. In corneas with the anatomical alterations described above, one has to conclude that grafting is a high-risk procedure, and we prefer to carry it out after tissue compatibility tests [8].

In cases where a conjunctival covering is applied, we, at least, have frequently noted a fairly marked increase in vascularization, a fact most unfavorable for the prospective graft. For this reason we decided when dealing with cases of loss of corneal substance with a fair amount of infiltration and vascularization, to treat by

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

corneal graft using fibrin sealant, and subsequently where necessary, by perforating graft after compatibility testing. The operative technique is the same as for a normal lamellar graft, the only difference being that here the fibrin sealant is used as a substitute for the corneal sutures. It consists essentially in creating a "bed," having its edges as clear-cut as possible and deep enough to accommodate a donated corneal section [9].

The preparation is quite easy if one is dealing with eyes having descemetoceles, or in which the anterior chamber is present (even if precariously) or can be reconstructed. Less simple is the preparation of the recipient bed when the anterior chamber is lacking, or worse still if the iris is implicated, or there is prolapse either of the iris or of the lens. In the various circumstances the surgeon has to create the best conditions for the lamellar graft by freeing the cornea from all extraneous tissue. He must also reconstruct an air-containing anterior chamber, essential at the moment of the application of the fibrin sealant, because the tissues to be glued together must be absolutely dry.

When the recipient bed has been prepared, by lamellar section a disk from a donated cornea is removed which has the same diameter and thickness as the bed to be covered. In the position prepared to receive the graft, by means of a syringe having a medium caliber needle, a few drops of the first component of the fibrin sealant – fibrogen in solution of 1:2 000 with aprotinin – are applied and spread in a single layer as thinly as possible using a tiny sable brush or a small hemostatic sponge.

Immediately after the application of the first component, the catalyst is applied in the same way, and within a few seconds the donated graft is placed in the prepared bed. With an iris spatula, slight pressure is applied to the graft so that it lies precisely in place and any surplus fibrin sealant is eliminated.

The catalyst is again applied in very small drops around the circumference to ensure a more rapid adhesion of the edges. Washing with physiological solution removes fragments of sealant and serves to dampen the corneal epithelium. The graft is kept in place by means of gentle uniform pressure for 3–4 min, this being the ideal time to create adhesion between the host and the donated corneas. Antibiotic ointment and bandaging which exerts a gentle pressure complete the operation.

As very few patients with this condition came under our observation, our case histories number only four. Of these, the first, who had a descemetocele, has regained vision of 3/10 and the second of 2/10 (Fig. 1). Of the other two, one with vision lower than 1/10 is awaiting a perforating graft, after eventual compatibility tests, while the other, monocular, with a remaining vision of 1/50 after treatment and having a highly vascularized and thickened cornea, subsequently underwent an odontokeratoprosthesis resulting in good visual recovery.

We have also treated, using fibrin sealant, loss of corneal substance caused by spontaneous or traumatic perforation, with or without limited vascularization, when the general health or fortuitous circumstances prevented an emergency perforating graft. On the other hand, in this type of case, with or without only slight infiltration and vascularization, we did not consider a conjunctival covering appropriate. The case reports number only two. One patient recovered 3/10 vision (Fig. 2) and the other 5/10.

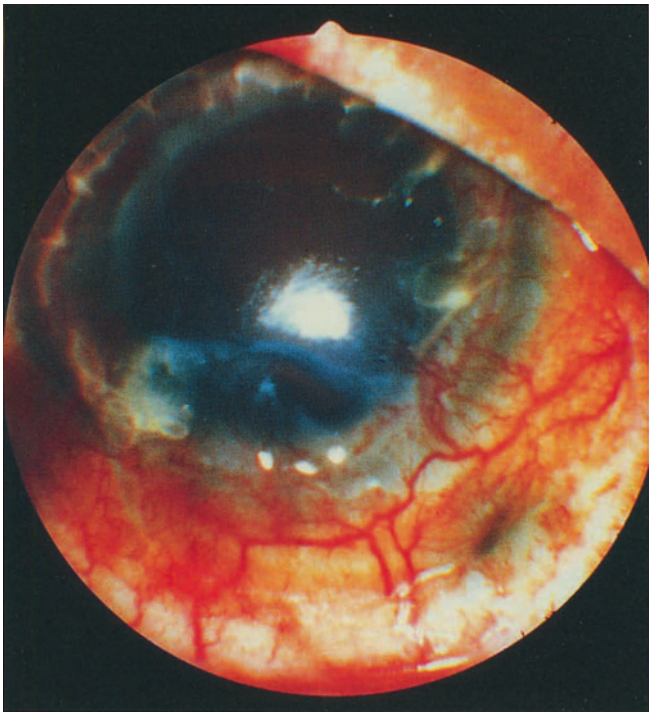

Fig. 1. Diffuse descemetocele in vascularized cornea treated by lamellar graft using fibrin sealant

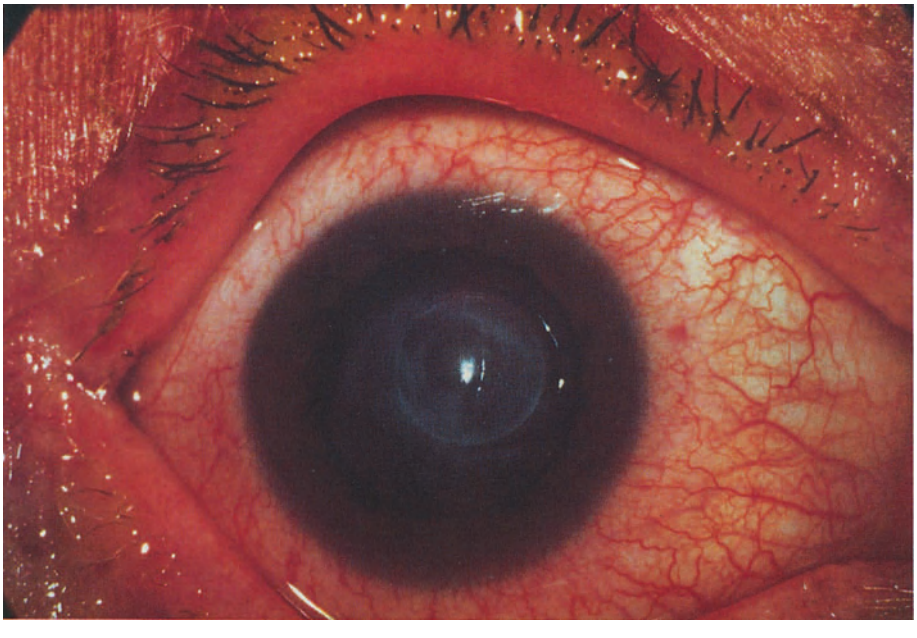

Fig. 2. Descemetocele with slight vascularization treated by lamellar graft using fibrin sealant

Let us now consider the application of Tissucol in keratoprosthesis. It must be admitted that notwithstanding continuous improvements in surgery, there are cases where keratoplasty is inevitably destined to fail. In these cases the cornea is extremely vascularized, or is severely altered in its anatomical structures; the conjunctiva, the lids, and the lacrimal apparatus are often involved. In such cases it is possible to perform a keratoprosthesis [10]. This comprises the substitution of the central part of an opaque cornea with a transparent acrylic cylinder.

The fundamental part of the keratoprosthesis is the support which serves to unite the transparent cylinder to the anterior segment of the eye. According to the nature of the support – whether it be of biological tissue or of alloplastic material – keratoprostheses are classified in two groups. The good quality of the support, whether biological or acrylic, and its correct application are the bases of the final result.

It is precisely for this reason, that is to improve the support itself and its cohesion with the anterior segment which receives it, that we have utilized Tissucol in keratoprosthesis both with nonbiological (polymethylmethacrylate), and with biological supports (osteo-odontokeratoprosthesis).

I have already described the use of fibrin sealant in keratoprosthesis with acrylic support [11]. I have described how it is employed to join the donated cornea placed over the support, and, again, sandwiched between the two divided parts of the opaque cornea. But we only use the acrylic support when it is not possible to carry out an osteo-odontokeratoprosthesis with biological support, an increasingly rare circumstance after our satisfactory results with cyclosporin, using teeth from consanguine donors. In osteo-odontokeratoprosthesis the support is derived from an osteodental lamina which maintains its vitality and is not subject to absorption. The lamina is formed from an ivory or dentine section with the dental alveolar ligament, and the bone and periosteum covering it. In the central hole executed in the lamina, using dentists' cement, the cylinder has been fixed (Fig. 3).

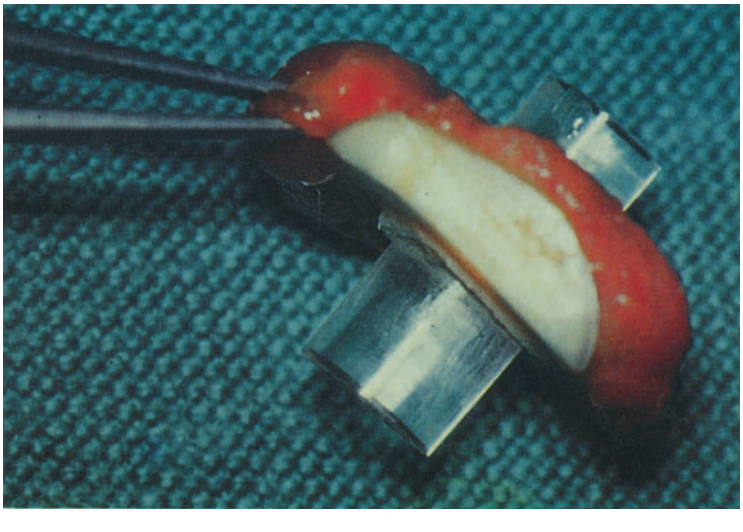

Fig. 3. Osteodental lamina with transparent acrylic cylinder fixed in the center

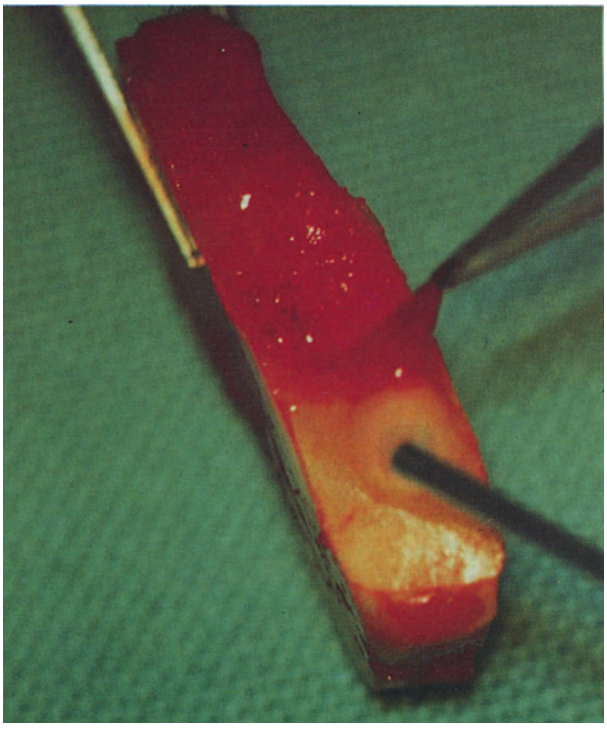

Fig. 4. Periosteum being glued to osteodental lamina with fibrin sealant

The lamina, before its insertion into the eye, has been buried for 3 months in a subcutaneous pocket. During this time the osteodental section may show signs of reabsorption. This often occurs if, during its preparation, the periosteum separates from the lamina.

Before Tissucol became available, we had discarded the cyanoacrylates because they had a toxic effect on small quantities of tissue. At that time we were using absorbable fine sutures but not always with satisfactory results. Since using fibrin sealant [11] we have had no indications of laminal reabsorption (Fig. 4).

To complete the operation the cornea is trephined in the center and the iris is removed, and the lens also if opaque. The transparent cylinder is inserted into the hole in the corneal center and sutured to it. The whole is then covered with the mucous membrane and sutured. After a few days the eye can see and the risks and complications are those possible in any serious kind of eye surgery.

In conclusion, we find Tissucol of great use in keratoprosthesis, the latter being the only possible means by which sight can be restored to patients with opaque corneas in whom it is not feasible to execute a graft.

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III. Neurosurgery

Chairmen: H. BRENNER
G. KLETTER

New Application of Fibrin Sealant in Neurosurgery

E. Benericetti, A. Dorizzi, and A. Taborelli

Key words: Fibrin sealant, arterial microanastomosis, surgical haemostasis, laminectomy membrane, encasement of cerebral aneurysms, CSF leakage

Abstract

The authors describe new utilizations of a fibrin sealant, Tissucol, in neurosurgery. Intraoperative haemostasis in the case of a chemodectoma of the glomus jugulare, the easy treatment of cerebrospinal fluid subgaleal collections, prevention of the so-called laminectomy membrane, the encasement of unclipped aneurysms, and the theoretical possibilities of preventing seeding from residual portions of medulloblastoma are discussed.

Introduction

Since Cushing and Dandy began the era of modern neurosurgery, operative results have been largely conditioned by the technological aspects, and, apart from differences caused by the particular skill and expertise of some surgeons, only improvement in the techniques of dissection, removal, suturing, haemostasis and clipping have increased the number of successful operations. The operative microscope, new ergonomic microinstruments, continuously improved microsutures, the recently developed laser and ultrasonic aspirator, together with improved microanatomical knowledge, have helped neurosurgery to make great progress.

One of the many technical aspects concerns glues and sealants. Owing to the potential risk of cerebrospinal fluid (CSF) fistulas in the case of an imperfectly closed dural layer, for many years surgeons have asked for sealants to facilitate their task. Another aim of researchers has been the repair of peripheral nerves without sutures. In 1940 Young and Medawar [13] described a nerve suture with a clotting derivative from human blood. Unfortunately, manufacturing difficulties, inconsistent results and inadequate strength of the seal resulted in a loss of interest in the method, and there was a large diffusion of acrylics, even though they were later introduced by Dutton (1956).

In an extensive review of different synthetic compounds, Handa et al. [5] in 1969 listed the characteristics of an ideal sealant. Using their theoretical model, the characteristics of fibrin and acrylic sealants are reported in Table 1. A clear superiority of natural clotting substances over synthetic compounds is apparent. Furthermore, considering the biological reactions induced by natural and synthetic compounds in different tissues, other obvious advantages of the fibrin sealant can be identified.

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

Table 1. Ideal characteristics of a sealant

Characteristic	Fibrin	Acrylic
1. High adhesiveness also on surfaces lined by fat substances and/or organic fluids	Yes[a]	No
2. Elasticity to avoid fractures when applied to elastic surfaces or necrosis of the wall when it is placed around the vessels	Yes	No
3. Time of hardening adaptable to different situations	Yes	No
4. Absence of acute or chronic toxicity	Yes	No
5. Absence of allergic reactions	Yes	No
6. Absence of carcinogenesis	Yes	No
7. Easy sterilization	No	Yes

[a] Even though not completely dry surfaces condition the differences in strength of an adhesion, it is possible also to seal not completely dry anatomical layers with Tissucol

The acrylics are not lysed or absorbed by natural processes, and since they are foreign bodies they have adverse effects on wound healing [3]. Synthetic adhesives have often been found to produce undesirable hypertrophic tissue reactions. Moreover, they may not always promote adhesion between anatomical layers, and the results in the treatment of CSF fistulas may not be as good as they appear. In contrast, the fibrin sealant is lysed and absorbed in times that can be controlled, and it promotes healing.

After a new successful approach by Matras et al. [9] in the 1970s, the fibrin sealant became a well-known method, with a consequent larger diffusion in clinical practice. New indications have been added to the old ones, so that it now has many fields of application.

Tissucol (Immuno, Vienna) is routinely used in the Neurosurgery Division of the Lecco Civil Hospital to reinforce the dural suture line, to fix dura to bone when a combined extradural and intradural approach has been used, to secure a vascular suture, and to fix tissues in the neck after carotid endoarterectomies. In most cases, Tissucol is applied with a Duploject (Immuno, Vienna) needle; Duploject may also be used with a spray head, or in other situations the sequential application may be used. In exceptional cases, Tissucol and Avitene (Avicon Laboratories, Fort Worth, Texas) (a microgranular haemostatic collagen) are blended to achieve encasement of the residual unclipped portion of intracranial saccular aneurysms or to secure clipping in an unstable position.

Methods

Table 2 lists the different uses of the fibrin sealant and alternative methods. The authors' judgment of the different alternative methods is also indicated.

Nerve Suture

The techniques and results of this field of application, which although the first is still one of the most debatable, have been described by many authors.

Table 2. Applications of fibrin sealants and alternatives

Fibrin sealant	Alternatives
Nerve suture[c]	Suture threads
Arterial suture[b]	Interrupted suture
	Continous suture
	Laser suture
	Tubes of PVA and cyanoacrylate
Reinforcing of dura[a]	Suture threads
	Suture threads and acrylics
Fixation of adipose tissue after laminectomy[b]	Adipose or other materials without sealing
Encasement of unclipped portion of aneurysms by Tissucol + microfibrillar collagen[b]	Biobond
Wrapping with muscle fixed by Tissucol of unclippable aneurisms[a]	Different tissues and acrylics
Haemostasis of vascular gaps by venous patch[a]	Suture threads
	Bicoagulation (sometimes)
	Laser sealing (argon)
	Biobond applied by metal shield (Sugita)
Intratumoral haemostasis by Surgicel then fibrin sealant[a]	Unknown by us
Treatment of CSF subgaleate collection[a]	In the *acute* phase: evacuate CSF, reduce subarachnoid pressure and apply compressive dressing
	In the *chronic* phase (when surfaces soaked by CSF become smooth and nonadherent): unknown by us
Encasement of residual portion of medulloblastoma on the brain stem to avoid cellular seeding in the interval before radiotherapy[b]	Laser removal of residual tumoral tissue (when possible)
	Nothing else known by us

[a] Excellent
[b] Good
[c] Indifferent

Arterial Suture

There have been many reports on this point [2, 8]. Here is described a case of deep microvascular anastomosis between the two anterior cerebral arteries. We performed an end-to-end anastomosis between a small vein taken from the dorsal region of the foot and A2 portion of the left side and then an end-to-side anastomosis between the vein and the corner of the right A1 and A2 portion. The schematic of the operation is shown in Fig. 1 and angiographic postoperative findings in Fig. 2.

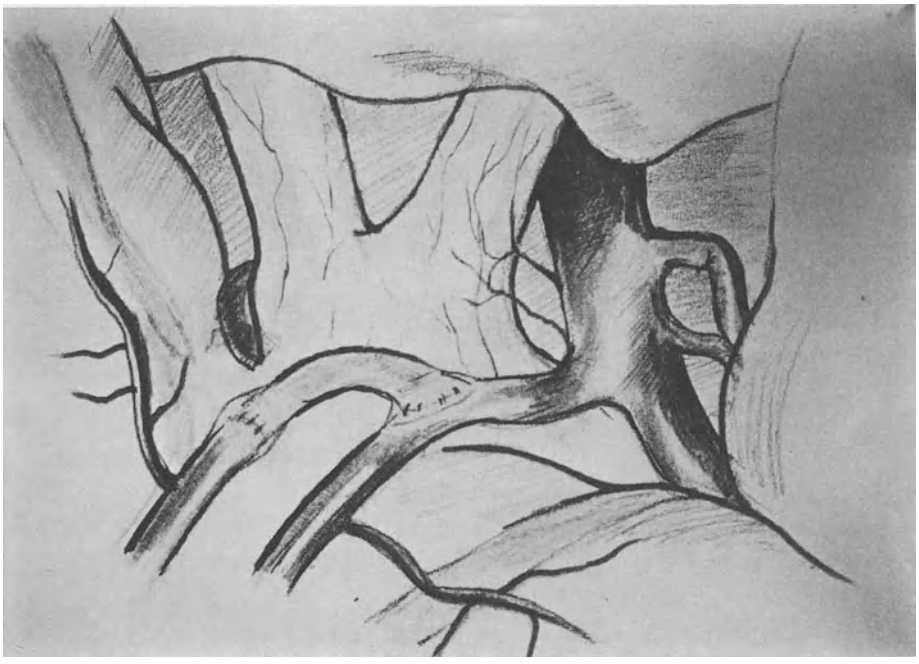

Fig. 1. Schematic of a microvascular graft between the A2 portion of the left anterior cerebral artery and the corner A1-A2 portion of the right anterior cerebral artery

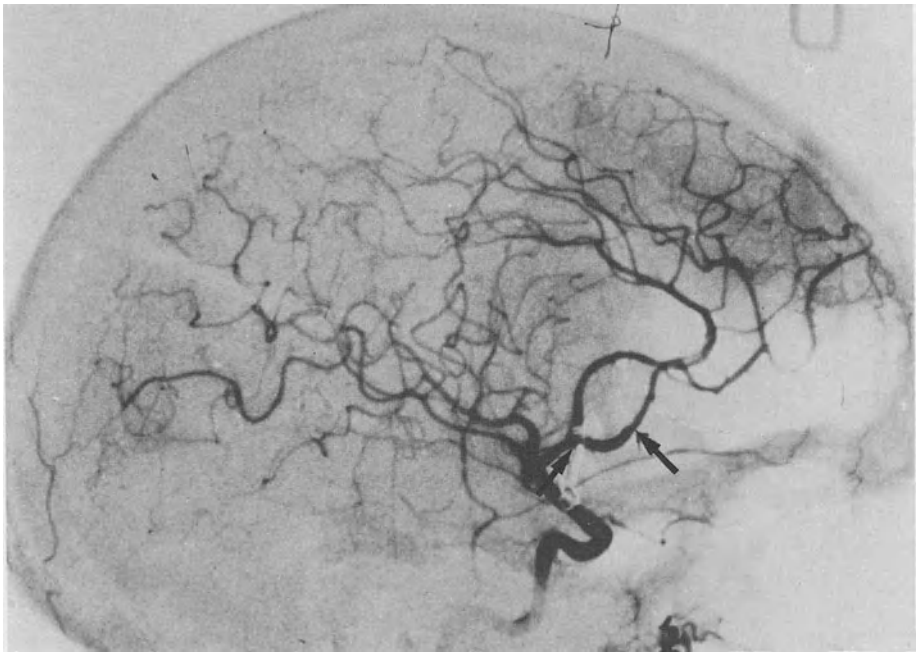

Fig. 2. Postoperative angiogram: the venous graft is indicated by the *two arrows*

The procedure was successful for the following reasons:

a) The use in the first step (end-to-end anastomosis) of a silicone tube inserted first in the vein and then in A2. The tube made the procedure easier because one of the major difficulties is the optimal recognition of the lumen to avoid taking the opposite wall of the vessel with the suture.
b) The use of straight, ergonomic microinstruments.
c) The obvious choice of a continuous suture.
d) Reinforcement of the suture line with Tissucol.

A perfect haemostasis in the posterior part of the suture is mandatory because of the impossibility of application of other stitches in the case of an imperfect haemostasis.

Reinforcing the Dura

Many reports have been written on this application, so it will not be dealt with here.

Fixation of Adipose Tissue after Laminectomy

This is a new application of the fibrin sealant. The so-called "laminectomy membrane", which is an unavoidable complication of intervertebral disc surgery and often the cause of failure of a standard operative procedure, has been described by many authors [4]. In some cases we have used adipose tissue fixed by Tissucol to the dura of the spinal nerve root. The purpose of this method is to avoid the failure of the application of autogenous fat, as reported by Mayfield et al. [10]. The failure may be due to infiltration of elements that produce granulation tissue and then scar tissue between adipose tissue and the dura layer. With the use of the fibrin sealant, if the quantity of fat is sufficient we try to reduce the production of scar tissue around the nerve root. In this application, Tissucol is used with a low concentration of aprotinin.

Encasement of the Unclipped Portion of Aneurysms

In this situation, the following method is used.

a) A microfibrillar collagen (Avitene) is blended with Tissucol. This step is time-consuming, and it should be done about half an hour before needed by a second instrument nurse. Small quantities of Avitene are blended with fibrinogen until a homogeneous compound is obtained. In this application, highly concentrated aprotinin is used with Tissucol.
b) A very small amount of the blended compound is applied to the surface of the aneurysm, which should be as dry as possible, and then highly concentrated thrombin is applied.
c) The procedure is repeated until the desired thickness is obtained.
d) The encasement can be reinforced with a thin layer of fibrin sealant applied with the Duploject needle.

Repair of Vascular Lesions

Another utilization of Tissucol is the haemostasis of vascular gaps. For instance, in our neurosurgical division, craniotomy rather than craniectomy (according to Prof. M.G. Yasargil, University of Zurich) is performed in posterior fossa operations. Even if lesions of the sinuses, particularly the sigmoid sinus, have been very rare, sometimes they do occur. The technique we used to repair them is the following:

a) Preparation of a very small piece of vein; a piece of dura would be a quicker patch, but owing to the high concentration of collagen it would be a target for thrombocyte aggregation and therefore dangerous for this procedure.
b) Application of a drop of thrombin on the vein patch.
c) Gentle release of jugular compression (sitting position) and a very quick application of Tissucol around the gap.

The patch is placed on the gap and the strength of the adhesion is checked after 3 min; the patch is then reinforced with Tissucol injected with the Duploject needle.

Intraoperative Haemostasis for Chemodectomas

This is one of the most interesting applications of Tissucol and it could be life-saving. Intraoperative haemostasis was used by us in a case of potential severely bleeding tumour (chemodectoma of the glomus jugulare). All surgeons that have operated on large non-chromaffin paragangliomas of this region know very well the danger of intraoperative haemorrhagic complications. In our opinion, this tumour is one of the most dangerous not only for the morbidity caused by its particular relationships with the cranial nerves and posterior fossa vessels, but for the potential mortality due to extensive vascularization through vessels arising from vertebrobasilar and internal carotid artery circulation, which obviously cannot be embolized preoperatively.

The technique described in Table 3 was used on a woman suffering from a severe syndrome of intracranial hypertension for a giant chemodectoma extending anteriorly to the paracavernous region and inferiorly to C3 in the neck (see Figs. 3, 4). After 2 years the patient was well and leading a normal life.

Table 3. Procedure used in a case of giant chemodectoma

1.	Preoperative embolization was carried out by selective catheterization of all filling branches of the extracapsular artery (ECA)
2.	Extracapsular dissection was performed and tumoural volume was reduced by bicoagulation
3.	To section a portion of the tumour since en bloc removal was impossible, the operating table was placed at a 30° angle to achieve a good venous drainage and mean arterial pressure was reduced to 65–70 mm Hg, squares of Surgicel (cellulose fabric) were folded several times, and Tissucol was prepared
4.	When the residual portion of the tumour bled, the Surgicel squares were placed in vascular lacunae and Tissucol was injected; perfect haemostasis was achieved

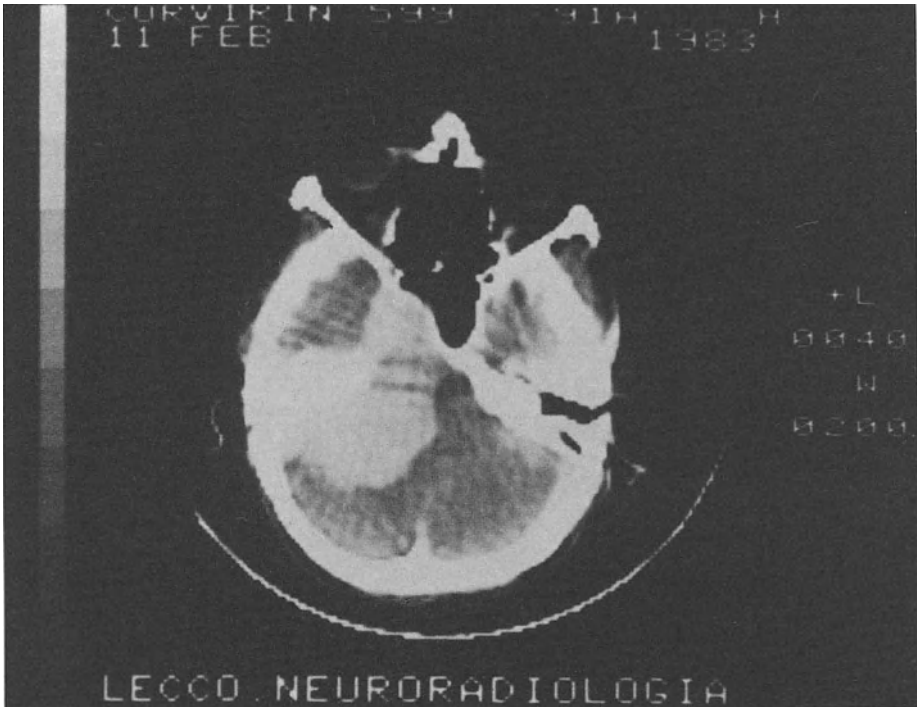

Fig. 3. Computed tomogram of a patient with a giant chemodectoma of the glomus jugulare

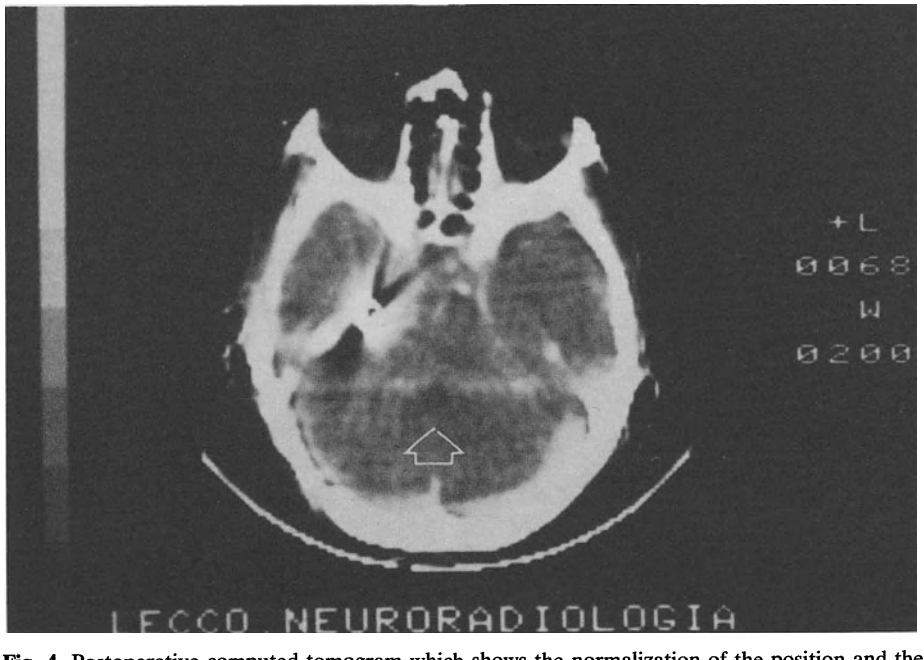

Fig. 4. Postoperative computed tomogram which shows the normalization of the position and the size of the fourth ventricle (same case as in Fig. 3)

Table 4. Procedure used in the treatment of CSF subgaleal collection[a]

1. The patient is placed in a sitting position
2. The CSF collection is withdrawn with a needle (positioned in the bottom of the collection)
3. After checking for complete removal of the CSF collection, Tissucol (3–5 ml according to the square centimetres of the detached area) is injected
4. A compressive dressing is applied for 2 h

[a] A low concentration of thrombin solution was used

Subgaleate CSF Collection

Another application of the fibrin sealant is in the treatment of CSF subgaleal collections. Unfortunately, many neurological surgeons have encountered this kind of complication, which sometimes needs reoperation to be resolved. The CSF turns the soaked surfaces into very smooth ones, making impossible the primary or secondary adhesion between two anatomical layers. Table 4 describes synthetically the simple, repeatable and, in the end, always successful technique (we have treated about ten cases).

Encasement of Medulloblastoma of the Fourth Ventricle

The last application proposed is the encasement of the residual portion of a medulloblastoma of the floor of the fourth ventricle to avoid cellular seeding. This complication is one of the most frequent and unresolvable in the management of children with a medulloblastoma and one of the major causes of failure of the surgical therapy [6, 7]. We treated a child with a tumour classified as T3B according to Chang et al. [1]. After 1 year the patient was doing well, and neither metastasis nor recurrence of the tumor was discovered. We are aware that the follow-up of this patient is too short to consider the case a clinical success, but we believe that it is theoretically a helpful example of another application of fibrin sealant.

Conclusions

Tissucol is a very useful tool to the neurosurgeon. It may be time-saving in many procedures, give better operative results, and make it possible to achieve results that would be impossible without the fibrin sealant. In particular cases, Tissucol is life-saving, such as in the one herein described of intraoperative haemostasis for a severely bleeding tumour. We believe that greater knowledge of mechanisms of adhesion strength in different conditions, of the time of reabsorption in different situations (i.e., in the CSF), and of the exact causes of late aneurysms in the site of end-to-end or end-to-side anastomosis will improve the results and extend the fields of application. We hope that in the future the use of polyvinyl-alcohol tubes, as proposed by some authors [11], and Tissucol will make it possible to achieve excellent results. Even more in the future, with the use of particular instruments

[12], it may be possible to repair bleeding vessels that today are sacrificed. All of the aforementioned applications of Tissucol are enough to justify introducing this substance in routine neurosurgical procedures, as we have done in our division.

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The Use of Fibrin Adhesive in Neurotraumatology

G. KLETTER

Key words: fibrin adhesive, frontobasal fractures, intracerebral hematomas, traumatic vascular lesions

Abstract

Adhesives have been used in neurosurgery ever since they were developed. There has never before been any adhesive which is absolutely non-neurotoxic and still has good adhesive properties. These requirements are absolutely met by human fibrin, which has led to its application in many fields of neurotraumatology. The present paper deals with the main fields of use, especially frontobasal fractures, sealing of dura in the spinal canal, and vascular lesions. In addition, the use of fibrin adhesive for sealing of fibers in the region of the cauda equina is reported.

Introduction

The use of medical adhesives is an important part of surgical technique. Until recently there was no appropriate substance which could be employed in the central nervous matter without concern. Tissue adhesives based on cyanoacrylate, for example, are strongly neurotoxic and cannot be removed after polymerization without damaging the brain.

Fibrin adhesive can be used without affecting the central nervous matter and it can also be removed shortly after application if placement has not been accurate enough. Both these qualities have made fibrin adhesive most useful for rhinosurgery and orthopedics, but particularly for neurosurgery.

Very soon it became evident that sealing with fibrin adhesive is most favorable in predamaged brain tissue, such as after stroke, in border zones of brain tumors, and in contused tissue. Therefore it has become widely applied, particularly in neurotraumatology. Patients with craniocerebral trauma not only show lesions of skin, skull, and dura but also damaged and vulnerable brain. All these impairments are combined in frontobasal fractures, where good cosmetic results are also required.

Frontobasal Fractures

To fix wide bone fissures in the base of the skull, particles of bone or muscle are used. Without suitable adhesive it would be problematic to fill these materials into a bone fissure just to leave them there. In our experience it has proved most

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

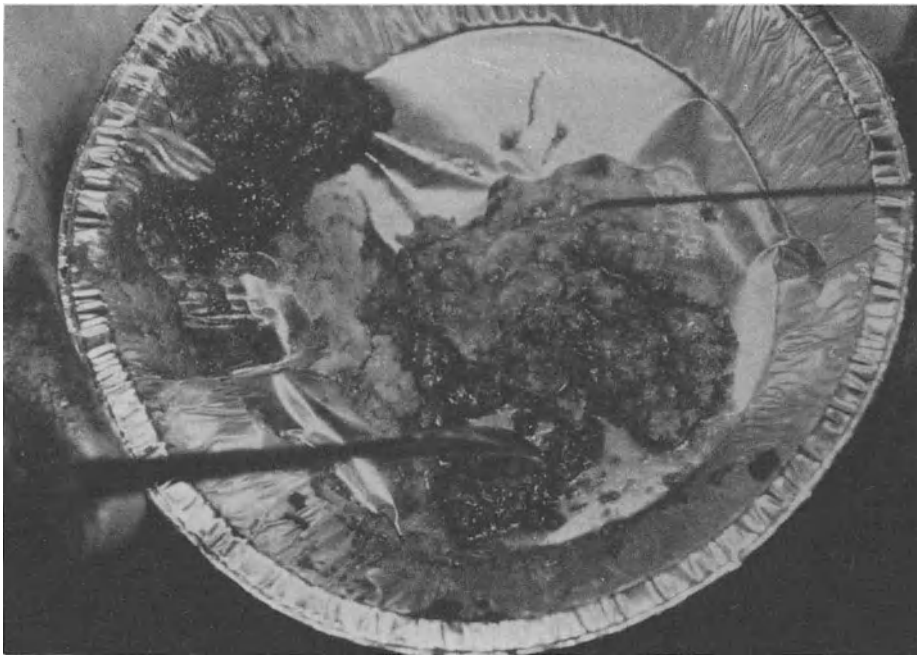

Fig. 1. Bone meal obtained in trephining is mixed with fibrin and thrombin and subsequently filled into bone matter defects

advantageous to take bone meal obtained from trephining (Fig. 1) to repair small defects. Such bone meal should be kept in small quantity in normal saline. It is stirred with fibrin adhesive to form a quickly hardening mixture which is then filled into the defect. In cases of major defects bone particles are sealed into the fissures and the remaining cavities are then filled with the mixture of fibrin and bonemeal. Rhinorrhea after surgery was avoided in all 126 cases fixed according to this method. There have been frequent reports (Bösch et al., 1977, Rupp et al., 1978, Sauer et al., 1977) on good blending of fibrin – bone mixtures with surrounding tissue. Successful results are also reported with dural sealing after frontobasal fractures. Our histological studies of the dura at the convexity, the base of the skull, and the vertebral canal in 50 animals have shown that waterproof dural sealing in the frontobasal area was achieved in 100% of cases, including those without sutures.

At the base of the skull, to which the dura is tightly attached, dural sealing is quite feasible by applying fibrin adhesive without sutures. This is of major interest in this area since dural defects are often situated so deeply that they cannot be satisfactorily fixed without substantial deplacement of the frontal flap. For small defects sealing with fibrin adhesive is sufficient. Wider defects of the dura require initial application of the galea – periosteal flap, which is fixed by some supporting sutures after which it can be completely sealed with fibrin adhesive (Fig. 2).

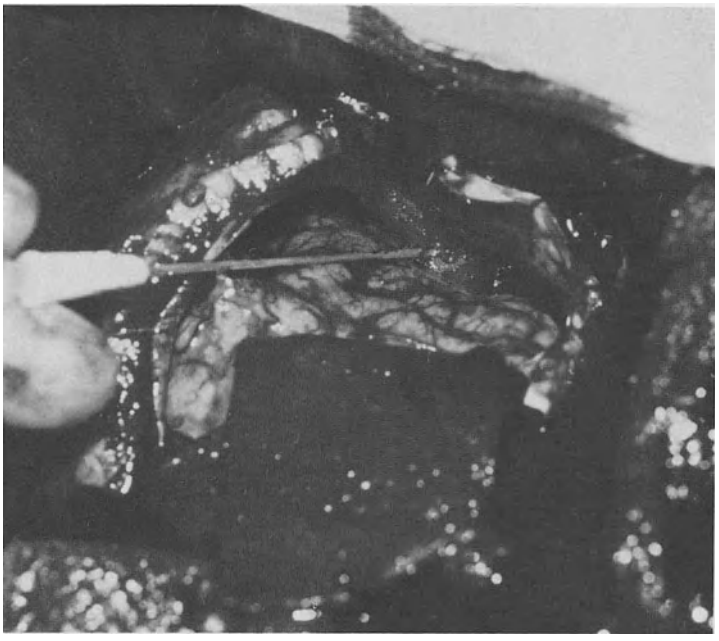

Fig. 2. Surgery of frontobasal fracture. Deep down, the galea-periosteal flap has already been fixed with two supporting sutures. Fibrin – thrombin mixture is applied to seal the galea – periosteal flap to the basal dura

Intracerebral Hematoma

Frontobasal lesions are often accompanied by malacic and contused parts of the brain. Hemostasis is problematic in such parts, particularly in cases of early surgery. The method of applying fibrin adhesive to a wider surface has proved very useful in these events and has already been adapted in surgery of large tumors and intracerebral hematomas of traumatic and spontanious origin. Walls of such hemorrhage cavities are also extremely vulnerable and even hemostasis with bipolar coagulation may prove difficult. Our method consists in sealing capillary hemorrhage and malacic tissue under the surgical microscope. Postoperative complications such as rebleeding have become quite rare since fibrin adhesive was introduced.

Sealing of Central Nerves

The author has operated on five cases of ruptured caudal fibers. One case was a 25-year-old man with paraparesis after falling from a window. Neurologically there was no immediate deterioration of his state so that conservative treatment with appropriate bedding and physiotherapy was applied. Three weeks after trauma paraparesis was getting increasingly worse. Myelography and CT scan showed signs of dural rupture and complete stop of radiopaque, so that surgery was indicated. During

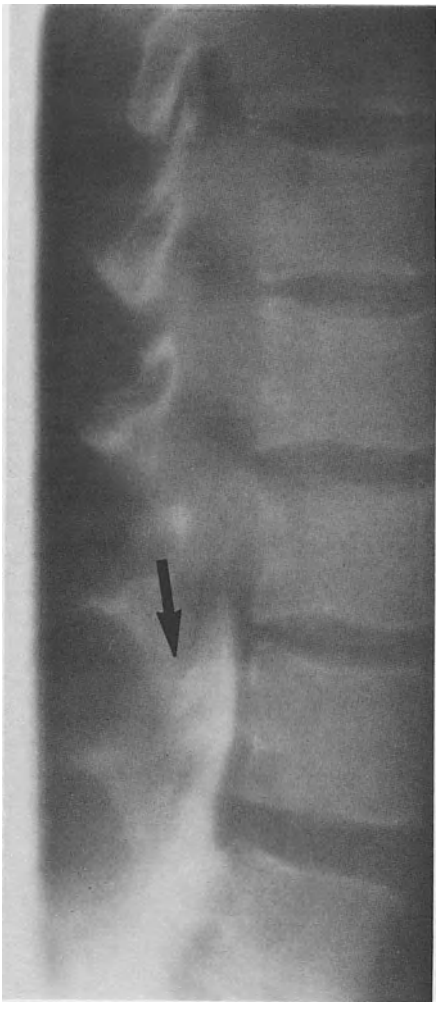

Fig. 3. Dorsolateral rupture of dura and some torn caudal fibers

operation rupture of the dura was confirmed and caudal fibers were found to be protruding (Fig. 3). Under the microscope the fibers were fixed with 10/0 sutures and sealed with fibrin adhesive (Fig. 4). Duraplasty was then carried out and secured with human fibrin adhesive. Postoperative progress of the patient was excellent. After surgery, paraparesis decreased to a certain extent and weeks after surgery it improved increasingly.

In the present five cases improvement can certainly be traced back to decompression, but in all similar cases reconnection of destroyed nervous matter should be performed. Results of surgery of peripheral nerves with and without adhesive justify this approach (Kuderna et al.).

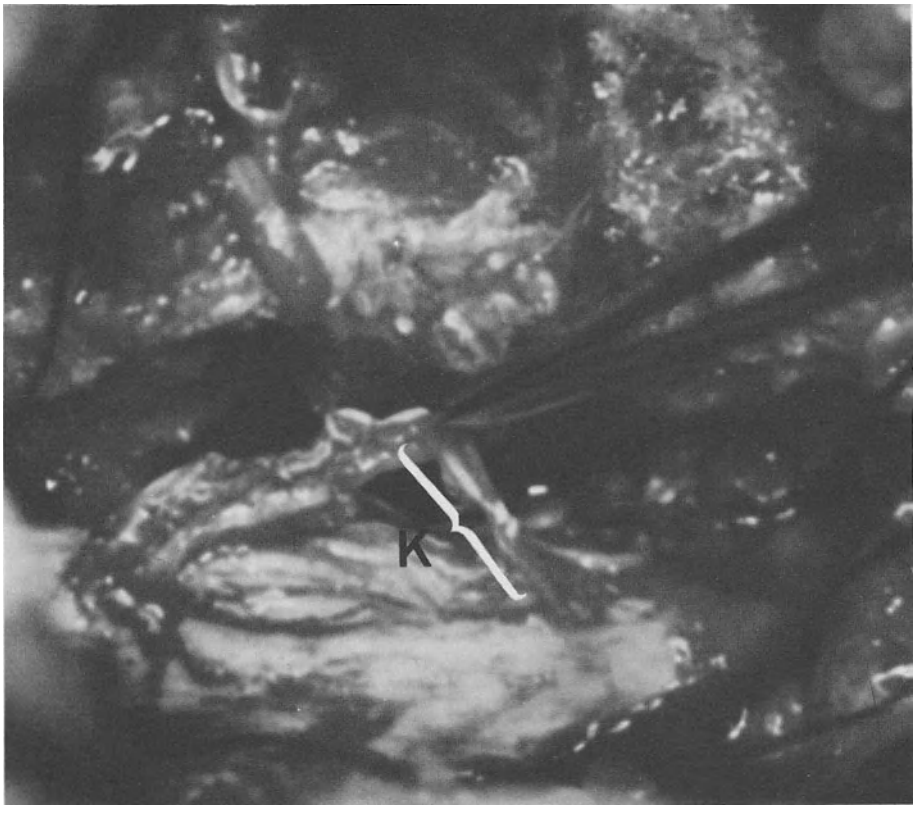

Fig. 4. Condition after adjusting caudal fibers (K'sealing area)

Traumatic Vascular Lesions

There are three indications for the use of human fibrin adhesive in traumatic vascular lesions:
Vascular occlusion
Traumatic aneurysms
Carotid-cavernous fistulas
Vascular occlusion affects the common carotid artery and surgery is only indicated in acute cases. We secure the sutured patch with human fibrin adhesive. If traumatic vascular occlusion occurred some time ago, external-internal bypass between the superficial temporal artery and the middle cerebral artery is indicated after decline of acute symptoms. Applying human fibrin adhesive has meant that the duration of surgery is considerably reduced and rebleeding is avoided.

Therapy of traumatic carotid-cavernous fistulas is still problematic to a certain extent, particularly if there are accompanying lesions, such as frontobasal liquor fistulas. If direct occlusion of a carotid-cavernous fistula is not feasible, the only way

to eliminate it is by ligating the internal carotid artery at the neck or perhaps by occluding the internal carotid artery in the intracranial area. These solutions also call for an external-internal anastomosis to bridge vascular occlusion. The duration of surgery is considerably reduced by applying fibrin adhesive.

More recent techniques such as the balloon technique permit sealing of the fistula by introducing a balloon without stenosing or occluding the internal carotid artery. Surgical methods by which fibrin adhesive is introduced into the fistula are still at an experimental stage.

They are problematic since there is a relatively high flow rate in the fistula which makes the application of adhesives seem inappropriate.

Conclusion

As already mentioned in the introduction, fibrin adhesive proved an asset in neurotraumatology not only because it paved the way for new sealing techniques but also because it is universally applicable in central nervous matter. It helps to make major interventions shorter and safer and in many cases also to improve late results with regard to physiology as well as cosmetics.

The present main fields of application of fibrin adhesive in neurotraumatology are:

1. Frontobasal fractures
   - Sealing and bridging of minor bone defects
   - Sealing of dura at the base of the skull
   - Hemostasis in malacic and contused tissue
2. Intracerebral hemorrhage
   Hemostasis in edematous brain
3. Sealing of dura in the spinal canal
4. Sealing of peripheral and central nerve structures
5. Treatment of traumatic vascular lesions
   - Sealing in reconstructions of intra- and extracranial vessels
   - Surgery of traumatic aneurysms
   - Surgery of carotid-cavernous fistulas

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Clinical and Angiographic Results in 47 Microsurgical Anastomoses for Cerebral Revascularization

G. PARENTI, and B. LENZI

Key words: Cerebral revascularization, cerebral ischemia, microsurgical anastomosis, giant aneurysm

Abstract

In 47 patients (43 with occlusive cerebral vascular diseases and 4 with giant aneurysms of the internal carotid artery) microsurgical anastomoses between the superficial temporal artery and a cortical branch of the middle cerebral artery were performed with the combined technique of interrupted sutures and fibrin sealant (Tissucol). The clinical and angiographic postoperative data were analyzed. Control postoperative angiography and Doppler sonography showed patency in 98% of the anastomoses.

Introduction

Extraintracranial anastomosis between the superficial temporal artery and a cortical branch of the middle cerebral artery, called the cerebral revascularization procedure, is a standardized microsurgery technique to avoid cerebral ischemia symptoms in patients with occlusive cerebral vascular disease or with other intracranial disorders. However, this form of treatment for ischemic cerebral vascular disease has not received universal acceptance. There is some skepticism in the neurological community about the beneficial value of this procedure [1, 2, 3, 4, 5, 6, 9, 11, 12, 13, 14].

The efficacy of the operation depends on the patency of the anastomosis and on the additional blood flow provided by the donor vessel. It is therefore important to avoid intimal thickening and the deposition of thrombi at the level of the suture line and on the surrounding endothelium areas which might cause occlusion or stenosis of the anastomosis and consequently insufficient collateral blood flow [4, 6].

There are many factors which influence platelet adhesion and intimal thickening, including the suture material in the luminal vessel, intimal damage due to perforation with microneedles, damage due to suture knots, handling of the arterial walls, pressure due to the application of clips and hemostasis through clamping.

Fewer stitches, hence reducing platelet aggregation and damage to the arterial walls, offer a greater chance of success with the anastomosis, also allowing increased cerebral blood flow.

In order to reduce the number of sutures needed to perform end-to-side anastomosis, we developed a combined technique of interrupted sutures and Tissucol;

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

we decided to use this procedure not only because of Kletter's reported experience, but also because of our own positive results achieved on experimental models of rat carotid [6, 8, 10].

We report here the angiographic and clinical data of 47 consecutive end-to-side anastomoses between the superficial temporal artery and a branch of the middle cerebral artery using interrupted sutures and Tissucol in patients requiring cerebral revascularization.

Material and Method

The patients were 8 females (17%) and 39 males (83%); ages ranged from 21 to 67 years, mean 50.54 years; follow-up was at least 6 months and averaged 2 years.

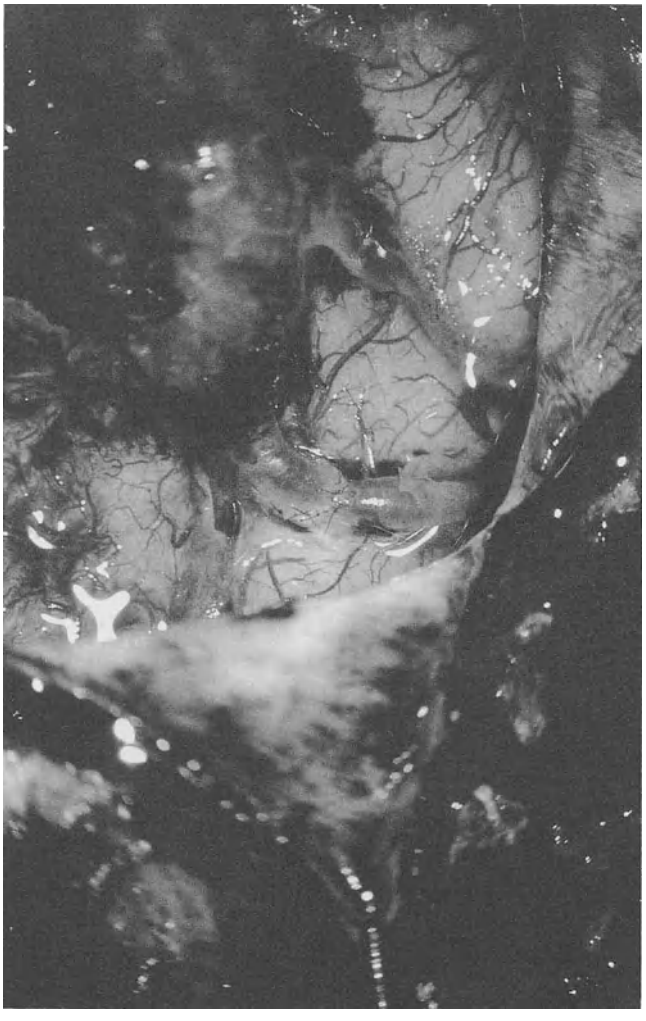

Fig. 1. End-to-side anastomosis performed with interrupted sutures

Twelve patients (25.5%) presented with transient ischemia attack (Tia); 14.8%) with neurological reversible deficit within 6 days (PRIND); complete stroke was present in 24 (51%), medium-mild stroke in 22 and severe stroke in 2; in 4 patients (8.5%) the anastomosis was performed to protect the brain prior to closure of the internal carotid artery because of giant aneurysms in the anterior circulation.

Occlusion of the internal carotid artery just at the origin was found in 24 patients (51%); 8 patients had additional stenosis in the opposite internal carotid artery.

Occlusion of the middle cerebral artery was found in three patients (6.3%). Fifteen patients (31.9%) had stenoses in a surgically inaccessible part of the internal carotid artery. One patient (2.1%) had bilateral stenosis of the carotid artery. Giant aneurysms were found in four patients; three in the ophthalmic-cavernous segment of the carotid and one in the supraclinoid portion.

The donor vessel was implanted at 45° into the cortical branch of the middle cerebral artery, applying two microsutures at 180° with 10.0 nylon monofilament.

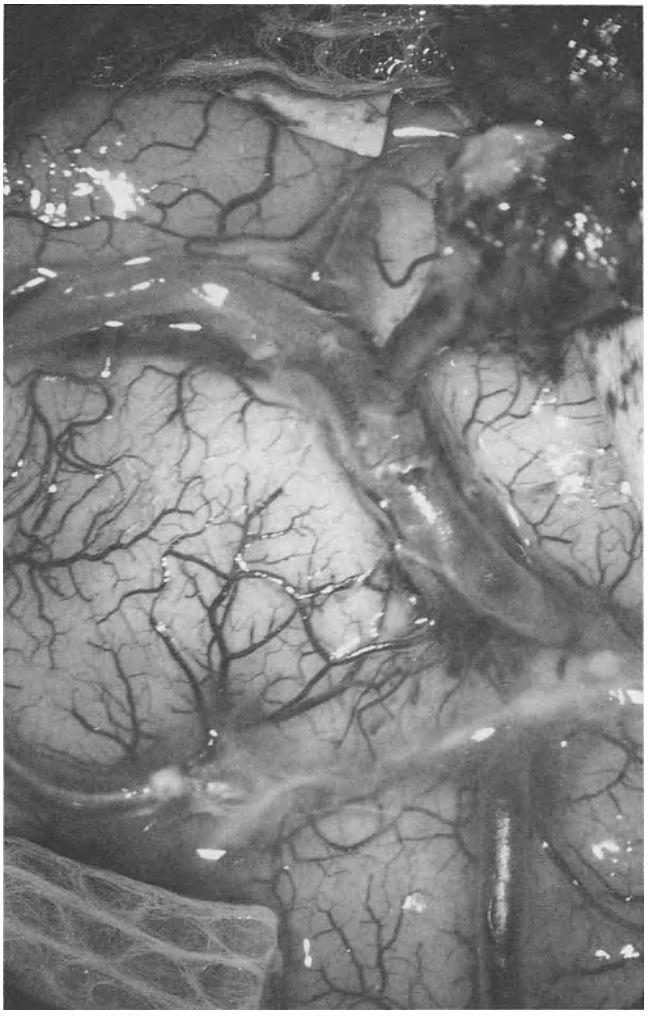

Fig. 2. End-to-side anastomosis performed with interrupted sutures and Tissucol

Afterwards, half of the stitches normally required were applied to the front and back wall in an interrupted sequence; holding back the vascular edges with microforceps, Tissucol was applied, making sure that the area was completely dry of liquor; the Tissucol was also spread over the donor artery to make it "watertight" to even the tiniest drop of blood; after 3 min the clips were removed, first from the cerebral artery and then from the donor vessel; no blushing was ever seen; the site of the anastomosis was covered in a whitish gel (Figs. 1, 2).

Results

Intraoperative and postoperative mortality was zero after 3½ years.

One patient with mild-medium stroke from occlusion of the internal carotid had an intracerebral hematoma 6 h after surgery; when this was drained surgically the site of the anastomosis, covered in the whitish gel, appeared intact; another patient with mild stroke due to occlusion of the carotid showed motory aphasia which lasted 5 days, though no parenchymal alterations showed up on the CT scan; nonexcerebral blood collection was seen in any of the patients.

Ninety-two percent of the TIA patients had no further complications; only one patient, who had several episodes of transient visual loss, continued to suffer from occasional episodes of visual TIA for about 7 months. One hundred percent of the patients who had presented with PRIND remained free of neurological disorders. Improvement was seen in 95% of the patients suffering from medium-mild stroke; one case worsened because of the onset of complications due to intracerebral hematoma; in two cases of severe stroke, one patient improved considerably, while the other showed only slight improvement. In the four patients submitted to progressive closure of the carotid following giant aneurysm there were no signs of neurological deficits.

Control postoperative angiography and Doppler sonography showed that only one of the anastomoses was not patent with a patency percentage of about 98%; the site of the anastomosis, when seen through angiography, proved to be in order, without signs of narrowing; moreover, in more than half of the patients there was good additional blood flow with donor vessel hypertrophy.

In 45 patients Doppler examination showed that the "encephalization" of the blood flow was good; in two patients the flow appeared to be worse: one because of occlusion of the anastomosis, the other due to lack of negative intracranial gradient; in this latter patient, the inaccessible tight stenosis of the carotid appeared to be well recanalized when angiography was performed 4 months after surgery and the anastomosis was filiform (Table 1).

Discussion

The clinical and angiographic data in our population permit a positive evaluation of the surgical technique whereby end-to-side anastomosis is performed with interrupted sutures and the association of Tissucol.

Table 1. Postoperative results

Data	Percentage of patients
Mortality	0%
Morbidity	4.2%
Neurological condition at the time of revascularization procedure:	
TIA	92% symptom free
PRIND	100% symptom free
Mild Stroke	95% improved
Severe Stroke	50% improved
Giant carotid aneurysms	100% no change
Control angiography and Doppler sonography	
Patency of anastomosis	98%
Donor vessel hypertrophy	>50%

TIA, transient ischemic attacks; PRIND, prolonged reversible ischemic neurological deficit

Table 2. Advantages of combined gluing and suturing technique in microvascular anastomosis

Reduction of number of sutures
Minor trauma to the vascular wall
Reduction of clipping time
Minor handling of vessel
Cover of donor vessel trunk, avoiding bleeding

The use of Tissucol offers the advantage of using less stitches, hence reducing platelet aggregation and avoiding intimal thickening at the level of anastomosis and the surrounding endothelial areas.

The fact that less stitches are used does not compromise the vascular anastomosis attachment; there were no extracerebral hemorrhagic complications in any of the patients; the use of Tissucol also had the advantage of "waterproofing" the vascular peduncle of the donor vessel against possible drops of blood (Table 2).

It is quite likely that once more is known about the adhesive properties of Tissucol it might be possible to reduce even further the number of stitches used, just as experiments on animals have in fact shown.

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Fibrin Adhesives in Intracranial Microvascular Surgery

G. KLETTER

Key words: fibrin adhesive, extra-intracranial Bypass, intracranial Aneurysms

Abstract

Histological examinations after experimental microvascular surgery have shown that around sutures and other foreign substances considerable scar formation develops and results in constriction of the lumen if vessels have an average diameter of 1 mm.

Using tissue adhesive made of fibrin and mixed with thrombin for coagulation means that the number of sutures and the hazards of long-term stenotic changes in microvascular anastomoses can be greatly reduced. The adhesive substance does not have any neurotoxic effects and is therefore applicable in cerebrovascular aneurysms where clipping is hardly possible or not possible at all.

Introduction

In surgery, there have been frequent efforts to replace sutures by tissue adhesive systems. However, use of such adhesives has almost always resulted in problems of crosslinking immediately after applying the substances, which then developed into hard, irremovable material. In addition, almost all adhesives proved to be more or less neurotoxic. The introduction of fibrin adhesion systems was vital for neurosurgery since they do not have any neurotoxic effects and are well applicable.

Microvascular interventions may still present surgical problems because the operating area is difficult to localize or because the tissue of the operating site is vulnerable in the presence of edema or after stroke. As even high-level microsuturing did not always have the desired results, discussion was started on replacing it by fibrin adhesion systems for microvascular anastomoses and other microvascular surgery.

Experimental Research

End-to-end anastomosis of the common carotid artery was performed on 100 Wistar rats weighing on average 300 g. Various already described techniques of end-to-side anastomosis were used on 100 additional rats. Two animals of each series were killed at intervals of 24 h for histological and selective electron-optical examinations of anastomotic areas. After 50 days, a continuous survey of the healing process of the

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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microvascular anastomoses was carried out. Healing of microvascular anastomoses was observed to follow a standard course – also typical of any vascular anastomosis of an average 1 mm in diameter: After 48–72 h, extensive and inevitable necrosis occurs in the sutured area (Fig. 1). In the course of 3 more days a strong reaction of connective tissue sets in around necrotic material and foreign body giant cells appear (Fig. 2). In about 30 % of all surgical cases this leads to narrowing of the lumen. During the last 3–4 weeks of the healing process new connective tissue is transformed into sometimes widespread and obstructing scar tissue (Fig. 3).

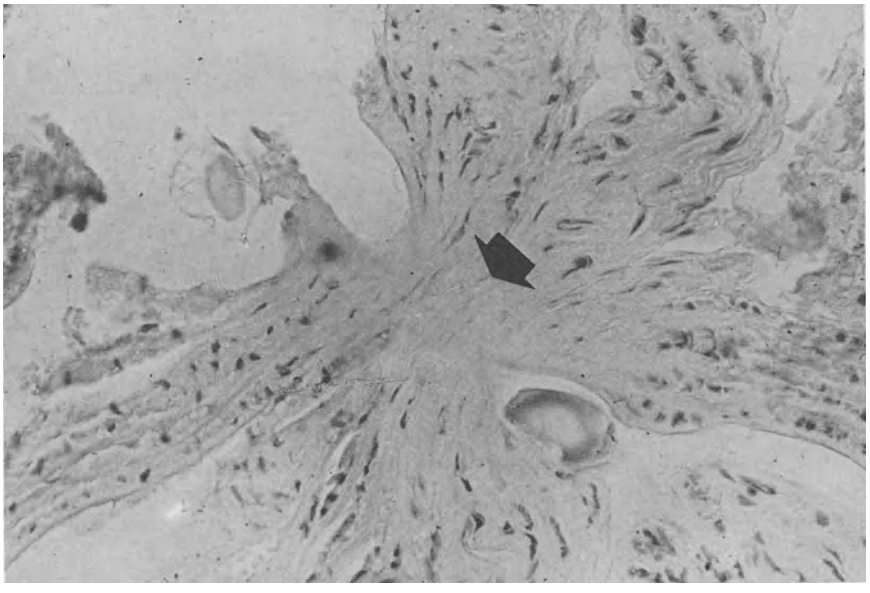

Fig. 1. Necrosis in the area of a microvascular suture (common carotid artery of rat 48 h after surgery)

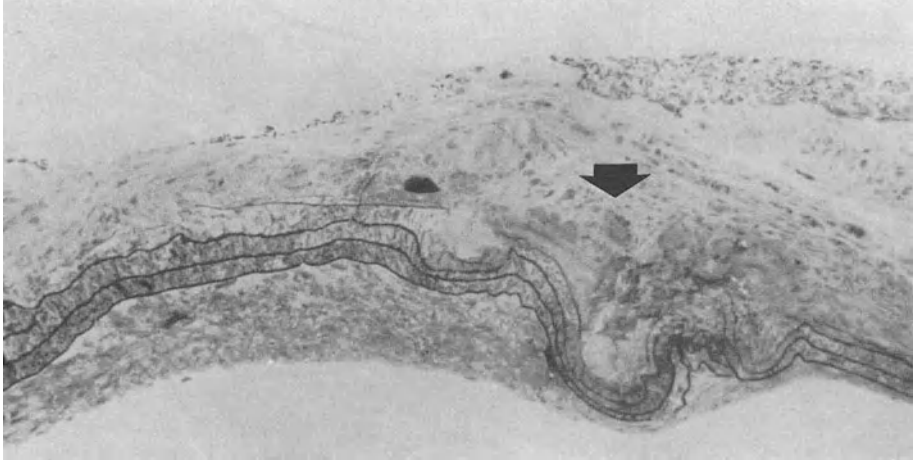

Fig. 2. Granulation tissue with foreign-body giant cells (common carotid artery of rat 14 days after surgery)

It was evident that in order to avoid considerable scar formation the number of sutures had to be reduced, which, however, meant enhancing the dangers of scar dehiscence and clot formation (Fig. 4).

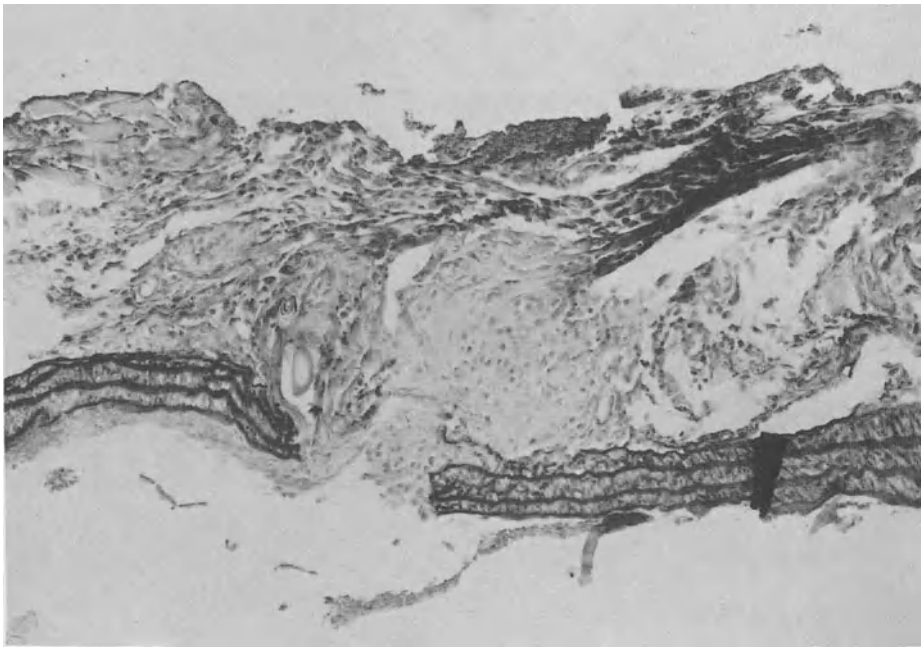

Fig. 3. Widespread scar tissue in the area of microvascular anastomosis (common carotid artery of rat 4 weeks after surgery)

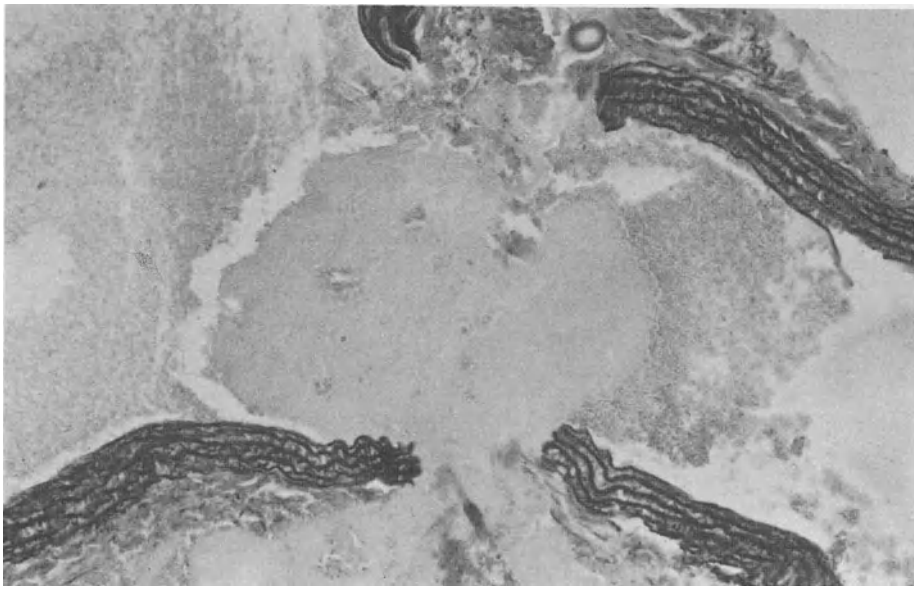

Fig. 4. Clot formation in the area of microvascular anastomosis and vascular wall dehiscence (common carotid artery of rat 12 h after surgery)

Since reduction of sutures still seemed the only viable method, another test series was carried out with the purpose of showing to what extent sutures could be replaced by fibrin adhesives systems or if anastomosis could be performed without applying any sutures at all. End-to-end anastomosis was performed in 50 rats and end-to-side anastomosis in another 50. Every second suture was omitted and the edges were sealed with fibrin adhesive. In ten further animals end-to-end anastomosis was tried without suturing, i.e., with sole application of adhesive. Animals were then sacrificed and examined as described above.

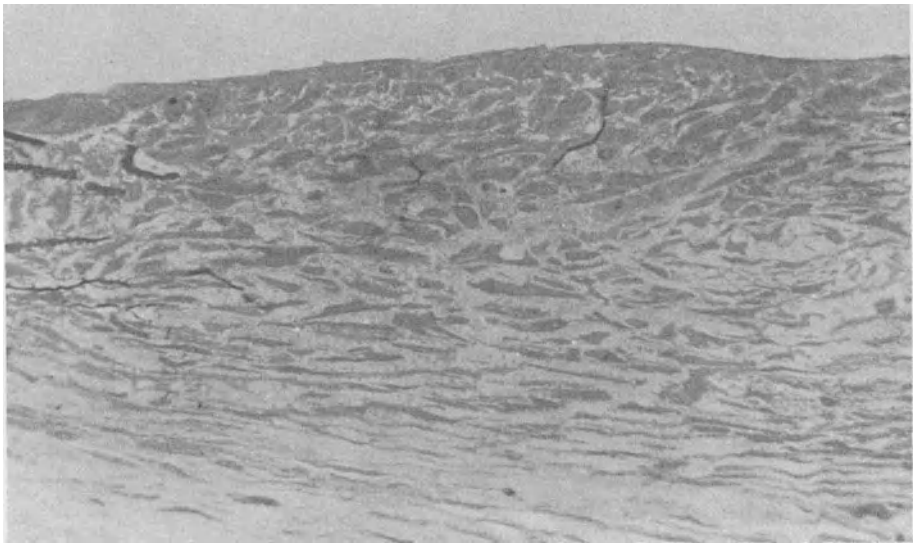

Fig. 5. Microvascular anastomosis sealed with fibrin adhesive without giant cells or scar tissue (common carotid artery of rat 2 weeks after surgery)

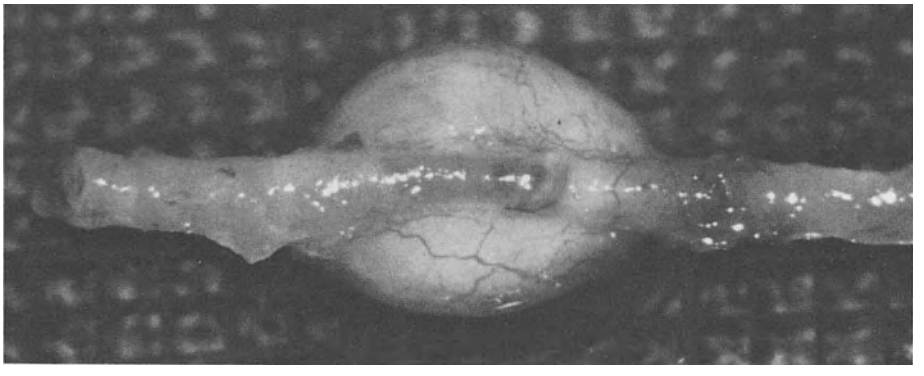

Fig. 6. Aneurysm formation in anastomosis sealed without supporting sutures (common carotid artery of rat 4 weeks after surgery)

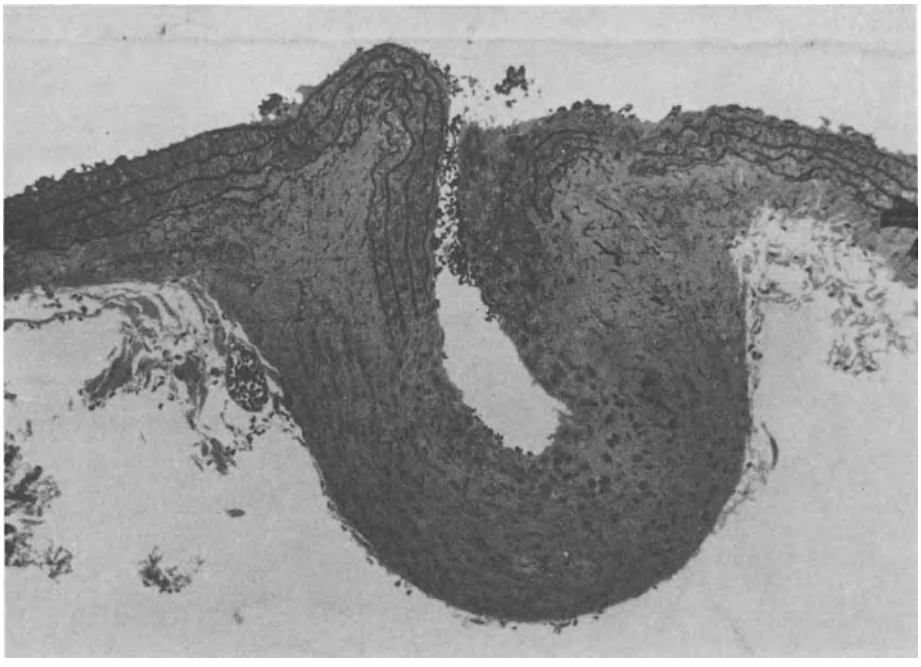

Fig. 7. Histological picture of Fig. 6

Histological findings clearly showed less scar formation as well as reduced necrosis if the number of sutures was reduced. Upon late examination, vascular walls had more regular structures, certainly as a consequence of absence of foreign body giant cells (Fig. 5). Along sealed edges there was also less intravasal and extravasal clot formation. Of the ten cases only sealed with adhesive, three developed aneurysms in the anastomotic areas (Figs. 5–7).

Clinical Application

The use of fibrin adhesives in intracranial microvascular surgery is indicated in extra-intracranial bypass operations and in intracranial aneurysms if clipping of the vascular malformation is not or only partly possible and if additional fixing of the clip is required.

Since 1975 more than 700 extra-intracranial bypass operations have been carried out at the Neurosurgical Department of Vienna University Clinic. In 336 cases a combined suture – sealing system was applied (Figs. 8, 9).

After preparing the temporal and cortical arteries both corners are sutured and the front wall is loosely closed with two to three stitches. Fibrin adhesive is then applied to the anterior wall, the sutured and sealed wall is checked from inside, and the posterior wall is sutured and sealed with adhesive as above. If hemorrhage occurs after clips are opened it is easy to close the clips again and reseal the anastomosis with fibrin adhesive.

Fig. 8. Microvascular anastomosis between superficial temporal artery (ATS) and middle cerebral artery – applying corner suture (63-year-old patient with carotid artery block and TIA)

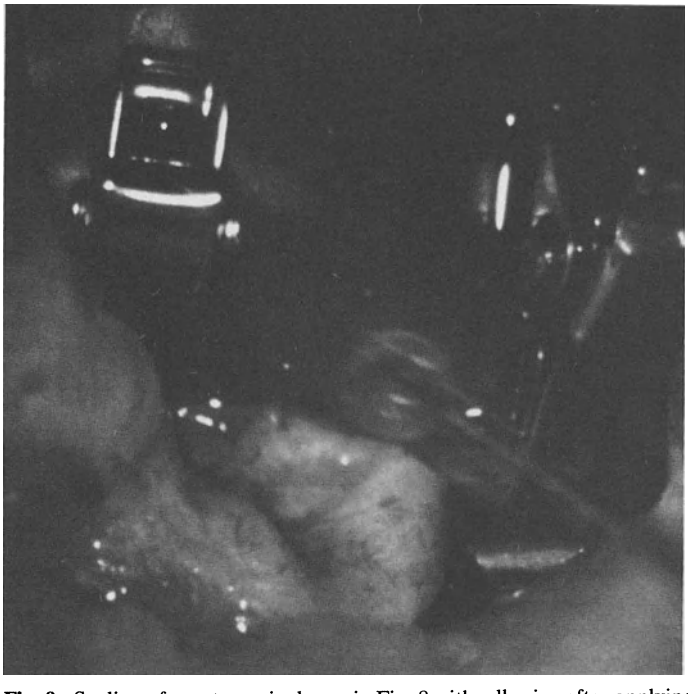

Fig. 9. Sealing of anastomosis shown in Fig. 8 with adhesive after applying two corner sutures and 2 sutures on both anterior and posterior walls

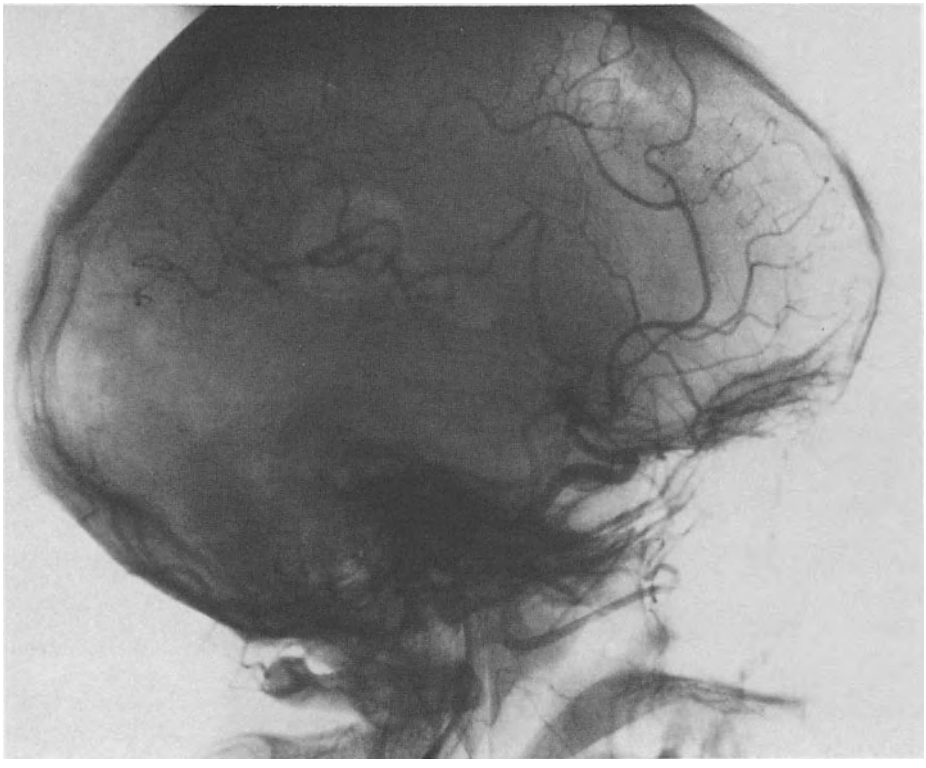

Fig. 10. External-internal by-pass on 30-year-old patient after completed stroke. Irregular outline of microanastomosis and sutured closure without sealing

Short-term patency of the mixed-type closure is equal to anastomosis which was only sutured, i.e., 98%. Angiography after 1 year shows regular structures if the anastomosis was closed with sutures and additional adhesive whereas walls that have only been sutured are often irregular (Figs. 10–12). One patient died 4 weeks after microanastomotic surgery because the posterior wall could only be sealed and not sutured for technical reasons. The result was aneurysm and subsequent rupture in the anastomotic area.

If direct clipping of intracranial aneurysms is not feasible, sealing of aneurysms by muscle or some other substance is applicable (Figs. 12, 13). The sealing method also helps to fix a clip to a certain position or prevent it from sliding.

Discussion

Tissucol is a tissue adhesive which consists of human fibrinogen and thrombin additive. Thrombin causes a hardening of the adhesive which corresponds to the physiological process. The adhesive substance is not neurotoxic and can be applied safely in areas of the central nervous system [2, 3]. If the adhesive is inappropriately

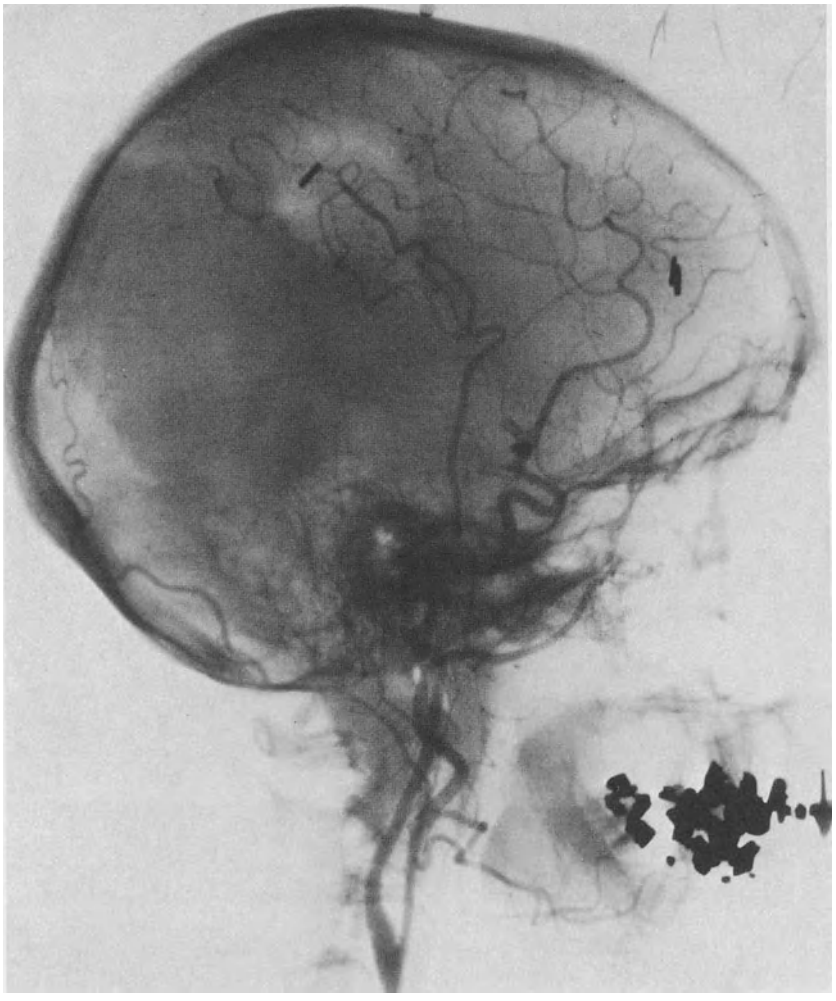

Fig. 11. External-internal by-pass on 32-year-old patient, regular outline of anastomosis after partial sealing with adhesive.

applied it can be removed without lesion of the tissue [1]. Histological findings show that the adhesive sealing leads to a better physiological healing process than sutures [1, 7], the only disadvantage being that it cannot withhold major pressure or tensile forces [2, 5]. So there has to be at least one additional suture in anastomosis in order to support vascular connection. Within 6 – 8 weeks a sealed closure obtains considerable stability since the adhesive acts as basic structure for continuous and regular germination of connective tissue.

Our results of intracranial vascular operations have shown that adhesive substance consisting of human fibrin and thrombin certainly helps to achieve better results and may in some cases make a very difficult surgical case successful.

Fig. 12. Aneurysm of middle cerebral artery after preparation

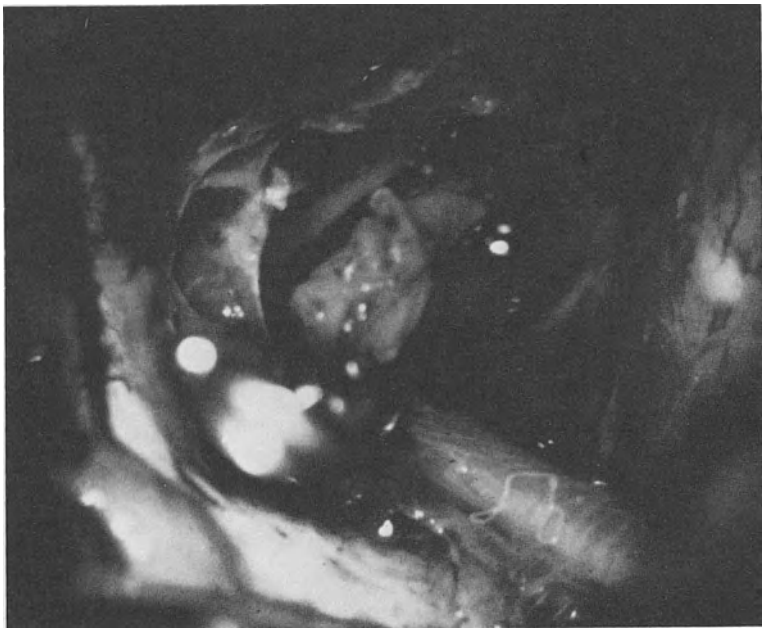

Fig. 13. Clipped and extirpated aneurysm. Clip is fixed with adhesive for protection against sliding

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Application of Fibrin Sealant in Microneurosurgery

H. R. EGGERT, W. SEEGER, and V. KALLMEYER

Key words: Fibrin sealant, microneurosurgery

Abstract

Based on the experience of more than 2000 microsurgical intracranial and spinal operations applications of fibrin glue are demonstrated. Applications with and without support material are distinguished between. Fibrin glue has proven to be useful in the preservation of venous vessels and stabilization of kinking veins and arteries as well as for fixation of implants. On the other hand, the use of fibrin glue cannot replace meticulous active hemostasis following brain operations. With regard to early reoperations economical use of fibrin glue within the subarachnoid space is recommended.

Introduction

The use of fibrin glue is usually recommended for several special indications, i.e., the repair of frontobasal fistulas [6, 7, 10], extraintracranial bypass operations [3] or nerve anastomoses [2, 3, 5]. The experience of more than 2000 microsurgical intracranial and spinal operations since 1981 has demonstrated a wide range of indications for the use of fibrin sealant. These, but also the hazards of the use of fibrin glue, are discussed.

Methods

We mainly used the commercially available frozen solution of coagulable plasma proteins and a thrombin solution, containing 500 IU thrombin and 3000 KIU aprotinin. Depending on the indication, mixing equal parts of both solutions takes place either out of the site of operation on an appropriate support material (cellulose gauze, collagen pads, etc.) or in the operation field directly on the tissue that is to be sealed (Table 1).

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

Table 1. Possible applications of fibrin sealant in microneurosurgery

1. *With support material* (cellulose gauze, collagen pads)		
a) Mixing of fibrinogen and thrombin on the support material		
	Sealing	Ventricles
		Venous vessels
		Small dural defects
b) Layer-by-layer application of fibrin glue and support material		
	Solidification of the dura sutures	
2. *Without support material*		
a) Premixed fibrinogen and thrombin solution		
	Plugging of CSF effusions	
b) Mixing of fibrinogen and thrombin solution on the site of adhesion		
	Plugging	Frontobasal fistulas, arachnoidea
	Coating	Brain wounds
	Stabilization	Veins
	Fixation	Arteries, implants

Applications with Support Material

Ventricle Defects

Intraoperative ventricle wall defects, if not closed temporally, can be a source of extensive, initially unrecognized hemorrhage into the ventricle system, which is only difficult to remove. Wall defects up to a size of 1 cm^2 can easily be closed by introducing fibrin glue impregnated cellulose gauze. In larger defects, temporary covering with cotton pads followed by wide fenestration of the tumor cavity into the ventricle seems to be more advisable.

Venous Vessels

There is a wide field of indications for the use of fibrin sealant in the control of hemorrhage, where the function of veins should be conserved. Bleeding from Pacchionian granulations after trephination of the skull can be closed rapidly and easily by fibrin glue impregnated cellulose gauze. In the same way, bleeding bridging veins with small wall defects can be controlled. The alternative is bipolar coagulation, which causes – if not total occlusion – significant stenoses of the vessels. Small sinus wall defects occurring during removal of meningiomas with sinus attachment may also be occluded by implantation of cellulose gauze or collagen pads.

Sealing with cellulose gauze is especially helpful in radical extirpation of meningiomas, which necessitate a resection of the outer layer of the sinus wall. Larger sinus wall defects, e.g., due to resection of a meningioma with sinus invasion, must be closed by suture. But by covering these with fibrin-impregnated cellulose gauze, stitches can be saved.

Dura Defects

Small defects of the dura, which cannot be closed by suture because of their difficult anatomical location, for instance above the wide-opened optic canal in sphenoidal meningiomas, where the dura had to be resected because of tumor infiltration, may be closed by implantation of support material, which preferably should be adhered at the inner margin. Insufficient dura sutures due to high tension can be sealed by application of fibrin glue first, followed by a layer of cellulose gauze and another layer of fibrin sealant. By this technique, extensive grafting procedures for closure of dural defects are avoidable in many cases.

Use Without Support Material

Subgaleal CSF-Effusions

Subgaleal CSF effusions after trephination – especially if frontotemporal – can be treated by local tap and evacuation followed by injection of 1 – 2 ml mixed (1:1) fibrinogen-thrombin solution through the same needle. A compression bandage should be applied for 2 days afterwards. This procedure can often avoid a long-lasting therapy of repeated local and lumbar punctures or continuous lumbar drainage.

Frontobasal Dura Grafts

Frontobasal fistulas, either due to trauma or after resection of large frontobasal meningiomas, are usually closed with pedunculated galea periost flaps. In contrast to other authors [10] we fix the graft by single sutures, since in our experience tension of the flaps cannot always be ruled out. The frontobasal dura defect is sealed by injection of fibrin glue under the periost flap.

Using microsurgical techniques, fixation sutures usually do not represent a problem including the region of the anterior clinoidal processes. However, in closing dural defects in the region of the tuberculum sellae, sometimes one must rely on the use of fibrin glue. But, also in this case, unintended tension of the periost flap can be reduced by lateral fixation sutures.

Arachnoidea

Opened gyrus' and the consecutively opened CSF space for resection of gliomas and metastases can be reclosed with fibrin sealant.

In the same way, we use fibrin glue for watertight closure of the arachnoidea in extraintracranial bypass operations in order to prevent subcutaneous CSF effusions.

Floor of the Sella

Closure of the sella is a problem after transseptal-transsphenoidal resection of intra- and suprasellar tumors. This can be done by implantation of an osseous or cartilagineous disk, excised from the nose septum, into the dural defect. If liquorrhea appeared during tumor resection, a free-fat transplant, fixed by fibrin glue, should be introduced into the empty sella first.

Brain Wounds

Larger brain wounds due to tumor resection can be mechanically protected by a coat of fibrin glue. Theoretically this can prevent postoperative hemorrhage caused by too high suction of the epidural drainage. On the other hand, there may be hemorrhage into the edematous brain substance behind the fibrin layer – an observation we unfortunately made several times. If, in these cases, the tumor cavity had not been sealed by fibrin glue, the blood would have been collected in the tumor cavity and could have been detected in postoperative CT control before it led to destruction of normal brain tissue. For this reason – in contrast to other authors [10] – we are of the opinion that meticulous, active hemostasis cannot be replaced by the use of fibrin sealant.

Stabilization of Vessels

If natural fissures (e.g., sylvian fissure, interhemispheric fissure, infratentorial supracerebellar space) are used for surgical approaches, bridging veins may be in danger of being obliterated by kinking or by being torn, even when they are primarily anatomically preserved. Coating of these veins with fibrin sealant increases the mechanical stability of the vessel wall and therefore may prevent accidental closure.

In the same way artery loops may hinder the surgical access. These can be mobilized and fixated by fibrin glue at surrounding structures (brain, dura).

Implants

With the intention of inducing reactive scarring and therefore reinforcement of the vessel wall, parts of aneurysms, which could not be clipped satisfactorily, are wrapped with different materials [7, 10]. Experimentally, cotton wool proved to be the most efficient material. Washing away by liquor and loss of contact between vessel wall and cotton wool is prevented by application of fibrin glue on the wrapped part of the vessel. It is not known whether application of fibrin glue alone leads to sufficient wall reinforcement.

The use of fibrin glue should be as economical as possible especially in this indication, because in the case of a reoperation, i.e., clip replacement, the fibrin clot may make the dissection extremely difficult.

In angioneurolysis of cranial nerves, i.e. trigeminal neuralgia or facial spasm, the affected nerve is freed from the vessel. A new contact between the nerve and vessel is permanently prevented by introduction of a buffer, i.e., silicone foam. Relapse occurs, when the buffer slips off. This can be prevented by fixation of the buffer with fibrin sealant.

Conclusions

In addition to the classical applications there are many other indications for the use of fibrin glue in microneurosurgery. Usually the surgical success cannot be related directly to the use of fibrin glue. But many steps of operations can be improved, technically simplified and may become safer. Therefore the use of fibrin glue is advantageous for the patient as well as for the surgeon.

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Importance of the Use of Tissucol (Tisseel) in the Reparation of the Dura

B. Lenzi, R. Cantini, and G. Parenti

Key words: Tissucol, CSF fistula, dural lesion, transphenoidal surgery

Abstract

We present favorable results obtained using Tissucol in 21 operations repairing lesions of the dura in various sites. In dural lesions of more than 1 cm^2 it is very useful to anchor the lyodura patch with some stitches. The characteristics of Tissucol proved to be undoubtedly superior to those of adhesives so far in use.

Introduction

A loss of cerebrospinal fluid (CSF) with a subsequent risk of infection is a frequent complication in operations repairing lesions of the dura. Suture alone is not always sufficient to guarantee a "watertight" seal against CSF loss. The introduction of adhesive materials such as cyanoacrylates raised hopes that the frequency of this complication could be reduced, but in practice the extent of their use was limited. These adhesives in fact proved to be neurotoxic and inelastic, and furthermore required a perfecly dry operating area for effective adhesion; the presence of CSF means that this is not always possible. Tissucol has some characteristics that permit a better result in operations repairing dural lesions, for this reason we use it in surgical practice. Our experience is described in this paper.

Cases

We report 21 cases in which we have used Tissucol to repair dural lesions resulting either from the removal of meningiomas of the calvarium (4 cases), from post-traumatic or spontaneous lesions of the base of the skull with CSF fistula (11 cases), or from the removal of spinal "hour-glass" neurilemmoma (1 case) or transphenoidal hypophysectomies (5 cases).

- On removal of a meningioma, four patients were left with a large dural lesion, up to approximately 4×8 cm, requiring the use of lyodura. In two cases, two adjacent fragments of lyodura were necessary, each of approximately 2×4 cm and held with four stitches each; the edges were glued with Tissucol. In the other two cases, one fragment of lyodura, 2×3 cm, was sufficient, anchored with four stitches and subsequently glued at the edges with Tissucol.

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

- Rhinoliquorrhea, resulting from a cranial trauma, was present in six patients. Neuroradiological examinations brought to light a fracture of the anterior part of the base of the skull. During the operation, the osteomeningeal lesion of the base of the skull could be identified both extra- and intradurally, holding back the frontal lobe with a Yasargil retractor (Fig. 1). The bone breach was filled with an acrylic material covered with a fragment of periosteum fixed with Tissucol. The dural lesion was subsequently repaired by glueing two corresponding fragments of lyodura from the outside and from the inside, and anchoring them with two stitches.
- In the case of one of our patients, a dural breach of approximately 3×2 cm with irregular edges remained after the removal of a cervical "hourglass" neurilemmoma. The reconstruction of the dura using a lyodura patch anchored with three stitches and subsequently glued with Tissucol provoked no difficulties (Fig. 2).
- Ten patients underwent transphenoidal dural reparation. In three of these the loss of CSF appeared after a cranial trauma due to a sphenoid fracture, and in a further two cases it was secondary to a chronic erosion of the sellar floor. In these cases, the reparation was effected by placing a lyodura patch in a position

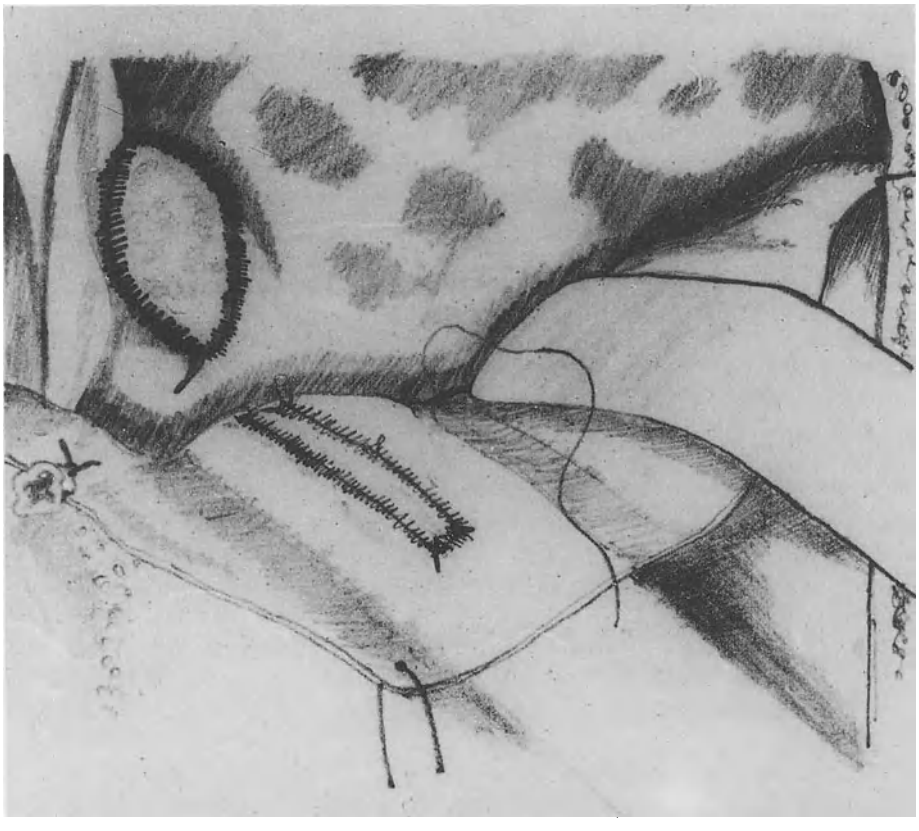

Fig. 1. Reparation of a CSF fistula of the anterior base of the skull

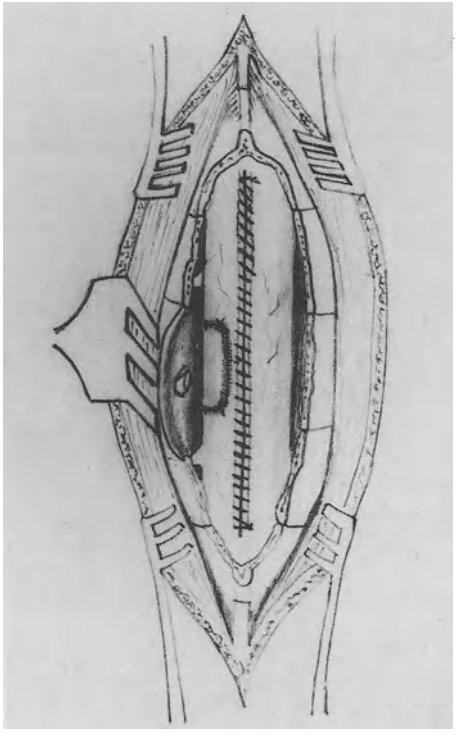

Fig. 2. Reparation of a spinal dural lesion resulting from the removal of an "hourglass" neurilemmoma

corresponding to the sellar floor and applying Tissucol along the edges; the sphenoid sinus cavity was subsequently filled with Surgicel soaked in acrylic material.

The remaining five patients who had undergone transphenoidal hypophysectomy for adenoma had a CSF loss from the sella at the end of the operation; the floor of the sella turcica was reconstructed using a lyodura patch glued down with a double layer of Tissucol. In these cases the sphenoid sinus cavity was not filled with Surgicel and acrylic material, so that a possible further operation would prove less complex.

Results

In our 21 cases, which include patients with lesions in various sites, no complications arose from the use of Tissucol. There was neither CSF loss nor subgaleal gathering of CSF in any case, and the surgical wounds healed well.

Discussion

The favorable results of our cases confirm the excellent adhesive and biological characteristics of Tissucol. In our opinion, Tissucol is an effective suture adhesive,

owing to its biocompatibility, its elasticity, its effectiveness in the presence of blood and CSF and, not least, because it activates the fibroblasts and is thus later replaced by a physiological reparation tissue. In dural lesions of less than 1 cm^2, Tissucol's adhesive action generally seems adequate to hold the dural patch in place. In lesions of more than 1 cm^2, we considered it necessary to anchor the lyodura patch with a certain number of stitches according to the size of the patch before proceeding to glue its edges; the distance between the stitches should not be greater than 3 cm.

Dural lesions located in the frontoethmoidal zone are generally not of wide extent, but repairing them often proves unsuccessful, perhaps because of the greater pressure exerted by the CSF. None of our patients suffered from a CSF fistula relapse. Spinal dural sutures often leave some residual openings from which there is an outflow of CSF, because a hydrostatic pressure is added to the normal fluid pressure; it is for this reason that we make use of the "watertight" properties of Tissucol in the majority of cases, even for simple, linear sutures. It is important to anchor the dural patch with a few stitches, otherwise there may be some dehiscence. Tissucol proved effective in assuring a "watertight" seal of the dura, even during operations, when the area was wet with blood or CSF.

The positive final result is not the only advantage of using Tissucol: this adhesive also makes for a more simple surgical procedure. A dural suture with stitches may sometimes prove complicated and require some time, both because of the fragility or scarcity of the dura. Suture by adhesion is a simple procedure.

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Use of Fibrin Glue for Sealing and Prophylaxis of Cranial and Spinal CSF Fistulae: Indications, Technique and Results

P. KNÖRINGER

Key words: Fibrin glue, CSF fistula, cranial and spinal

Abstract

Fibrin glue was used in 612 neurosurgical operations from January 1980 to August 1985. A frequent and important indication for glueing was secure closure of the cranial and spinal CSF space for sealing or avoidance of a CSF fistula.

Twenty-five patients with rhinogenic CSF fistula could be treated with lasting success. The diagnostic technique and the microsurgical procedure are described. The bone meal-fibrin glue plastic operation for closing bone defects in the region of the base of the skull and the frontal sinuses is described.

The indication, technique and results of percutaneous fibrin glue application in 17 postoperative subcutaneous or subgaleal CSF fistulae are reported.

In the domain of prophylactic application, important indications for fibrin glue are above all in operations in the posterior cranial fossa, in primary treatment of frontobasal injuries, in occlusion of the sellar floor after transphenoidal hypophyseal operations and in the early treatment of myelocele malformations of neonates. Further indications for prophylactic glue application are indicated and listed in Table 2.

Introduction

In view of the author's positive experience with fibrin glue application since 1976 in the Department of Neurosurgery at the Rechts der Isar Medical Center at the

Table 1. Fibrin glue in sealing of manifest CSF fistulae

Rhinogenic CSF fistulae	
through	
Sinus frontalis	
Cellulae ethmoidales	open
Sinus sphenoidalis	surgical
Tuba Eustachii	
Postoperative CSF discharge	
after	
Cranial operation	closed percutaneous
Spinal operation	or
	open surgical

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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Table 2. Fibrin glue in prophylaxis of CSF discharge

Frontobasal injury
Transsphenoidal operation
Posterior cranial fossa operation
Lyodura + Palacos resin
Lyodura + osteoclastic trepanation
Lyodura + prior irradiation
Myelocele operation
Untight dura suture
Inaccessible dural gap

Technical University of Munich, fibrin glue has been employed routinely in an increasing number of indications since 1980 in the Division of Neurosurgery of the University of Ulm in the Günzburg District Hospital.

From January 1980 to August 1985, fibrin glue was employed in 612 neurosurgical operations. An important indication for glue application was secure occlusion of the CSF cavity for sealing (Table 1) and prophylaxis (Table 2) of cranial and spinal CSF fistulae.

Application of Glue to Seal CSF Fistulae

Rhinogenic CSF Fistulae

In the presence of a rhinogenic CSF fistula, the fistula orifice was localized precisely before the operation in order to be able to carry out a pinpointed operation which is as small as possible and nevertheless achieves secure sealing. Escape of fluid from one nostril, the Dextrostik test, the smell test, X-ray tomography and computer tomography gave pointers with regard to the lateral localization. CSF indium scintigraphy proved to be an exceedingly sensitive and effective method of detection.

If the lesion could be localized with certainty, only the affected side was exposed after a coronal incision. If this was not possible, or there were bifrontobasal dural fistulae, the frontal base of the skull was inspected on both sides. In this case, two frontal bone lids were formed and a bony bridge in the form of a handle was left behind over the upper longitudinal blood conductor. In this way, the surgical procedure could be simplified and damage to the superior sagittal sinus could be avoided with certainty.

Afterwards, an exclusively intradural microsurgical procedure was applied subfrontally from lateral [5].

The most frequent fistulae were found in the olfactory canal in the region of the cribriform lamina. In these cases, olfaction was usually disturbed unilaterally in the sense of a hyposmia or anosmia. If anosmia occured in the presence of a fistula in the olfactory canal, the olfactory bulb was resected. Defects were filled by glued-in collagen sponges or bone meal. The gap in the dura was glued in two layers with

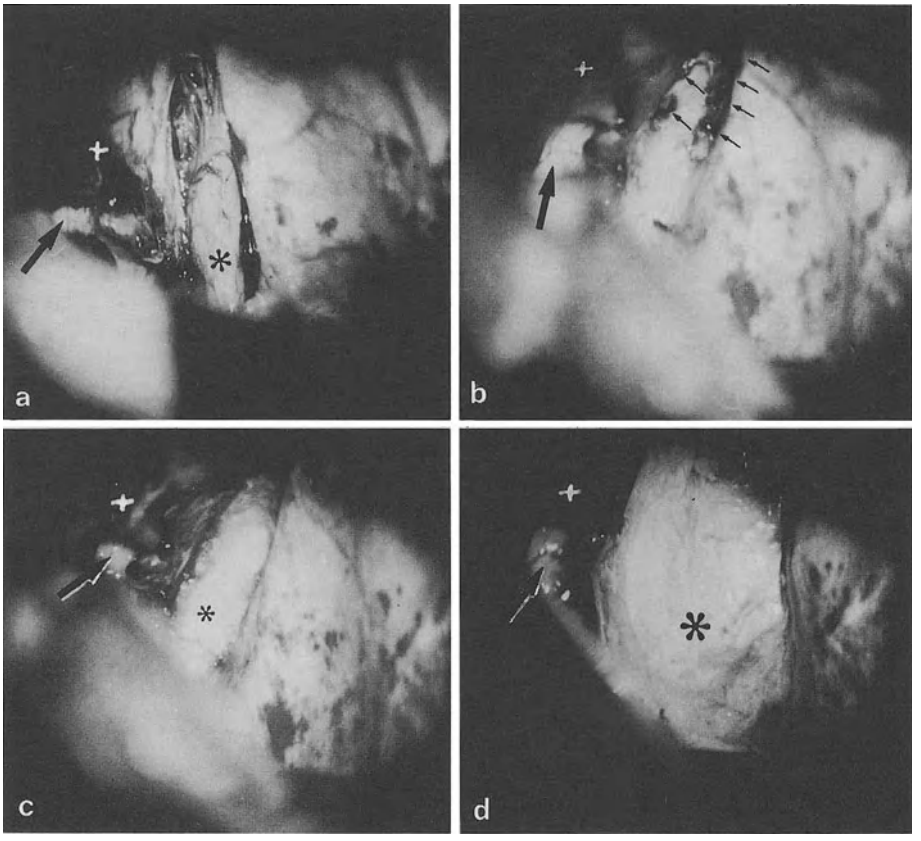

Fig. 1a–d. Frontobasal operation site in nasal CSF discharge on the right. Clinically, there is anosmia on the right and a normal olfaction on the left. The right olfactory bulb is atrophic; all olfactory nerves are torn and no longer detectable due to autolysis. The olfactory bulb (*) is mainly damaged rostrally. **b** The olfactory bulb is resected, and several small dural openings (about six = *small arrows*) are visible in the olfactory canal. They correspond to the sites of passage of the destroyed olfactory nerves. **c** The olfactory canal is filled with a glued-in collagen sponge (*). **d** The olfactory canal is glued over with a periosteal fragment (*) which overlaps it on all sides (→, left olfactory nerve; +, falx of cerebrum)

overlapping periosteal, fascial or more rarely lyophilized dural fragments (Fig. 1a–d). If the olfactory nerve was intact in fistulae in the cribriform lamina, and its function was undisturbed or only partly disturbed, then care was taken to preserve the organ in that only the fistula opening was glued and further sealing was performed by periosteal or fascial fragments which fitted exactly and which were glued onto the olfactory bulb with fibrin glue (Tissucol) (Fig. 2a, b).

In fistulae opening into the frontal sinus, which were usually associated with large dural gaps and bone defects, a frontal sinus revision avoiding damage to the olfactory nerve with subsequent filling with a bone meal-fibrin glue was performed (Fig. 3a–d). The dural defect was covered as already described above [5].

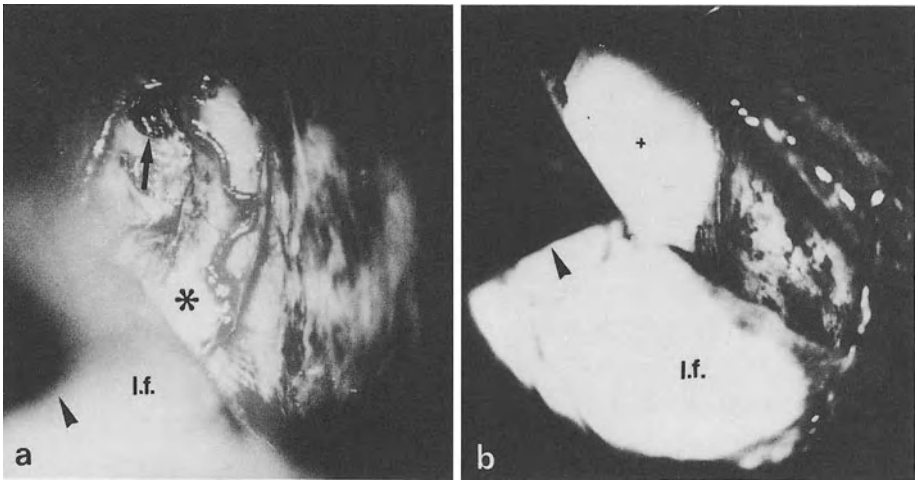

Fig. 2a, b. Operation site of the right olfactory canal in nasal CSF discharge on the right. The olfactory capacity is reduced on the right (hyposmia) and is normal on the left. The olfactory bulb (*) appears intact, but the olfactory nerves are partially damaged. On the left, beside the bulb, a fistula opening (→) can be discerned which corresponds to the site of passage of a torn and now autolytic olfactory nerve. An organ-conserving procedure is possible in that only the fistula opening is glued and care is taken to avoid damage to the olfactory nerve. **b** In order to increase security of sealing, the olfactory bulb is glued over with a fragment of lyophilized dura, which is directly modeled to fit. At the present time, we would give autologous material such as periosteal and fascial fragments preference compared with Lyodura (*, right olfactory nerve; +, Lyodura; *l.f.*, retracted lobus frontalis; ↑ self-retaining brain retractor)

In a patient whose prolactin-producing macroadenoma of the hypophysis melted away under Pravidel therapy, massive nasal CSF discharge developed on both sides, since the tumor had also grown infrasellarly into the sphenoid cavity up to the pharyngeal mucosa. The CSF discharge could be successfully treated by filling the sellar and the sphenoid cavity with glued-in muscle and fat tissue after removing the tumor remnants.

In a further patient, an ipsilateral rhinogenic CSF discharge occurred after an operation on an acoustic nerve neurinoma in which the inner auditory canal had to be bored out via the eustachian tube to remove a tumor cone. The fistula opening could be permanently closed by a glue-in muscle plug, and the CSF discharge could thus be eliminated. Since then, the opened mastoid cells and the dural defect are glued prophylactically when the inner auditory canal had to be bored out to remove a tumor cone. In all cases treated in this way, there was no longer any postoperative CSF discharge.

In 25 patients who were treated in the manner described, no complications were observed. There was suspicion of fresh nasal CSF discharge only in one patient. However, this could be refuted by the diagnostic measures then instituted and the further clinical course.

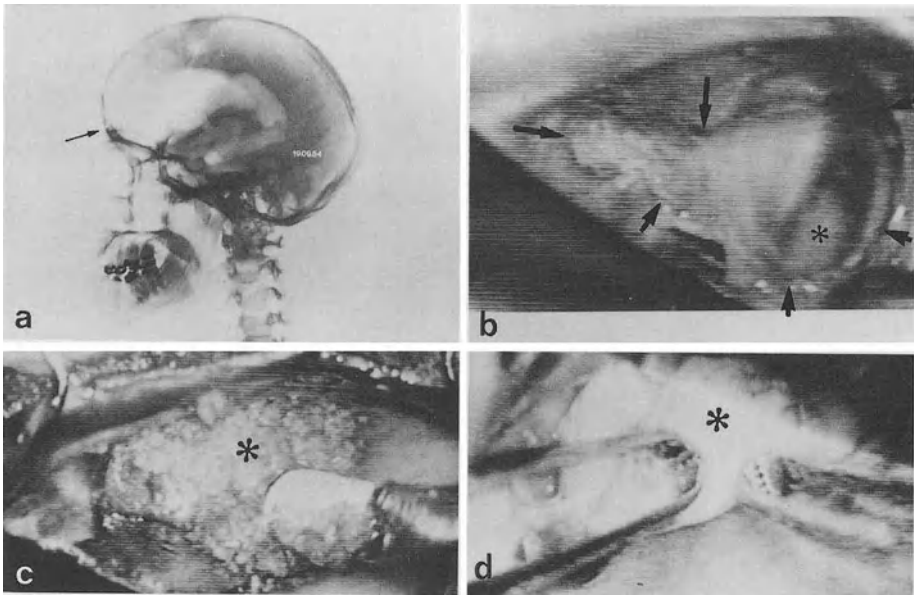

Fig. 3a–d. Pneumatocele and pneumencephalus. Clinical manifestations of brain pressure with highly somnolent state of consciousness, meningism and massive nasal CSF discharge on the left. Bone defect visible in the region of the posterior wall of the sinus frontalis (→ = fissure). **b** View into the left frontal base of the skull. This is possible due to the pneumatocele without insertion of brain spatulae. A dural and bone defect measuring about 1.5×1.5 cm is visible. A view into the sinus frontalis up to the efferent duct to the nose is possible. *Arrows*, dura and bone defect; between the arrows on the left is the sinus frontalis, * = efferent duct to the nose). **c** The sinus frontalis is largely filled with bone meal-fibrin glue. The bone meal was collected in making the boreholes for the trepanation. (* = bone meal-fibrin glue).**d** The dural defect is closed with a glued-on periosteal fragment (*). (Fig. 3a–d derives from the videofilm *Complications and their surgical treatment in a frontobasal injury with primary conservative treatment* [5])

Postoperative CSF Fistulae

We have glued CSF fistulae percutaneously after operation on the brain or spinal cord when there were no other reasons for surgical revision. Here, the subcutaneous or subgaleal or epidural CSF cavity was exactly punctured out percutaneously. Through the needle which was still in position, a glueing of the tissue layers was brought about with occlusion of the fistula opening itself by distribution in the crevice spaces (Fig. 4a, b). In order to bring about as even as possible a distribution before the onset of the glue effect, we have always preferred slow glueing. In 17 patients whom we treated with this method, only one failure was to be recorded. However, double applications were necessary in six of these patients and triple applications in four cases [3, 4]. We consider that the advantages of percutaneous fibrin glueing consist in the rapid and simple performance, the possibility of applying it in outpatients, and above all in the avoidance of a fresh operation.

As already mentioned, patients who have to undergo a secondary operation for other reasons (e.g., CSF congestion in not sufficiently eliminated blockade, raised

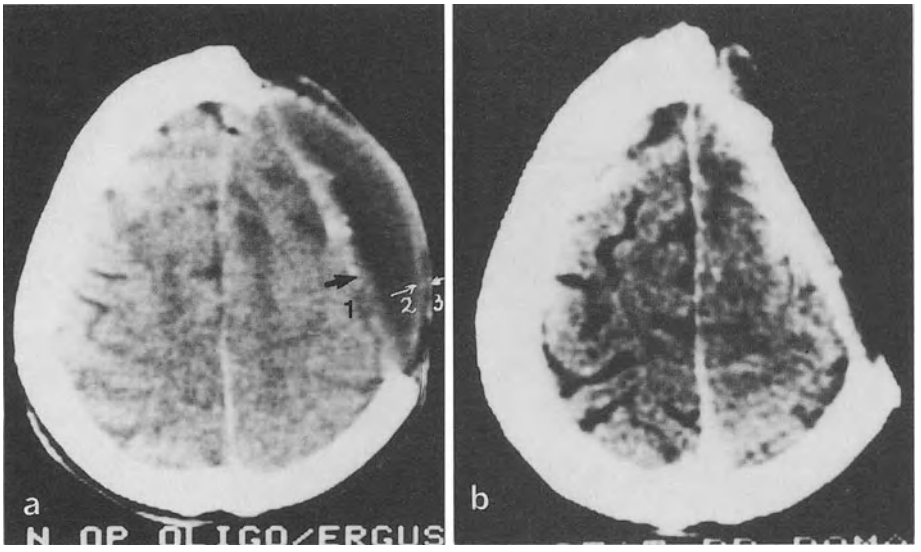

Fig. 4a, b. Epidural CSF cushion after osteoclastic retrepanation to remove a recurrent glioma. Despite several local punctures, combined with pressure bandages and lumbar punctures, the CSF fistulation recurred. *Arrow 1*, dura; *2*, galea; *3*, cutis.**b** After exact puncture out of the epidural CSF cushion (110 ml), glueing of the dura with the galea and occlusion of the fistula opening itself was performed via the cannula, which was still in position. After manual compression for 5 min, a light pressure bandage was applied for 2 days. A lasting closure of the CSF fistula could be attained by a single glueing procedure. Reoperation could thus be avoided

CSF pressure in malabsorptive hydrocephalus, gravely overstretched skin of a fistula wall, manifest infections, persisting or intensified radicular or medullary symptoms in spinal operations) must be excluded from this kind of treatment. In this group, elimination of the cause and closure of the fistula opening is necessary. The possibility and the technique of closing by means of fibrin glue a dural gap which is inaccessible or only accessible with difficulty to suturing is demonstrated by a case with caudal fiber herniation and CSF fistula in the region of the ventral side of the dural sac after operation on an intervertebral disk prolapse (Fig. 5a–d).

Glueing for Prophylaxis of a CSF Fistula

In 52 frontobasal injuries, we were able to avoid secondary CSF fistula by consistent prophylactic sealing by application of the glue in the first surgical treatment.

In 108 cases, the sellar floor and the sphenoid cavity was prophylactically sealed with glued in collagen fleece. There was a brief nasal CSF discharge postoperatively in only two cases: this could be healed up with conservative measures.

There is a pronounced tendency to postoperative CSF fistula in operations in the region of the posterior cranial fossa, in operations on the skull, when a dural patch and a Palacos graft was employed, and in osteoclastic trepanations in which a dural

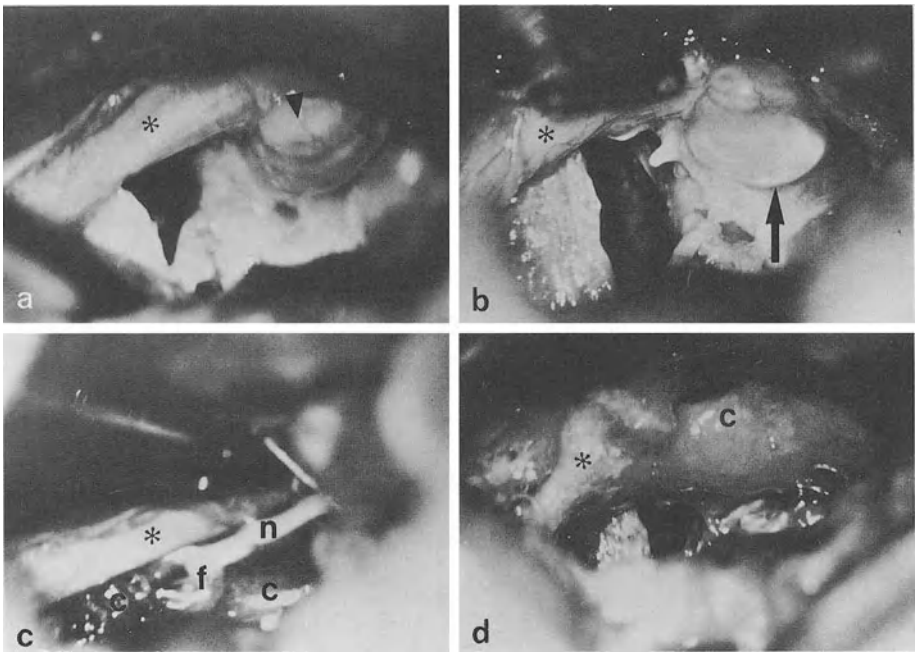

Fig. 5a–d. Operation site in a case of CSF fistulation after operation of an intervertebral disk prolapse. Postoperatively, the patient was initially free of symptoms, and from the third postoperative day severe radicular pain and a subcutaneous CSF fistulation occurred. **a** As cause of CSF fistulation a dural gap and as cause of the radicular pain a herniated root filament at the root origin cranial to the cleared intervertebral space was found. **b** The filament is repositioned and the arachnoid sac is still prolapsed. **c** A collagen fleece is pushed under the root; fibrin glue (slow glueing) is applied and the root is enveloped with this. This part of the figure shows the application of the glue to the collagen fleece pushed under the root. **d** The glued collagen fleece is modeled exactly on the root. In this way not only the CSF fistula could be closed, but the radicular pain could also be abolished by the reposition of the herniated caudal fiber. Since the injury was mainly on the lower side of the root, it would have been accessible to suturing only with extraordinary difficulty. Even if this had been successful, it would have led to a stenosis of the root origin and thus probably to irritation of the root with consequent persistent radicular pain. The simple handling, which significantly shortens the operation time, must be regarded as a further advantage (*, root; ▸, herniated root filament; →, prolapsed arachnoid sac; *f*, Tissucol applied with Duploject; *n*, cannula; *c*, collagen fleece

defect had to be closed by a patch, above all when there was prior irradiation. In these constellations (Table 2), we sealed the suture sites prophylactically with fibrin glue and collagen fleece (Tachotop) and were able to attain in this way a significant lowering of the rate of postoperative CSF fistulae.

In 12 myelocele operations, we sealed the sutures of the various layers with fibrin glue. A subcutaneous CSF cushion did not occur postoperatively in any patient. This had formerly been the case relatively frequently without additional glueing of the suture sites and then mostly required repeated puncture treatments up to implantation of a shunt system. In the first two patients of this group, we glued a suture row with artificial dura and collagen fleece. In these two cases, the wound healing was protracted due to a fistula formation, whereas it was undisturbed in the remaining

cases. For this reason, only the glue alone without further foreign material should be used to seal the suture rows in plastic closure of a myelocele, which is to be regarded as a contaminated wound.

In many of these operations, we applied here the components of the glue separately and preferred rapid glueing. Recently, we have increasingly applied the glue simultaneously with Duploject to improve the glueing effect by optimal mixing of the components and with use of slow glueing [2, 11, 12].

Discussion

The sealing of the CSF space after accidents with dural rupture, above all in the frontobasal region as well as after cranial and spinal operations, is an important and numerically frequent indication for fibrin glueing in neurosurgery [1, 5, 6, 8, 9, 10, 14].

Compared with the method of closing frontobasal dural fistulae by nailed on periosteum, fascia or lyophilized portions of the dura, fibrin glueing not only has the advantage of being a simpler operation, but also of greater safety since no gaps are left behind as between the nails. In contrast to nailing, postoperative neuroradiological investigations are not disturbed in glueing. In particular cases, chiefly when the occurence of traction or shearing forces must be reckoned with postoperatively, nailing can be combined with glueing and is then meaningful. Glues with an acrylate base (Histoacryl) are hardly employed any longer today in view of possible toxic side effects and the poor glueing effect in the moist CSF medium.

Recently, an alcoholic prolamine solution (Ethibloc) has been recommended for closure of frontobasal CSF fistulae [7]. In our opinion, this method is only suitable to close small defects, since this solution displays solidification which is too slow and the substance only displays a low glueing effect. Moreover, a small fissure space may easily be left behind between the prolamine and the defect. Fibrin glueing appears to be markedly superior to this method with regard to secure sealing and above all in view of the possibilities of combination (fibrin bone meal grafting, spongiosa-fibrin glue grafting [13], glueing of autologous tissues such as periosteum and fascial fragments).

In view of the considerations mentioned, fibrin glueing is at present the safest and simplest technique for sealing and for prophylaxis of cerebral and spinal CSF fistulae.

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Bone Meal – Fibrin Sealant Plasty in the Neurocranium: Technique and Indications

P. KNÖRINGER

Key words: bone meal, fibrin glue, cranioplasty

Abstract

A method is described to cover small to medium bony defects of the skull base and cap by a plasty of bone meal and fibrin sealant. The material is obtained during trepanation; a second intervention in another part of the body is thus unnecessary. Performance of the operation is rapid and simple. Further advantages are the watertight closure of the skull defect, good moldability, resistance to deformation, and restoration of vital bone tissue. The low primary solidity of the plasty does not play a decisive role, as this method is applied in parts of the skull that are not exposed to greater mechanical strain in the initial stage. Indications are pointed out and illustrated.

Introduction

Bone meal obtained in trepanations has proved an excellent material for filling drill holes. It is frequently applied in frontal craniotomies, for it helps avoid the disfiguring shrinkage and depression of scar tissue which appear in open trepanation holes. In general, osseous vital incorporation takes place with slight shrinkage. This has been confirmed repeatedly in recraniotomies, e.g., in recurring tumors.

As vital incorporation of the bone meal was clinically confirmed in this indication, it seemed obvious in the management of CSF fistulas to close bone defects reaching into the facial sinus system by filling the holes with a mixture of bone meal and fibrin sealant. The dura fistula was usually closed microsurgically on the intradural side by glued-on periosteal or fascial patches [4, 5]. Based on our positive experience with this method since 1980, the indications could be extended. These indications and the surgical procedure are reported on here.

Materials and Methods

The bone meal obtained in trepanations is collected and preserved in an isotonic Ringer's or NaCl solution enriched with an antibiotic (e.g., Nebacetin, Refobacin). When drilling, physiological saline should be used to an extent that the bone meal is not washed away or excessively heated and damaged in the course of the drilling

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

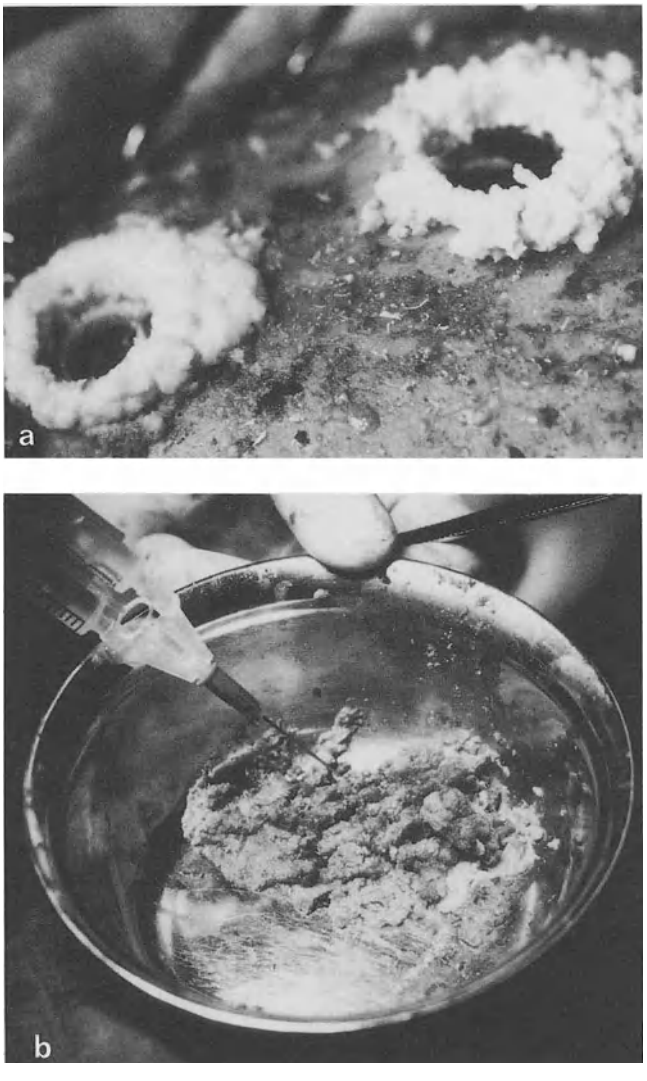


Fig. 1a, b. When drilling, physiological saline should only be applied to such an extent that the bone meal is not washed away nor excessively heated or damaged during the drilling procedure. **b** The surplus antibiotic is squeezed out of the bone meal; the two components of fibrin seal are then put on the bone meal in layers by means of Duploject and well mixed

procedure. For preparation of the bone meal – fibrin sealant plasty, the excess antibiotic solution is decanted and then gently squeezed out of the bone meal that has been placed between compresses. To produce the combination, bone meal is evenly mixed with 1–2 ml Tissucol. Both components of the sealant are applied simultaneously with Duploject; it is important to use the slow sealing effect so that there remains enough time for further processing (Fig. 1). The mixture is molded into the bone defects formed.

If necessary, a procedure in several steps is possible. In this case the bone meal–fibrin sealant plasty is prepared step by step from fresh and fitting portions. With this method even more complicated structures may be formed successively or corrections made. After setting of the sealant (approximately 5 min after application), the bone defect is closed watertight by a tough elastic plasty.

Indications

Bone defects at the frontal skull base can be sealed watertight with immediate and lasting effect by partial filling of the sinus concerned (sinus frontalis, cellulae ethmoidales, sinus sphenoidales, cellulae mastoideae) (Fig. 2). This method represents a considerable improvement in the therapy and prophylaxis of rhinogenous CSF fistulas.

Closing the defect with a vital layer of bone eliminates the risk of an ascending infection with meningitis or meningo-encephalitis, which would exist in the case of an open bone defect.

If the sinus frontalis or cellulae mastoideae have been opened unintentionally during trepanation, biologic closure for prophylaxis of infection or CSF fistula is possible by combination of bone meal, fibrin sealant, and antibiotic. This procedure seems safer and more physiological than the usual occlusion with bone wax.

Smaller to medium defects of the skull cap can be well closed by a plasty of bone meal and fibrin sealant. Trepanation holes outside of the hair, especially in the frontal area, or a partial or complete bald head can be taken into consideration. If the bone meal is mixed with fibrin sealant, the shrinking procedure, which usually takes place in the incorporation stage, appears to a lesser degree than if bone meal alone is used.

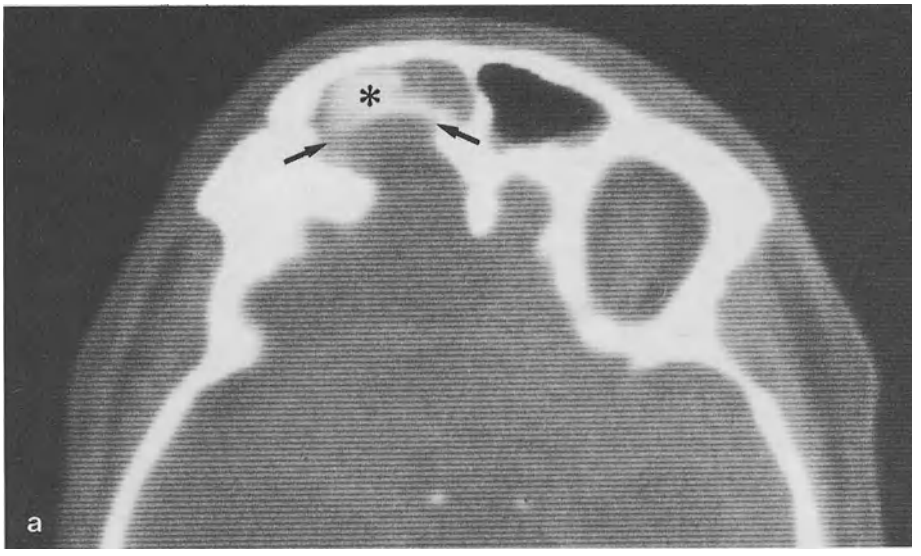


Fig. 2a–d. Bone meal – fibrin sealant plasty (marked with a *star*) in the area of the skull base with partial filling of the sinus concerned with rhinorrhea for closure of various defects:
a A large frontobasal bone defect (between *arrows*) reaching into the left sinus frontalis. **b** Dura and bone fistula reaching into the left sinus sphenoidalis. **c** A bone defect reaching into the dorsal medial cellulae ethmoidales. Behind the bone plasty the big sinus sphenoidalis with septum is visible. **d** Sagittal reconstruction (magnification of a detail) of **c.** *Arrow*, sinus frontalis; *star*, bone meal – fibrin sealant plasty (between four small *arrows*) in the area of cellulae ethmoidales; extensive sinus sphenoidalis in front of and under the sella turcica (*cross*). Change of the whole frontobasal anatomy due to preceding trauma

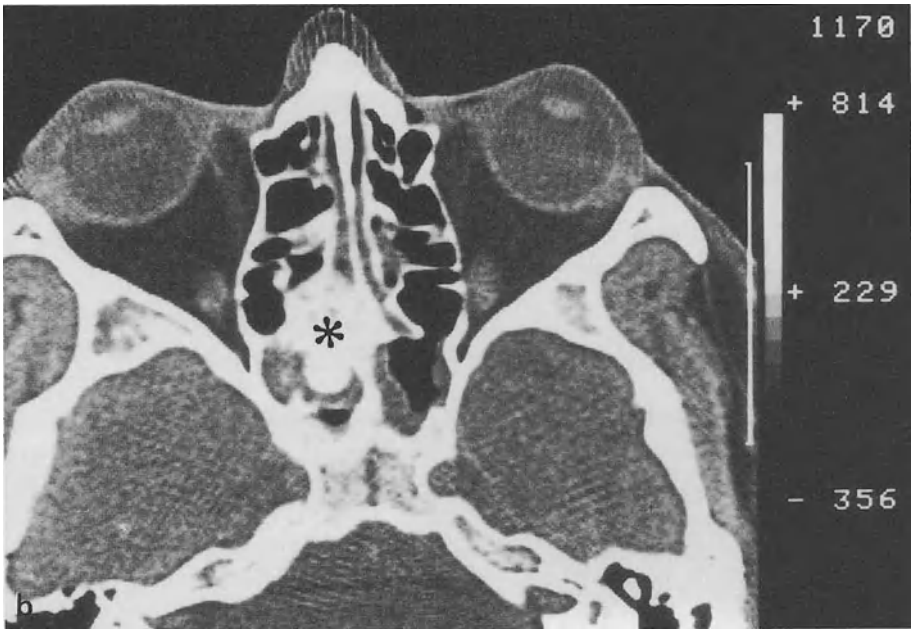
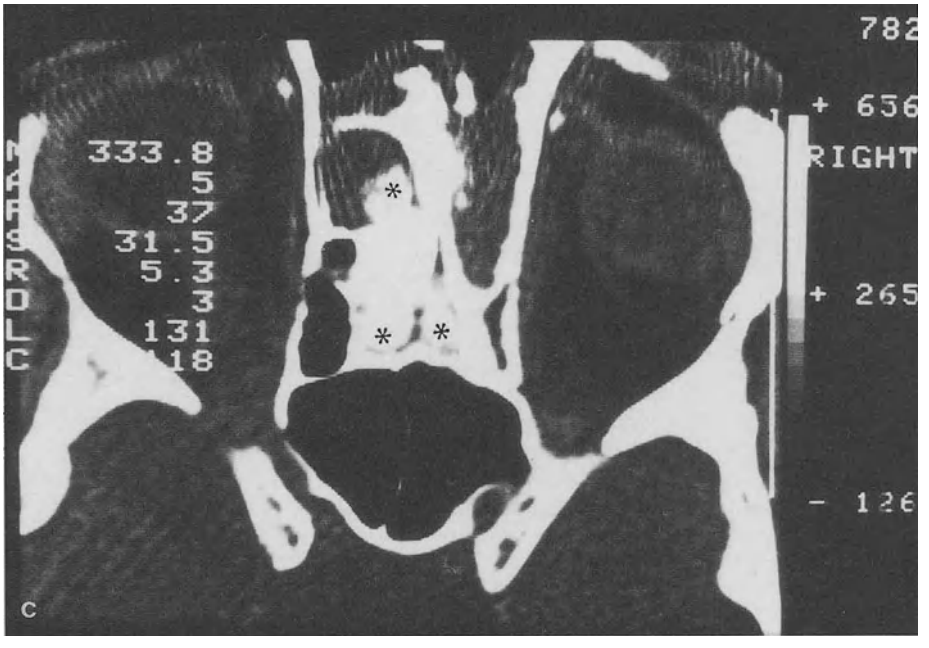

1170
+ 814
+ 229
- 356
b

Fig. 2b


782
+ 656
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333.8
5
37
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3
131
18
C

Fig. 2c

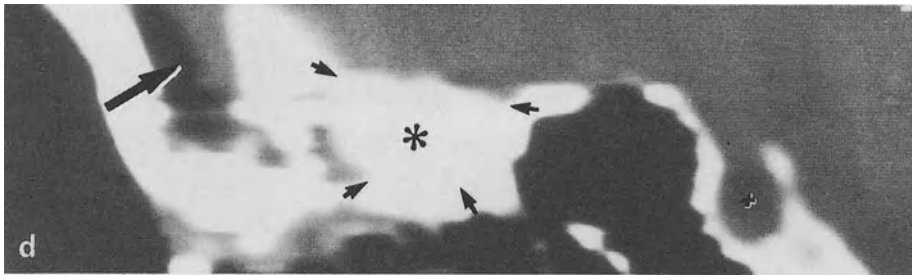

Fig. 2d

Another indication is the closure of a cloverleaf trepanation opening after removal and drainage of a chronic subdural hematoma. If the defect remains uncovered there is usually deep shrinkage after surgery. This may be a psychic strain for persons who already suffer from partial alopecia. For this reason, secondary closure of such defects had to be performed at our clinic in several cases. Primary closure by the method indicated helps avoid these secondary interventions.

This method has proved successful in closure of trepanation defects in EIAB operations as well. This indication has the advantage of improved closure of the CSF space without restriction of the donor vessel.

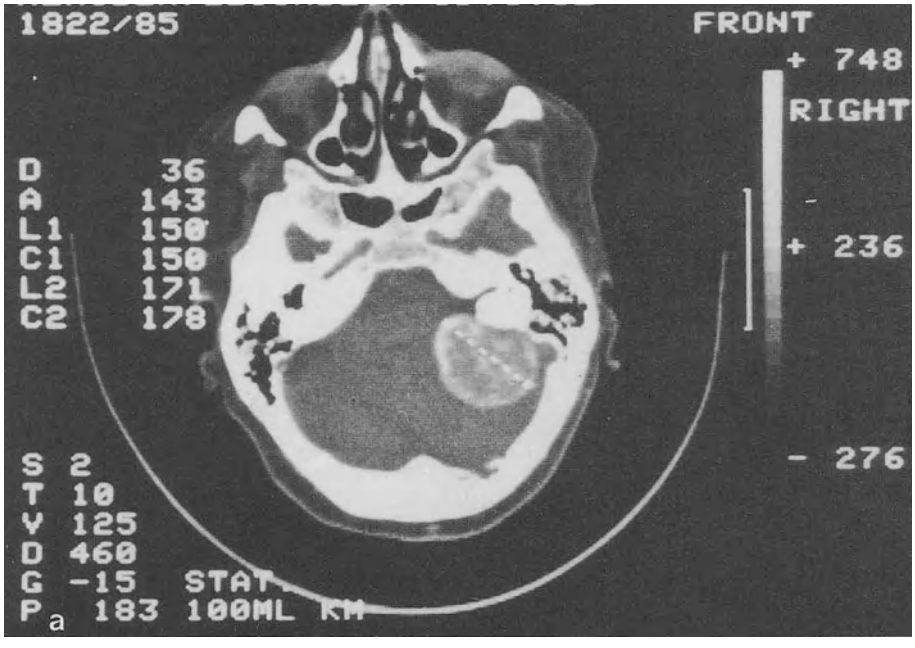

Fig. 3a–d. Meningioma of the posterior cranial fossa in the area of the right cerebellopontile angle. **b** Osteoclastic trepanation defect closed with bone meal – fibrin sealant plasty (between *arrows*). **c** Operative site with modeled bone meal – fibrin sealant plasty. **d** Plasty seen through operation microscope for demonstration of tight sealing of bone gap

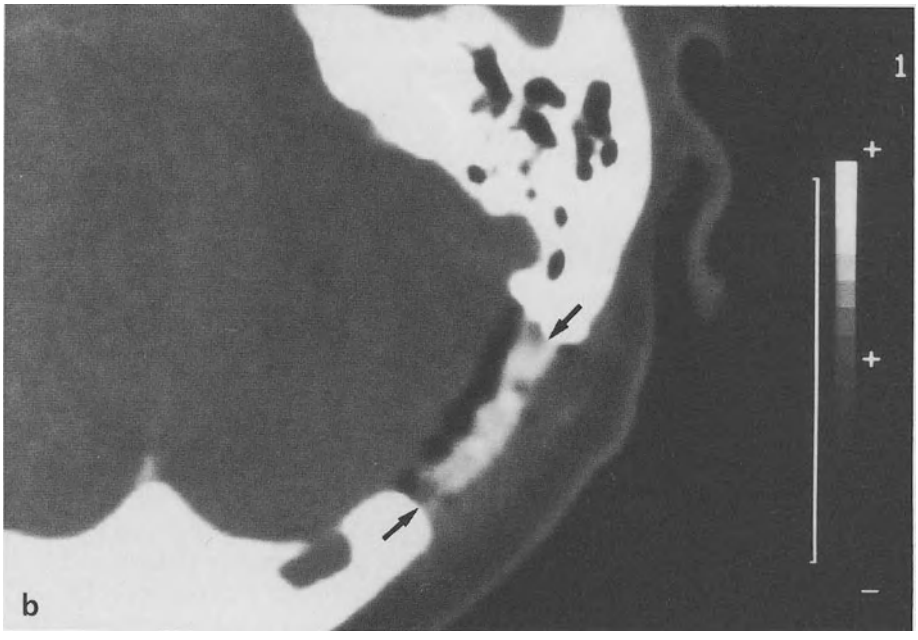

Fig. 3b

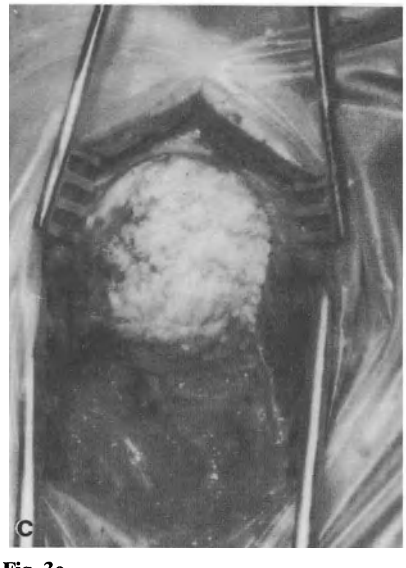

Fig. 3c

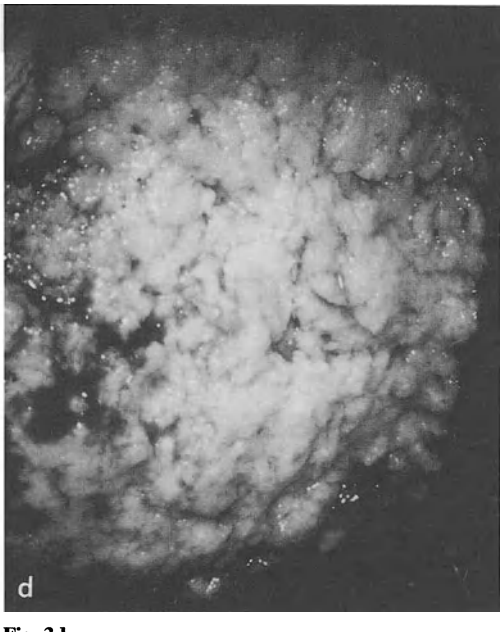

Fig. 3d

It may happen that the trepanation hole has to be extended osteoclastically, e.g., for removal of a tumor. The defect remaining after reimplantation of the sawn-off bone cover can be sealed without complications with the bone meal – fibrin sealant plasty.

There is a relatively high risk of CSF fistula formation in the area of the posterior cranial fossa. The median, paramedian, and lateral routes of access are particularly concerned, as there is less soft tissue to cover the trepanation hole. If the dural suture is sealed with collagen fleece and the trepanation defect filled with a bone meal – fibrin sealant plasty, the appearance of CSF fistulas is rare. Another advantage of this technique is the decreased vulnerability of the cerebellum by restoration of a bony closed posterior fossa. In lateral interventions for removal of benign cerebellopontile angle lesions or for microvascular decompression of a cerebral nerve, and especially after placing a PICA anastomosis, this method is absolutely indicated (Fig. 3)

If an operation has been performed in the orbita from a subfrontal or frontotemporal approach for removal of an orbital tumor or management of an injury, the roof and wall of the orbita should be reconstructed. This helps restore the physiological conditions in the orbita and effectively prevents the transmission of cerebral pulsations to the bulbus oculi (pulsating ex- or enophthalmos). Filling with a bone meal – fibrin sealant plasty is a simple method for achieving good reconstruction with autologous bone tissue that can be molded easily (Fig. 4).

In this and similar cases fibrin sealant helps maintain the desired form until the bony graft is stabilized and incorporated.

The method of choice for the closure of larger bone defects of the skull is still the plasty of autopolymerizing synthetic material (Refobacin Palacos R), to which has been added an alutable antibiotic for prophylaxis [3, 7] or treatment [2] of an infection. If the defect to be closed reaches into a sinus, especially the frontal sinus,

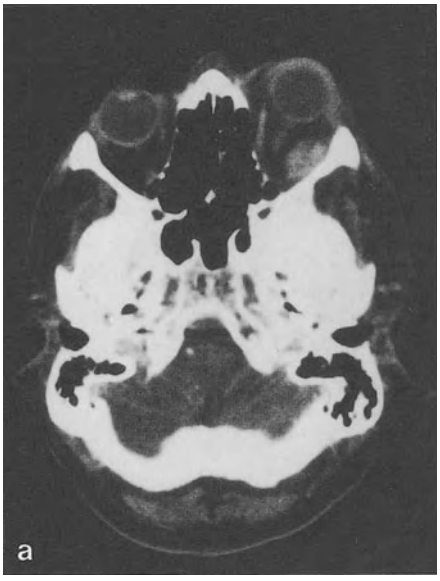

Fig. 4a–e. Right orbital tumor with considerable protrusio bulbi. **c, d** Condition after microsurgical removal of the tumor with extradural, right lateral, frontotemporal access. Reconstructed right lateral orbital wall (*arrows*). **e** Coronary reconstruction for demonstration of orbital roof and wall reconstructed with bone meal – fibrin sealant plasty (*arrows*).

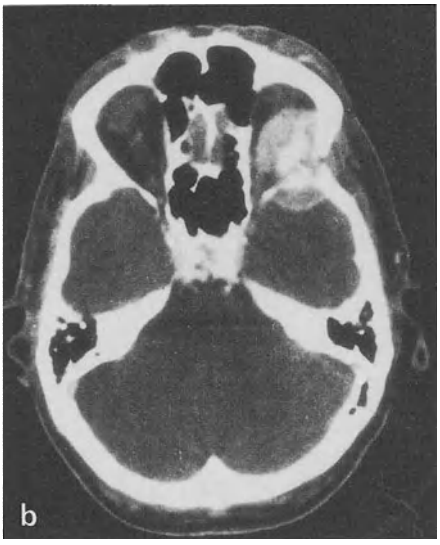

Fig. 4b

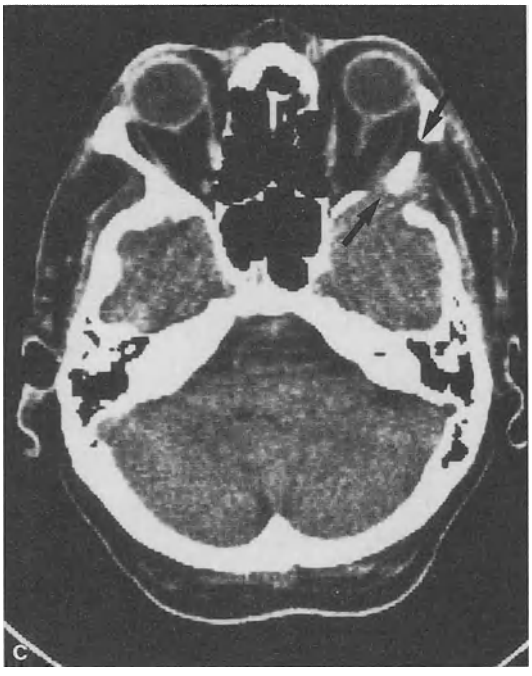

Fig. 4c

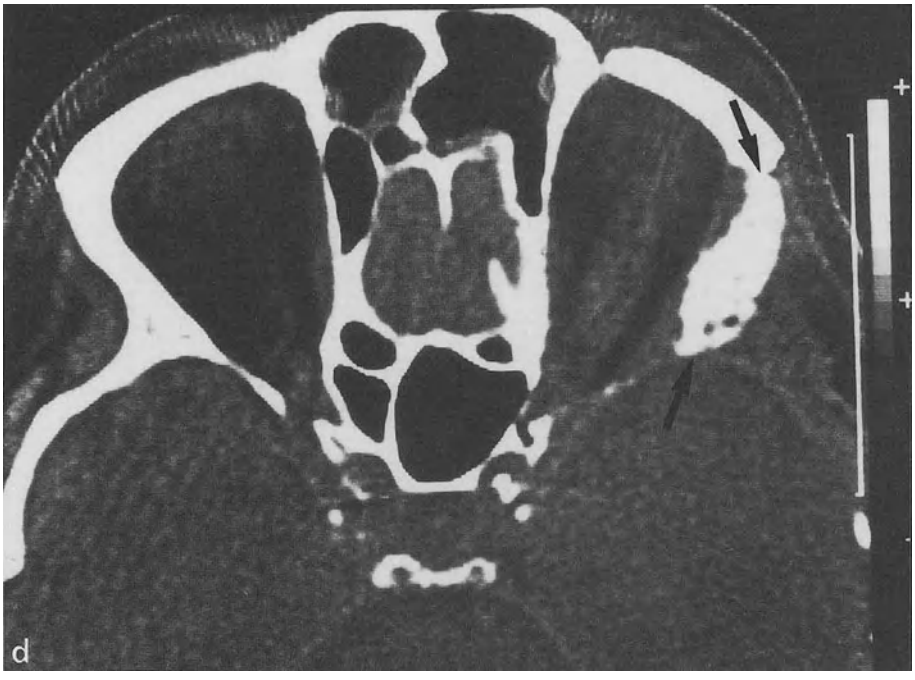

Fig. 4d

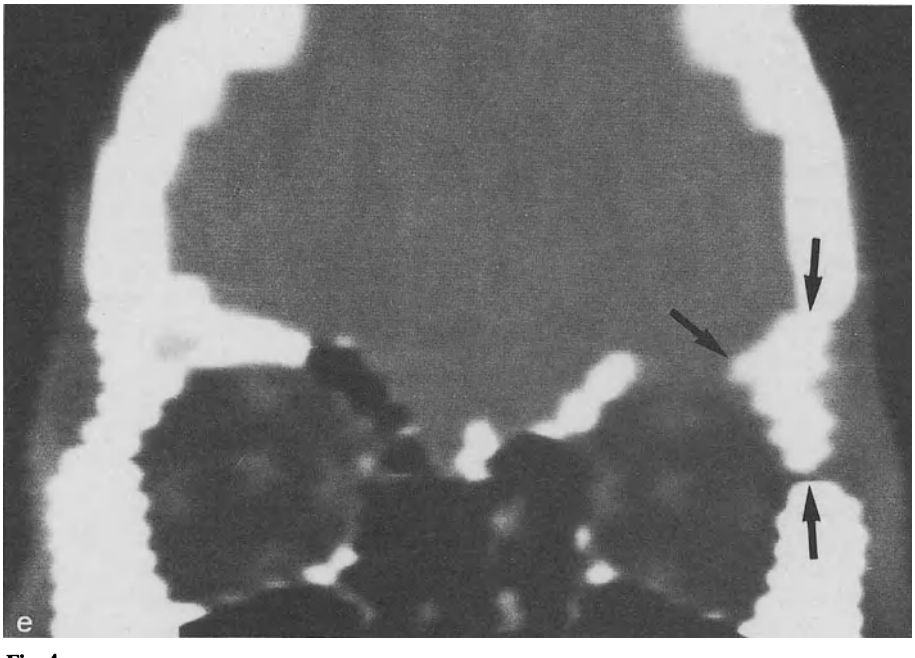

Fig. 4e

Table 1. Indications for closure of small to medium bone defects by a bone meal-fibrin sealant plasty

Bone defects of the skull base to the facial sinus system
Closure by partial filling of the
– sinus frontalis
– cellulae ethmoidales
– sinus sphenoidalis
– cellulae mastoideae
Bone defects of the skull calotte
Trepanation holes outside of the hair
– in the frontal area
– in cases of alopecia
Defects after osteoclastic trepanation
– clover leaf trepanation – chronic subdural hematoma
– trepanation hole – EIAB
– osteoclastic extension hole in cases of too small bone cover
Osteoclastic trepanation defects of the posterior cranial fossa
Especially with lateral access:
– benign cerebellopontile angle procedures
– microvascular decompression of cerebral nerves
– PICA anastomosis
Reconstruction of the bony orbita
– after tumor surgery
– after management of frontobasal injuries with involvement of the orbita
Restoration of a vital layer of bone tissue between nasal sinus and alloplasty of the skull
– after trauma
– after tumor surgery
– after osteomyelitis and sinusitis frontalis

and is filled with a plasty, there may be a late infection of the plasty following elution of the main substance of the antibiotic after approximately 3 years by communication of the sinus with the outside world. A late infection of the Palacos plasty can be safely avoided if a vital bone barrier, 1 cm large, of bone meal and fibrin sealant is erected between the sinus and the alloplasty, to interrupt the communication mechanism described [6]. The bone material required is obtained by drilling two or three holes on the edge of the bone gap. This minor extension of the bone defect is closed in the course of the Palacos grafting. The main indications for a bone meal – fibrin sealant plasty are listed in Table 1.

Discussion and Conclusion

Small to medium bone defects of the skull base, calotte or posterior cranial fossa can be sealed perfectly with a bone meal – fibrin sealant plasty. This method prevents postoperative shrinkage involving negative psychic and cosmetic consequences, the risk of cranial injury is eliminated by the formation of autologous bone in the closed defect, and pressure variations that influence the brain in lying and standing positions with an unclosed defect are avoided.

By mixin g bone meal with fibrin sealant, defects can be closed in a watertight manner with immediate and lasting effect. The modeled form is preserved better and the postoperative shrinking procedure of the plasty is less serious than if bone meal alone is used.

According to experimental and clinical studies by Stübinger [8], transplanted spongiosa is vascularized more quickly and thus vitally incorporated if combined with fibrin sealant. Its even mixture is a prerequisite, the sealant acting as a binding agent in a very thin layer and not as spongiosa replacement.

Addition of an antibiotic serves as prophylaxis of infection. The antibiotic is released from the combination in protracted steps and acts in the border area [1].

The bone plasty described is thus a giant step forward in therapy and prophylaxis of CSF fistulas in the area of the skull base, the auricular sinus system, and the posterior cranial fossa. The bone meal – fibrin sealant plasty seems to be the method of choice in the reconstruction of the roof or lateral walls of the orbita. It is more simple than other techniques and is the only one that leads to formable biologic restoration of the bony orbita.

As the bone meal is obtained during the operation, a second intervention in another part of the body is not necessary. Vital incorporation of the graft in the recipient eliminates a late repulsive reaction.

It is not of great importance that the plasty is not very stable in the beginning, as the spots on the skull where this method is indicated are not exposed to great mechanical strain in the early stage.

The bone meal – fibrin sealant plasty described is simple and does not require more time than other methods as regards the preparation and processing of the material. The results are good and the indication can be handled generously. In the future, more indications will certainly be added.

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Fibrin Sealant in Surgery of the Lumbal Intervertebral Disks

J. STROHECKER

Key words: Lumbar disk operation, postdiskectomy syndrome, interbody fusion, fibrin sealant

Abstract

Thirty-two cases of herniated lumbar disks were operated on performing lumbar disk excision, followed by interbody fusion simultaneously from the same approach. The interbody fusion was done by taking autologous bone chips combined with fibrin sealant. All but one are symptom free without any sensation of pain.

Introduction

Comparing the results after lumbar disk operations in literature, one can see various differences concerning the postoperative outcome. Compared with 90% of the patients working again in their previous job without any troubles, some authors write of only 60% of cured patients and 33% of improved patients. After microsurgical lumbar diskectomy, only 39,2% patients had a complete recovery, in 33,6% a good result, 18,8% a satisfactory result, 6,2% an unsatisfactory result, and 2,2% a poor result [1, 5, 8, 10].

The so-called postdiskectomy syndrome is a real problem for the postoperative treatment, not only for the patient being operated on, but also for the operating surgeon [11]. To understand the "postdiskectomy-syndrome", knowledge of the moving segment of Junghanns including the special innervation is of fundamental importance [2, 7].

The dorsal part of each intervertebral disk, the anulus fibrosus, the dorsal longitudinal ligament, the dura mater, and the intervertebral joint are innervated by the ramus recurrens of the sinu-vertebralis nerve. The ramus recurrens, a branch of the spinal truncus, generally conducts pain put also has proprioceptive and sympathetic nerves. As a result of vertical reduction of the intervertebral space an irridation of these neuronal structures can be started with pain sensation. The same occurs by postoperative arthrosis of the intervertebral articulations after lumbal diskectomy; the nerve is also regularly decompressed.

By interbody fusion performed simultaneously the vertical reduction of the intervertebral space can be stopped; the intervertebral articulations are released respectively. In cervical diskectomy simultaneous interbody fusion is the method of choice in operative technique [4]. Otherwise in lumbar disk surgery interbody fusion will only be done in cases of postoperative instability, usually performed as a second

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

operation [6], although the axial overloading is at its maximum at the lumbar spine, producing a reduction in the intervertebral space.

Material and Method

Proceeding on the above-mentioned assumption I operated on 32 patients suffering from herniated lumbar disks, 22 men and 10 women, performing lumbar disk excision followed by interbody fusion simultaneously. The mean age was 40 years, ranging from 33 to 71 years. Repeated operations were necessary on 11 patients to remove a sequester. Nine patients showed intraoperatively besides the herniated disk, an instability of the intervertebral segment; two patients had herniated disks and a derangement; one patient also had spondylolisthesis. The most frequent disk involved was that between the 4th and the 5th lumbal vertebral bodies, followed by L5/S1 (Figs. 1 and 2).

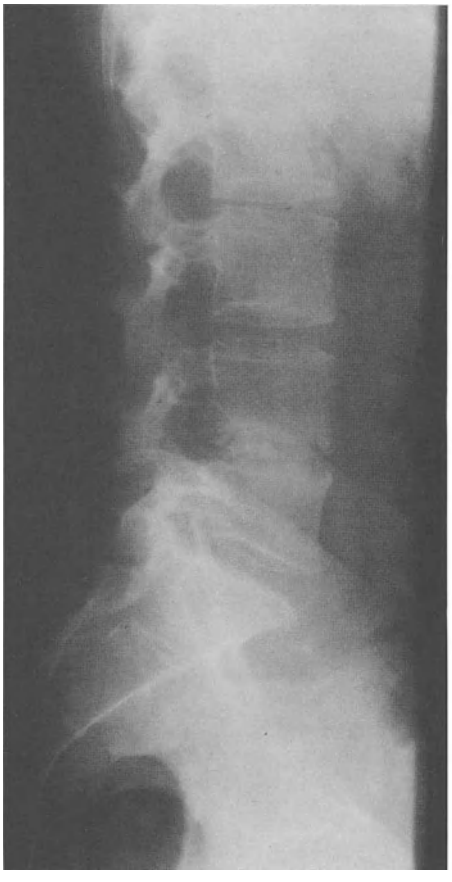

Fig. 1 Postoperative lateral view showing interbody fusion by taking autologous bone chips between L IV and L V (one week postoperative)

Fig. 2 Lateral view 6 months postoperative. The interbody fusion is consolidated, no signs of bone resorption

Twenty-nine patients underwent interlaminar diskectomy; three were treated by laminectomy with extensive decompression because of narrowing of the lumbar spinal canal. In seven patients excision of two lumbar disks was performed followed by interbody fusion.

The operation technique was standardized: In the lateral position of the patient the paravertebral muscles were bluntly shoved off down to the dorsal vertebral arches using the dorsolateral access. The ligament flavum was excised by fenestration. Subsequently the proximal and distal parts of the dorsal arches were partially resected. After exposure of the dura mater and the nerve root of the respective segment the dorsolateral part of the anulus fibrosus was exposed. After oval excision of the ligamentum longitudinale posterior and of the anulus fibrosus the intervertebral space was curetted as far as possible. When curettage was considered to be complete the vertebral cover plates were even curetted. From the partially resected dorsal arches and the neighboring spinous processes, cortical spongiosa chips were taken by filling the intervertebral space with a plug of the cortical spongiosa fibrin sealant [3, 4, 9]. To prevent sticking together of the dura mater and the fibrin sealant plug, surgical cotton soaked in H_2O_2 was introduced temporarily.

Results

All patients were mobilized on the fourth postoperative day without a supporting corset. For all patients follow-up has been obtained. The observation period ranged from 1 year to 20 months. So far 29 patients are symptom free with no neurological deficit or sensation of pain (low back pain, postdiskectomy syndrome). Three patients showed for some weeks to 4 months at the most similar pain symptoms to those caused by postoperative diskitis especially in prolonged standing or rotation. But there were no signs of inflammation either clinical or radiological. All but one returned to work or to their former activities if over the retirement age. The last one is now symptom free and receiving an early social pension.

Discussion

The rather discouraging outcome in patients with only lumbar nucleotomy, independent of macro- or microsurgical procedure, led us to modify the operation technique. We have to do everything to prevent the postdiskectomy syndrome or postoperative low back pain. Thus I mean it is logical to perform interbody fusion immediately when the disk is removed working like a shock absorber. I can state that surgery of lumbar herniation followed by interbody fusion on the whole gives good results. The operation takes only 10 min longer than usual and the advantages are the following:

1. Autologous bone chips are taken from the neighboring arches and spinous processes, and not from the pelvic bone by an additional operation.
2. Interbody fusion is performed immediately after nucleotomy. A further operation is unnecessary.

3. The use of fibrin sealant prevents not only the bone chips from protruding into the spinal canal, but also persisting intervertebral disk sequelae. A beneficial side effect of the fibrin sealant is a smaller scar formation.

Finally, in spite of the limited number of patients, I consider lumbar diskectomy followed by interbody fusion to be a therapeutic procedure specific for low back pain and the so-called postdiskectomy syndrome respectively.

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Experimental and Clinical Use of Tisseel (Tissucol) and Muscle to Reinforce Rat Arteries and Human Cerebral Saccular Aneurysms

J. HAASE, E. FALK, F. FIND MADSEN, and P. STUBBE TEGLBJÆRG

Key words: Cerebral aneurysms, fibrin sealant, extravascular reinforcement of aneurysms

Abstract

Muscle wrapping of cerebral saccular aneurysms has been used since 1933. It seems that a gap always occurs between the muscle and the aneurysm wall and therefore the method of muscle wrapping has not found widespread use. In a rat model we have used rat-Tisseel to produce an immediate effective contact between pieces of muscle and the rat femoral artery wall, confirmed by histological examinations up to 1 month postoperatively. The use of Tisseel in human cases of muscle wrapping of giant aneurysms or broad-based, nonclippable aneurysms is introduced.

Introduction

Human intracranial saccular aneurysms develop based on a combination of developmental and degenerative disturbances in the vessel wall [8]. Blood velocity in the cerebral arteries is high and this combined with hypertension may be the pathophysiological factors most important for the development of saccular aneurysms and their subsequent rupture [8].

The neurosurgeon often observes that aneurysms having bled several times have caused severe arachnoiditis around the aneurysmal sac and thereby have created an external layer of fibrous tissue, which may have hindered subsequent, fatal intracerebral or subarachnoidal bleedings.

Material and Methods

In an experimental model, using Wistar rats, the femoral arteries on both sides were dissected and pieces of muscle, gauze, Gelfoam, and Surgicel were placed around the artery on one side, with and without the aid of rat fibrin sealant (Tisseel). In each series at least two animals were evaluated. Tisseel was used with a high concentration of Thrombin and Aprotinin for fast coagulation. Histological investigations showed that Tisseel disappeared within 7 days, and 1 month following the operation no difference could be detected between the explored artery and the treated artery. There were no signs of external fibrosis or compression of the artery. Gelfoam and Surgicel created no reaction of significance and had disappeared within

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

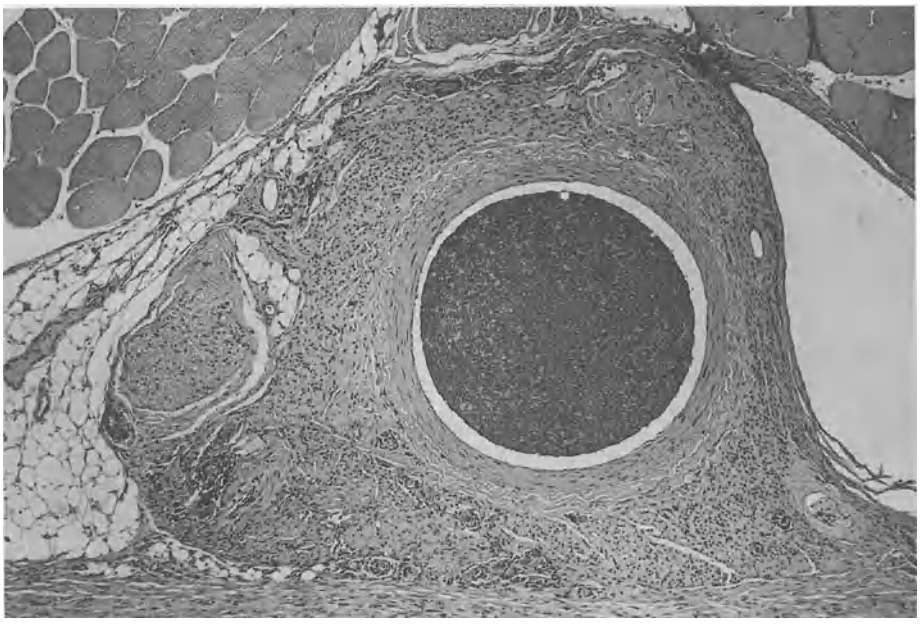

Fig. 1. Rat femoral artery surrounded by degenerated muscle tissue 1 month following application with fibrin sealant. Note close adherence to the vessel wall

1 month. Gauze created a very severe reaction with inflammation and 1 month following the operation a giant cell reaction with a high number of macrophages was demonstrated.

Using Tisseel and muscle, 2 days following the operation the muscle was closely adherent to the vessel and a slight inflammatory reaction had disappeared within 7 days. At the same time muscle fibers started to degenerate and at 1 month follow-up the vessel was surrounded by atrophic muscle and collagenous tissue. There were no signs of inflammation. The fibrous tissue was adherent, but not incorporated in the adventitial layer of the vessel. There were no signs of compression of the vessel wall (Fig. 1). Postmortal angiography was carried out in all cases and no thrombosis or external stenosis was demonstrated.

Discussion

The neurosurgical interest in reinforcement of aneurysm walls dates back to Dott, who in 1931 packed an aneurysm in loose muscle during an operation, where the aneurysm suddenly bled [2]. Subsequent examples showed that packing with muscle was not a satisfactory treatment [5] and in two experimental studies using dogs, Dencker [1] and Sahs [7] found that muscle in itself did not create a satisfactory development of fibrous tissue around the internal carotid arteries. Several polymerizing plastic compounds have been used since then with doubtful results due to

severe reaction in the brain with necrosis of both brain substance and vessel walls [3, 5, 6]. Microsurgical technique, clipping of aneurysmal neck, in combination with development of special clips and endovascular embolization are so far the most succesful methods in the treatment of aneurysms today [4]. However, there are still a few cases where reinforcement of the wall of the aneurysm would be the method of choice or an important adjuvant to the clipping.

During the operative intervention of an 80 mm giant aneurysm, arising directly from the posterior cerebral artery, the aneurysm was found to be extremely thin walled. It was considered unsuitable for clipping or resection due to its position, the high age of the patient and generalized severe arteriosclerosis. Instead hammered muscle was fixed to the aneurysmal wall with fibrin sealant, which was used to coat the entire aneurysm. Control CT scan 1/2 year later revealed the same size of the aneurysm and the patient is still living now 2 years following the operation with no signs of repeated bleeding or brain stem compression. Another type of aneurysm is the thin-walled, broad-based aneurysm, where part of the parent vessel is included in the aneurysm and is thus not suitable for clipping without causing severe stenosis of the artery as demonstrated in Fig. 2

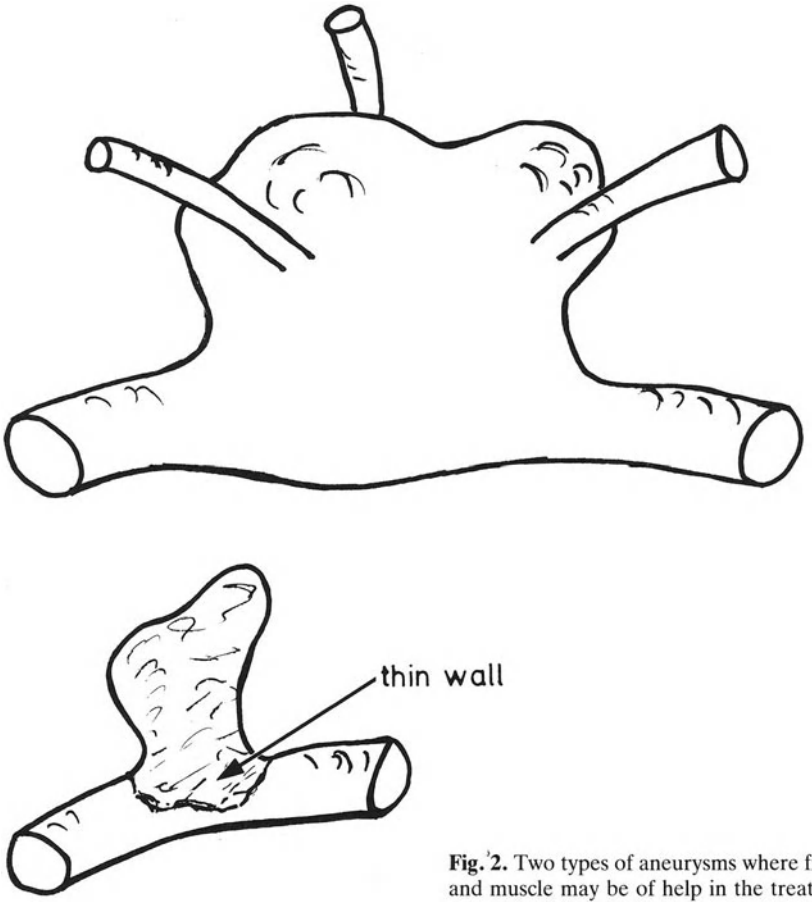

Fig. 2. Two types of aneurysms where fibrin sealant and muscle may be of help in the treatment

We may conclude that fibrin sealant makes it possible to adhere muscle closely to the aneurysmal wall in human cases and in animal experiments. It is suggested that fibrin sealant (Tisseel) will hinder dissection of the muscle from the vessel wall by CSF and that the correct concentration of the sealant will introduce sufficient fibrosis to make muscle a helpful initiator of an external fibrotic layer around aneurysms.

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The Use of Fibrin Sealant in the Neurosurgical Treatment of Lesions of the Base of the Skull

H. WALDBAUR, and R. FAHLBUSCH

Key words: Fibrin sealant, base of the skull, liquor fistula, pituitary tumours, transsphenoidal operation

Abstract

In the Neurosurgical Clinic of the University Erlangen-Nürnberg fibrin sealant has regularly been employed since December 1976 in the surgical treatment of frontobasal liquor fistulae. In the period from December 1976 to April 1984, 171 such interventions were carried out using this fibrin sealant. Closure of the defect was always, intradural, occasionally extradural, in addition. Hereby, we employed dura obtained from dead bodies, fascia lata or galea-periosteum flaps. In the six cases that subsequently developed a leak, the patients had originally presented with severe head injuries, resulting in extensive dural defects; two of these cases developed inflammatory complications in the region of the paranasal sinuses.

In about 600 transsphenoidal operations to remove pituitary tumours, some carried out in Munich, some in Erlangen, fascia lata and fibrin sealant were employed to seal off the defect in the region of the sphenoidal sinus. A liquor fistula occurred postoperatively in only 1% of the patients, as compared with a general incidence of such fistulae of between 3% and 5% in the period before the introduction of fibrin sealant.

Finally, the use of fibrin adhesion in the surgical treatment of other types of neoplastic processes involving the base of the skull is reported. The fibrin sealant was also employed in the closure of defects remaining after the removal of meningiomas of the cribriform plate, aesthesioneuroblastomas or carcinomas of the ethmoid bone. Here, too, the sealant was used with good success.

In our clinic, fibrin sealant is routinely employed during the surgical treatment of traumatic liquor fistulae and in the sealing off of liquor spaces in transsphenoidal interventions.

Liquor Fistulae

Material and Method

Between December 1976 and April 1984 – that is, over a period of more than 7 years – graft closure of frontobasal liquor fistulae was performed in 171 patients, fibrin sealant being employed to fix the graft in place in all cases. In the case of moderately

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

severe head injuries, we perform the operation 10–12 days after the accident. Severe traumatization sometimes enforces a longer delay; in cases with mild trauma, we often operate in the 1st week, in particular if marked liquorrhoea is present. If an extensive open injury or a frontal haematoma requires a primary operation, simultaneous treatment of basal dural defects is also attempted, but not if marked oedema of the brain presents. Roughly one-third of our patients also suffered middle third fractures of the face. As a rule, any oral surgery required is done at the same session – after neurosurgical repair. In about 75% of our patients with a liquor fistula, exposure was usually bilateral. Especially in the case of severe injuries, we give preference to bilateral exposure even when, preoperatively, a liquor fistula can be found on only one side.

In recent years, we have abandoned our former practice of sawing out a bifrontal bone flap. Instead, after preparing a bifrontal scalp flap, frontal craniotomy is now carried out on both sides, with a wide central piece of bone being left between the craniotomies. This procedure is less massive and suffices to permit the inspection and repair of the frontobasis. The exposure and closure of the dural defect, of which there were frequently several in one and the same patient, was always done intradurally (Fig. 1), occasionally extradurally in addition. The material used for the closure was dura obtained from corpses, fascia lata or galea, the latter either as a free graft or as a flap. Dura was applied in 159 patients, fascia lata or galea in 12 patients. On the use of fibrin sealant– in particular in the operations on liquor fistulae – the following points should be mentioned: the dural defect must be clearly exposed, the graft bed must be kept dry immediately before positioning of the graft to which sealant has been applied, and accurate placement of the graft, which must extend well beyond the margins of the defect, must be ensured, as also firm contact between the graft and graft bed.

Results

In the case of the patients reported on here, at least 1 year has elapsed since the repair of the frontobasal liquor fistulae. In six out of the 171 patients operated on (that is, in 3,5%), liquorrhoea subsequently recurred (in the patients treated with dura grafts the recurrence rate was 3,1%). In one of these patients, a revision procedure was carried out several weeks after the initial operation to treat a repeated intracranial collection of air. On this occasion, however, no leak was to be found. Possibly, a temporary leakage had occurred. In two patients who had suffered severe injuries in the region of the paranasal sinuses, a suppurative inflammation of the paranasal sinuses occurred postoperatively together with sloughing of parts of the graft.

The three remaining patients showed very extensive dural defects that were difficult to close, and extensive bony defects in the cribriform plate and posterior wall of the frontal sinuses, so that it proved difficult to find a stable substrate to which the graft could be glued.

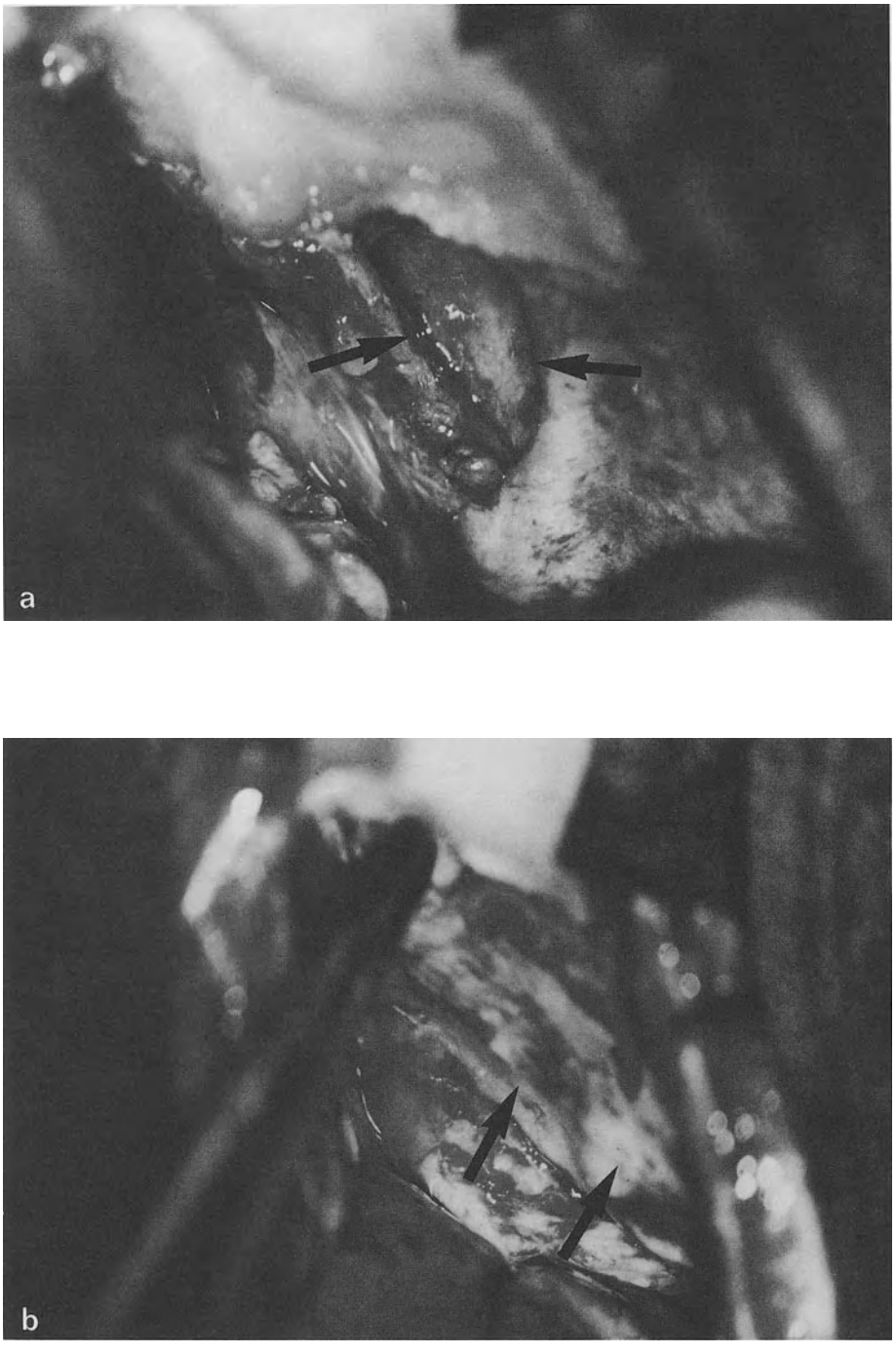

Fig. 1a, b. Graft closure of a frontobasal liquor fistula. **a** Dural defect in the region of the lesser wing of the sphenoid; **b** closure of the defect with a galea-periosteum graft fixed into position with fibrin sealant

Discussion

If the results and also the unfavourable courses are considered critically, we may be inclined to be "generous" in establishing the indication for an additional otorhinolaryngological intervention – shortly after neurosurgical management – in severe injuries including crush fractures and haemorrhage in the region of the paranasal sinuses. Thorough clear-out of the paranasal sinuses and creation of a good drainage to the nasal sinus reduce the danger of inflammatory complications arising. Naturally, the postoperative courses in which a suppurative colliquation led to a recurrent liquor fistula cannot be ascribed to unsatisfactory adhesion of the fibrin sealant. Even when these details are not taken into account, a recurrence rate of about 3% following surgical treatment of liquor fistulae must be considered favourable. In the relevant literature published prior to the introduction of the fibin sealant, considerably less favourable results are found in almost every report. With the exception of the series reported on by Dietz [2], who, in 110 surgically treated frontobasal injuries, observed only two failures, the recurrence rates were between 6% and 20% (Tönnis and Frowein [7], Colas et al. [1], Paillas et al. [5], and Probst [6]).

Pituitary Tumours

In our clinic, fibrin sealant is also routinely used in transsphenoidal operations for pituitary tumours. After removal of the tumour, sealing is effected with fascia lata introduced within and outside the plane of the floor of the sella, together with abundant fibrin sealant (Fig. 2). With the exception of patients with microadenomas located at a distance from the diaphragm of the sella, closure with a fascial graft is regularly practised. At surgery a discrete flow of cerebrospinal fluid may be

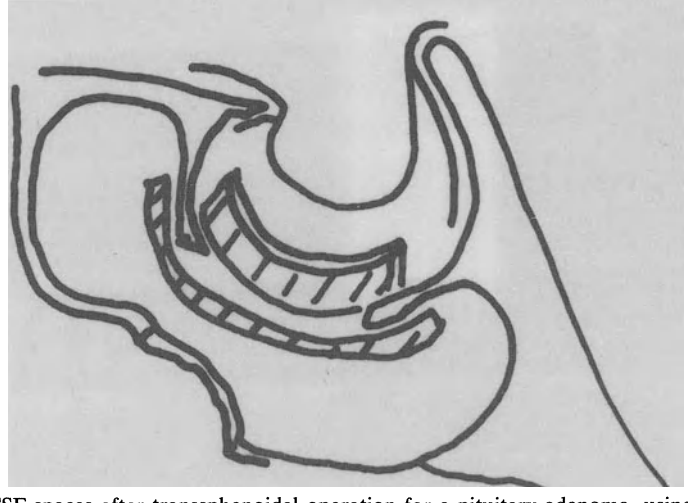

Fig. 2. Closure of the CSF spaces after transsphenoidal operation for a pituitary adenoma, using fascia lata. The fascia is introduced in two layers in the plane of the floor of the sella (Fixation with fibrin sealant)

observed in 10% – 20% of the patients. Most of these cases have hormonally active microadenomas, and a thorough exploration of the sella is carried out. If the flow of CSF is marked, and in cases in which the tumour had given rise, preoperatively, to a liquor fistula, a lumbar drain is placed and left in position for about 2 days. To date, fibrin adhesive has been employed in about 600 transsphenoidal operations on the pituitary. In only six patients, that is in 1%, did a liquor fistula requiring operative treatment develop postoperatively. In the series of Erlangen liquor fistulae were observed in 0,54% (in two out of 368 patients). In comparison, the rate of liquor fistulae observed in such interventions in the period prior to the introduction of the fibrin sealant was 4% [4].

Other Tumours of the Base of the Skull

In surgery on the base of the skull, the fibrin sealant has proved a useful aid in another area, too. We refer to graft closure following surgery for meningiomas invading the cribriform plate, for aesthesioneuroblastomas (n = 3) and carcinomas of the ethmoid bone. The last two types of tumour can massively infiltrate the base of the skull and grow into the cranium as well as into the sinuses and into the cavum nasi. These interventions are carried out in cooperation with ear, nose and throat (ENT) specialists from the Otorhinolaryngology Department of our university. The removal of the tumourous tissue can result in extensive defects that are difficult to close. In such a situation, we have repeatedly made use of the fibrin sealant with good results, although the number of operations and the postoperative observation periods are still inadequate to permit any definitive assessment to be made.

Summarizing, it may be stated that, owing to its ease of use, its biotolerance and good adhesive properties, fibrin sealant represents an important adjunct in surgical treatment of lesions of the base of the skull. In consequence of the reduction in the incidence of liquor fistulae, the danger of developing meningitis, which even in the age of antibiotics still represents a life-threatening complication, is further diminished. Hepatitis was observed in none of our patients.

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Results of Nerve Grafts with Tissucol (Tisseel) Anastomosis

D. V. EGLOFF, A. NARAKAS, and C. BONNARD

Key words: Fibrin, Tissucol, nerve, graft, microsurgery

Abstract

Between 1980 and 1982, 56 peripheral nerve repairs were made with Tissucol. For technical reasons combined anastomoses were chosen in brachial plexus repairs (23 cases) and main nerve trunk repairs (17 cases), Tissucol alone being used in most other cases (8 free flaps, 8 digital nerves). A preliminary report was published in 1983. The results compared well with the so-called classical repair methods using stitches. The same cases were reviewed for long-term follow-up, which confirmed the conclusions of the preliminary report:

1. The glue applied as a kind of sleeve around the neurosynthesis site creates a regeneration chamber protecting the nerve stumps against invading neighboring tissues and restricts axon sprout escape
2. There is no impairment of axonal growth through the single (mainly free toe transfers) and the proximal anastomoses
3. There is an impairment of axonal growth through the distal anastomosis proportional to the length of the graft
4. The main advantage is the gain in operative time
5. It is necessary to use slightly longer grafts than with classical methods, to avoid any tension which would be deleterious
6. The other advantages are: hemostasis, stabilization and solidarization of small grafts allowing sharp division, atraumatic direct muscle neurotization

Since 1983 a slight change has been introduced to the technique: using a few stitches, in most cases, before Tissucol is applied so as to maintain orientation and coaptation during the glueing process and the first postoperative hours.

Introduction

Using human fibrin's adhesive properties in peripheral nerve repairs is not new. Young and Medawar [1] and then Tarlow and al [2] mentioned it as early as 1940 and 1944 respectively. Matras et al. [3, 4, 5, 6, 7] get the merit for having studied them further first experimentally, then clinically. Their research has been completed by van der Werf et al. [8], Ventura et al. [9], Duspiva [10] and Kuderna [11], the latter reporting on the largest clinical series.

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

In 1980, we started using Tissucol instead of sutures, to fix nerve grafts. A preliminary study was made in 1982 on the first 56 cases [12]. This was thought necessary to assess the reliability of the method. Since then 110 other cases have been treated, using Tissucol. We report on our first 56 cases only, for which we have an adequate follow-up.

Material and Method

In these 56 cases, Tissucol alone was used in free flaps, and digital nerves. A combination of anastomosis, stitches and Tissucol was used in most brachial plexus and nerve trunk cases. The injured nerve and the grafts are prepared as for a classical repair with stitches. The nerve stump is cut with Meyer's instrument. The grafting is usually effected by fascicular groups. The grafts are fixed with one stitch of 10°. Three to four drops of fibrinogen are placed on the suture line and a rubber band (a fragment of surgical glove) is folded over it for 2–3 min. We always use the highest concentration of aprotinin.

Evaluation

For the motor recovery, results were considered:
- Good if the muscle response was M4 or M5, i.e., complete recovery or contraction possible against resistance
- Average if the muscle recovery was M3
- Bad for M2, M1 and Mo

Regarding sensory recovery, we used the following scheme:
- Good result for S4, that is discriminative sensation
- Average result fo S3, i.e., protective sensation
- Bad result for S2, S1 and So

Results

Table 1 shows the overall results in 1982 and 1985. The difference is essentially due to the fact that all cases now have an adequate follow-up and can be studied. Some cases improved from bad to average or from average to good. No case scored less on the 1985 evaluation than compared with the 1982 control.

Table 1. Overall results

56 cases	1982	1985
Good	17	23
Average	15	16
Bad	6	10
?	18	7

Table 2. Free flaps (eight cases)

	1982	1985
Good	4	4
Average	2	3
Bad	–	–
?	2	1

The survey on free flaps covers six toe transfers, one pulp transfer and one muscle transfer (Table 2). This is a particularly interesting group, set aside since the cases it contains have been dealt with one single suture or sealant application, tension being avoided in dissecting the nerves of the free flap at a great enough distance. We had no poor results in this category. This should be the proof that Tissucol can be used in similar cases and for proximal anastomosis. Since 1982, 15 other toe transfers have been performed using Tissucol to anastomose the plantar collateral as well as the dorsal nerves, with consistently positive results.

In 1980, Tissucol was used for brachial plexus repair in approximately one case out of five (Table 3). Whereas we were aware that 2–3 years would be necessary to make a valid assessment of the final outcome, the experience we acquired, particularly in handling the glueing material as well as the first encouraging results obtained, induced us actually to use Tissucol in all cases, at least for the proximal anastomosis. This technique has many advantages, particularly in brachial plexus repair: it makes possible the anastomosis of the proximal end when rupture is located at the foramina, the anastomosis of the intercostal nerves, direct muscle neurotization, etc. We think that the quality of the results has remained consistent ever since we began to use Tissucol. In any case, the gain in operative time in itself makes us consider the use of fibrin as progress.

Tables 4 and 5 show the results of the main nerve trunks and digital nerve repairs.

Table 3. Brachial plexus (23 cases)

	1982	1985
Good	5	10
Average	2	2
Bad	3	7
?	13	4

Table 4. Main nerve trunks (17 cases)

	1982	1985
Good	5	6
Average	7	7
Bad	3	2
?	2	2

Table 5. Digital nerves (eight cases)

	1982	1985
Good	3	3
Average	4	4
Bad	–	–
?	1	1

Discussion

It is always very interesting to study failures. They can be related to technical errors, inadequate indication or to the method itself – since the method can of course be questioned. But two facts prove the value of the gluing technique: the consistently good results of the free flap cases already mentioned and the regular absence of the Tinel sign at the site of the proximal anastomosis.

As was already apparent in the preliminary report and confirmed by the second control, the problems arise with long grafts, and the outcome of the glue at the distal anastomosis; the Tinel sign is frequent there. However, this does not seem related to the method but more to the length of the graft.

Among technical errors, several are possible:

1. A prerequisite in the use of Tissucol is the absence of even the slightest tension. Proximity of joints must be taken into consideration.
2. Too much Tissucol can isolate the graft from the surrounding tissue and compromise its revascularization or be interposed between the repaired nerve and the graft, forming a sclerotic diaphragm which the growing axons will not be able to cross.
3. Not enough Tissucol will have a deleterious effect as well, because a gap can occur between the nerve and the graft.
4. Sometimes also, Tissucol has not been prepared according to the instructions and does not glue.

Finally, the indication might be wrong. One failure concerned a digital nerve which was repaired, whereas the trauma had occurred 15 years before and the digit had remained painful ever since. The failure must certainly be attributed to cortical fixation and not to a peripheral cause.

Success is due to several factors related to the use of Tissucol. The main one is that Tissucol should be used only if and where there is no tension at all. This is a fundamental rule for successful nerve repair and need not be emphasized, although it is probably too often transgressed when using stitches.

Tissucol goes with an atraumatic technique; no handling, no pinching is necessary. No sutures are forced through a small perineurium and a lot of axoplasm. Also, we believe that a regeneration chamber is created, which protects the nerve stumps against invading neighboring tissues and restricts the escape of axon sprouts.

Hemostasis is another advantage. It is useful in nerve repair either at the end of the operation, which is obvious, or during the process of dissection. It is particularly

helpful in brachial plexus repair where oozing is frequent. Tissucol also enables grafts to be bundled, which can be thus fixed together to match the size of the nerve, fascicle or group of fascicles to be grafted, or eventually resected into a better or neater surface.

Grafts can be thus also stabilized in their bed, isolated from one another to ensure a better revascularization. They can be set on the desired course if, for instance, a scarry area has to be avoided. And muscle neurotization can be performed almost atraumatically as well.

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The Use of Fibrin Sealant in Nerve Adhesions (Peripheral Nerves and Plexus Brachialis)

S. PALAZZI

Key words: Nerve suture, peripheral nerves, human fibrin glue, nerve grafts

Abstract

From March 1984 to April 1985 we operated on 53 patients presenting nerve lesions using fibrin sealant in nerve repair. The technical advantages of fibrin sealant are discussed. No local or systemic complications have been found in this group. It is too early for a complete evaluation of the results; nevertheless, nervous regeneration follow-up by means of the Tinel sign shows results at least as good as those from using classical suture techniques. As this paper is a preliminary report, further experience is necessary to achieve significant clinical results.

Introduction

The accurate adaptation of the fascicles of a severed nerve is a must in peripheral nerve repair. Otherwise regenerating axons cannot properly reach the distal perineural tubes. Microsurgical techniques have improved the accuracy of peripheral nerve repairs by perineural sutures. Stabilization of the stumps or grafts with clotting substances such as a fibrin cuff around the junction was described by Young and Medawar [7] in 1940 and Tarlow [6] in 1943. Further experiences by Matras [3], Duspiva [1], Kuderna [2] and Egloff and Narakas [5] have shown the effectiveness of the method. As this International Symposium is dedicated to fibrin sealant, I will not describe the glueing principles or the biochemical properties of the components. Since March 1984, 53 nerve repairs by fibrin adhesions have been made.

Technique

The preparation of the nerve stump is the same as for classical microsurgical repair as described by Millesi [4]. The Epineurium is removed. Single fascicles or fascicle groups are isolated and the neuroma is resected. The grafting is done regarding the fascicular pattern. Sometimes an isolated graft is made, mostly in assembled bundles. Once the fatty tissue and epineurium are removed, the nerve grafts are glued together and then sharply resected using the Viktor Meyer neurotome, to obtain a "polifascicular" nerve made by glued grafts (Figs 1, 2). The graft and the

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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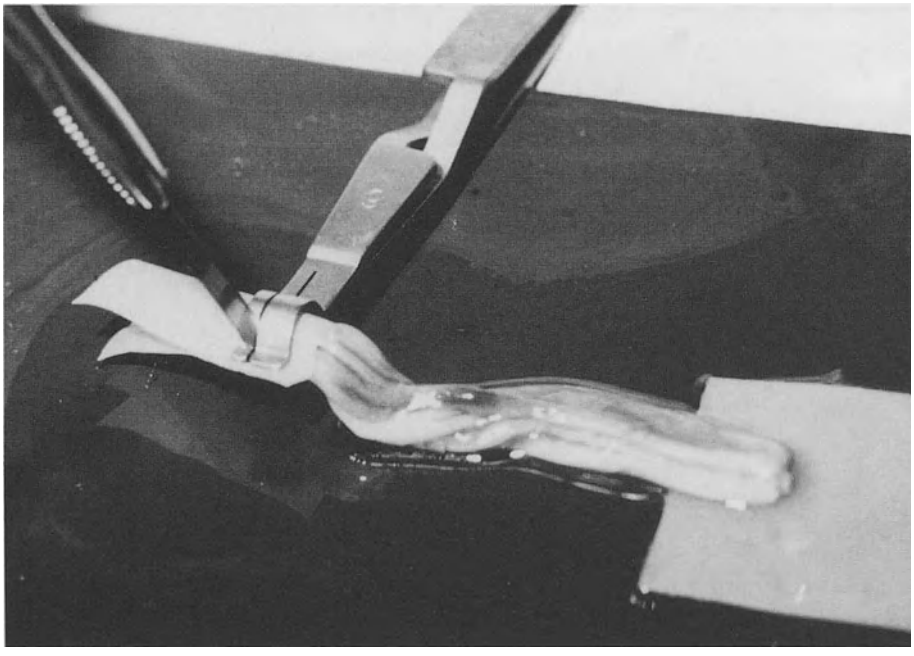

Fig. 1. Nerve autograft glued in a bundle of fascicles. Cut by means of Meyers's neurotome

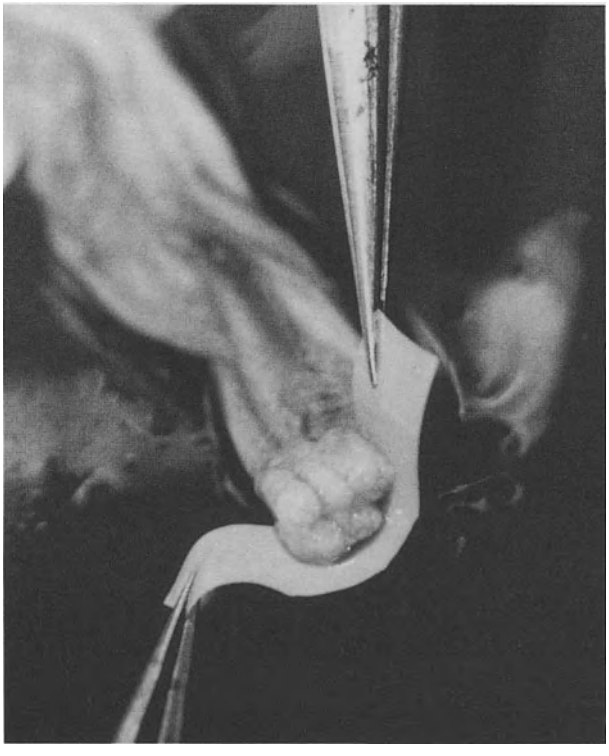

Fig. 2. Transverse surface of the graft. Note a very thin layer of fibrin sealant

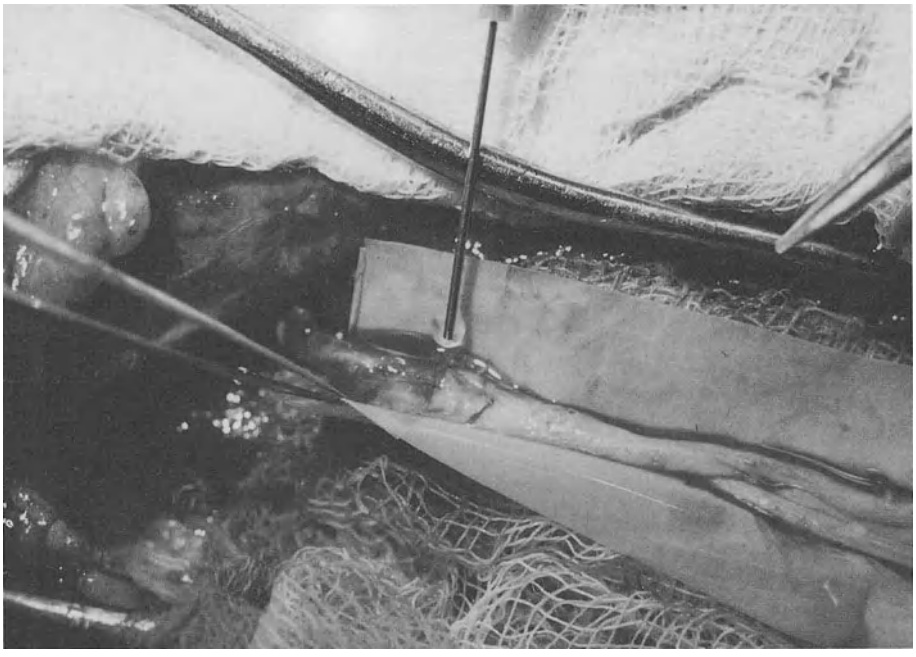

Fig. 3. Complete division of the musculocutaneous nerve repaired with a three-tailed nerve graft. Moment of glueing

Fig. 4. Final aspect of the proximal stump of the musculocutaneous nerve repair

Table 1. Fibrin sealant nerve repair (alone and combined)

Brachial plexus	30	Grafts 30	Sutures Primary	Sutures Secondary
Nerve trunks				
musculo-cutaneous	3	2		1
Median	4	3		1
Ulnar	5	3	1	1
Radial	2	2		
Sciatic	1	1		
Peroneal	3	3		
	18	14	1	3
Colateral nerve	5		4	1
Total	53	44	5	4

Table 2. Scale of results

– Good	=	discriminative sensibility and/or good motor function (M4, M5)
– Fair	=	protective sensation and/or M3 motor function
– Nil	=	failures

repaired nerve are wrapped with a rubber sheet (preferably transparent) so the fibrin sealant makes a kind of artificial epineurium with the well known hemostatic and tensile properties. (Figs. 3, 4). Actually we always employ fast thrombin and a concentration of aprotinin (100 KIU). The graft is left in plaster for 3 weeks.

Clinical Materials

Our 53 cases are divided into three groups: brachial plexus, main trunks and collateral nerves (Table 1). As you can see 80% are grafts. The results are evaluated schematically in a simple scale in good, fair and failures (Table 2). It is obvious that in 1-year follow-up no definitive results can be seen, especially in brachial plexus surgery. But the initial results in repairs close to effective organs (motor or sensitive) are encouraging (Table 3, Figs. 5, 6).

Table 3. Preliminary results (March 1984 to April 1985)

	u	Average (years)	Follow-up (months)	Results Good	Fair	Nil	Lack of follow-up
Brachial plexus	30	29	9	1	2	1	26
Nerve trunks	18	36	8	4	3	2	9
Colateral nerve	5	25	6	3	1	–	1

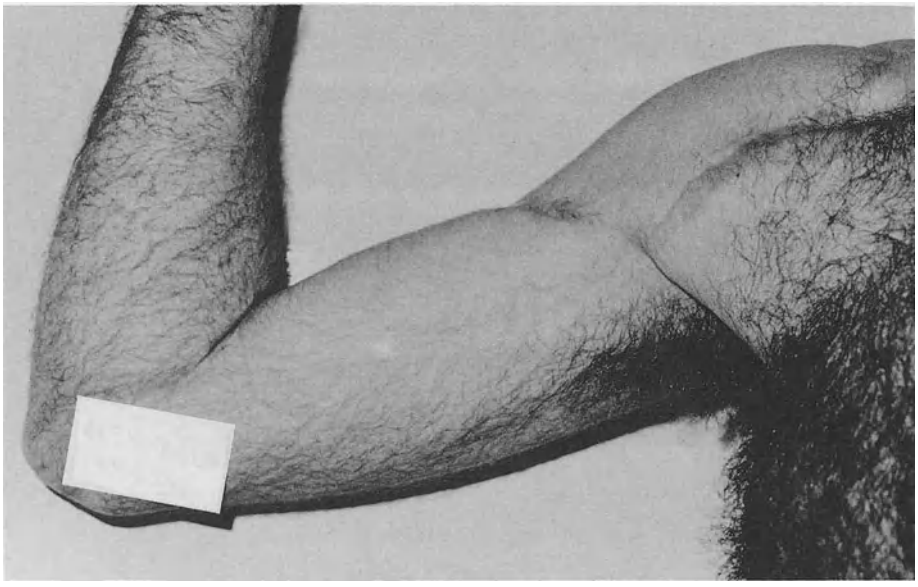

Fig. 5. Clinical result after 12 months

Conclusions

The Conclusions we can draw from this preliminary report are:

1. Fibrin sealant in nerve anastomosis is an effective technique.
2. Again in operative time and precision of placing and stabilization the grafts are some of the advantages.
3. The nerve junction must be free of tension. Therefore in primary repair nerve glueing is used as a reinforcement of the suture.
4. In monofascicular grafts we use fibrin sealant alone. In polifascicular or "package" grafts we use the glue together with monofilament stitches.
5. No serum hepatitis or postoperative infections have been observed.
6. In spite of the too short follow-up of our cases, initial results are encouraging.
7. Further experiences on human nerve reaction to aprotinin and the possible immunological toxicity of the fibrin are needed.

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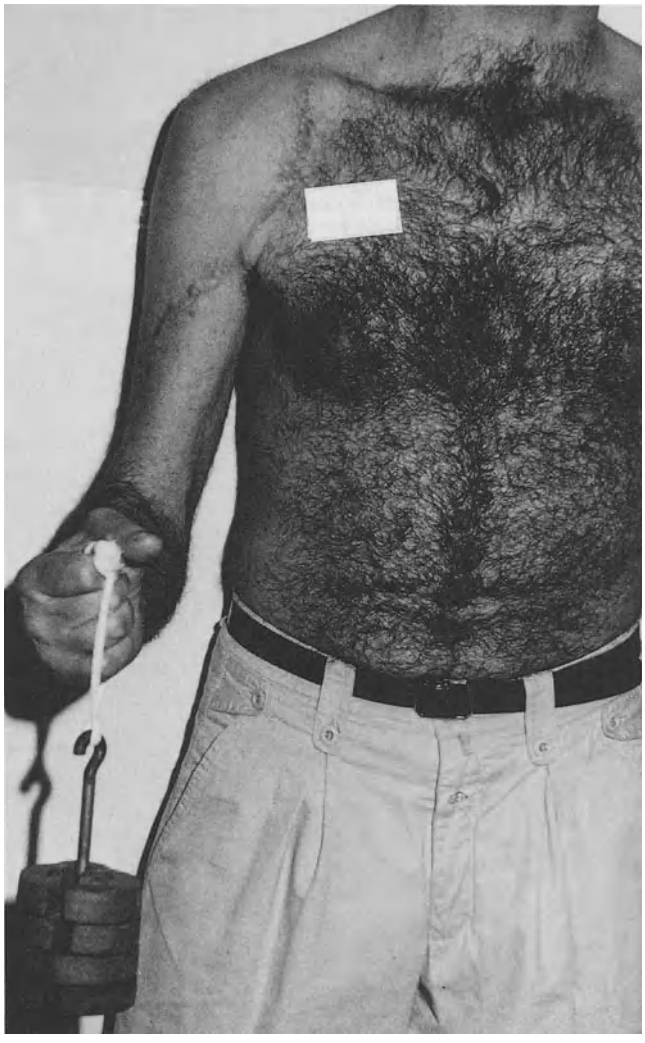

Fig. 6. Clinical result after 12 months

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The Use of Fibrin Glue in the Treatment of Painful Neuromata

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Key words: Fibrin glue, neuromata

Abstract

As a result of traumatic or surgical division of nerves, the occurrence of painful neuromata remains a challenge to therapy. The use of fibrin sealant (Tissucol) we present here is another possible therapeutic approach, in addition to the already numerous available methods to handle such problems.

The technique consists of:

1. Resecting the neuromata
2. Fixing the nerve stump in nonscarry tissue with Tissucol

The results on 27 neuromata (17 patients) were studied using objective and subjective criteria, with 78% of recoveries and 22% of failures.

Unfortunately the method is no panacea, but appears as a worthwhile alternative which compares favorably with other proposed treatments. It has three advantages: It is atraumatic, simple and quick. There are no contraindications.

The occurrence of a symptomatic neuroma after traumatic or surgical section of a sensitive peripheral nerve remains a problem not yet really well solved, as can be seen from the numerous different publications treating this subject. Indeed, the functional limitation resulting from the traumatic pathology of the hand – mutilating in itself – is further intensified by the problems linked to an amputation neuroma.

The large number of techniques described in the literature attests to the problems met in trying to elaborate a single and preferential way to solve them.

These various methods include prophylactic means and treatments such as tapping (or patting) [16]; infiltrating such substances as formol [1], phenol [2], alcohol [3], cephalorachidian liquid [4], and corticoids [5]; electrocoagulation, ligature or resection and burying into various tissues such as muscle [7], vessel [8], bone [9–11], another nerve [12–15] or also into inorganic materials like tantalum [16], gold [17] or silicon [18–20].

Since 1981, we have used a technique based on creating a "muff" or sheath to protect the nervous end with fibrin glue, which at the same time helps to set it in the best possible site and position, and this atraumatically.

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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Method and Means

Between June 1981 and December 1983 we operated on 17 patients with 27 painful neuromata in the hand. In all cases the following technique was used: skin incisions made through previous scars, providing whenever possible flaps which gave easy access to the nerve and covered the glued nerve stump at its new location. The nerve and its neuroma were isolated from the surrounding tissues, often sclerotic, and from the collateral artery. The latter was ligated. The neuroma was sharply resected with the surgical knife and the new nerve stump was placed in a nonpressure zone, either dorsally or laterally, and fixed in that position with Tissucol.

All of our above-mentioned cases resulted from trauma sequelae with shortening of the skeleton. They included ten men and seven women, with an average age of 40 years, ranging between 15 and 64 years. Morbidity was twice as acute on the left side, with a marked predominance of P2-type [7] amputations, rather than P1 [4] and P3 [1] types.

Symptoms prior to the operation had been felt during 8 months on average, i.e., 3 months at the shortest, to a maximum period of 44 months. The postoperative follow-up averaged 24 months, i.e., between 11 and 35 months.

Assessment Criteria

We used objective as well as subjective criteria:
Subjective criteria were:
1. Pain
2. Functional hindrance

The scale adopted concerning the assessment of pain was:
Level I : no pain or sporadic pain
Level II : moderate pain (permitting normal activities and work)
Level III : acute pain impeding or preventing work

The scale covering functional hindrance was:
Level I : no functional hindrance, no impediment in professional life
Level II : some functional hindrance, however, work still performed with normal results
Level III : condition unchanged or worsened
Objective criteria were:
1. Tinel sign
2. Retraction sign

The scale regarding the Tinel sign was:
Level I : no sign
Level II : moderate sign, not impeding work
Level III : acute sign impeding work

As for the retraction sign, the scale was:
Level I : no sign
Level II : positive under tapping (patting)
Level III : positive under palpation or to the merest contact

Table 1. Subjective criteria

	Level I	Level II	Level III
Pain	62.9%	14.8%	22.2%
Functional hindrance	77.7%	0%	22.2%

Table 2. Objective criteria

	Level I	Level II	Level III
Tinel sign	62.9%	14.8%	22.2%
Retraction sign	77.7%	0%	22.2%

Results

Subjective and objective results respectively are shown in Tables 1 and 2. In addition, we assessed the sensitivity of the neuroma site with the following results:

1. Protective: 37%
2. Hypoesthesic: 33%
3. Hyperesthesic: 22%
4. Dysesthesic: 4%
5. Anesthesia: 4%

The percentage of failures corresponding to the various levels III amounts to 22%, without possibility for cumulation since the same cases are always found in those levels III, whatever the assessment criteria.

In relation to patients rather than neuromata, our figures are slightly different: 82% recovery and 18% failure.

This variation may imply the influence of a psychological factor or a different limit to pain feeling.

Discussion

Treatment of painful neuromata through surgical resection and fixation of the new nerve stump at the proper location through Tissucol has proven useful. It makes possible a high percentage of recovery and, furthermore, a significant improvement in cases previously treated in vain by other means.

Although it certainly is no panacea for the cure of all painful neuromata, this technique has several advantages, the main one being the fact that the new nerve stump can be set in the right position without resorting to stitches, which would inevitably require a further handling of the nerve, thus causing more trauma and often incurring a deleterious inflammatory reaction. Besides, it also ensures hemostasis and isolation of the nerve from surrounding tissues – which are two other pro factors.

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Use of Fibrin Seal (Tisseel/Tissucol) in Peripheral Nerve Surgery: An Experimental Rat Model

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Key words: Peripheral nerve regeneration, fibrin sealant

Abstract

In an experimental situation with nerve grafting using rat ischiatic nerves, rat fibrin sealant was evaluated for glueing purposes. It was noted that the sealant provided a good medium for nerve sprouting, but was unable to provide sufficient fixation of the nerve ends and grafts. Sole use of fibrin sealant (Tisseel) cannot be advocated.

Introduction

In cases of completely transected nerves the aim of peripheral nerve surgery is to secure the nerve so that axons may sprout from the proximal to the distal end of the nerve and thereby establish neurophysiological function. How this happens and by what means is completely unimportant, if only function is established. The scientific and clinical results reported by Haase [1], Millesi [4, 5] and Samii [7] have demonstrated that the fibrous tissue, which is derived in the suture line from the epineurium, is the most important factor hindering eventual neurophysiological function, either by impeding sprouts from reaching the distal part of the nerve or by secondary compression of already regenerated nerve fibres. Tension has been shown to be the most important factor concerning scar tissue development [6]. Based on these facts, nerve grafting with multiple, thin, freely floating autologous grafts was introduced in clinical practice and has demonstrated its superiority to epineurial suturing ever since [1, 5, 7].

Material and Methods

We wanted to evaluate the possible role of fibrin sealant (Tisseel) using either end-to-end sealing or cuff sealing combined with exact adaptation and incorrect coaptation in a rat animal model. A 5-mm nerve graft from one ischiatic nerve in the rat is transferred to the other nerve and the nerves are glued with fibrin sealant in different concentrations. A large series using different techniques was prepared, but preliminary studies and evaluation showed that all our grafts showed complete disadherence, with significant gaps between the nerve grafts and the distal end of up to 4 mm despite using the cuff technique or the end-to-end gluing technique. On the

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
Edited by G. Schlag, H. Redl

other hand nerve fibres seems to sprout nicely through the graft without significant fibrotic changes within the graft or the proximal suture line. Due to these preliminary results our study was stopped and has been redesigned with the concomitant use of microsuturing.

Discussion

It has been postulated that fibrin sealant should create a better adaptation of severed nerve stumps and grafts compared to microsuturing [2, 3]. However, by restudying the anastomosis in previous experimental papers [3] a rather fibrotic reaction could be seen. As fibrin sealant increases fibroblast function and thereby connective tissue development and as this is precisely what we want to reduce [4, 6], the use of fibrin sealant seems apparently not relevant in microsurgical treatment of peripheral nerve lesions so far. However, it may be anticipated that fibrin sealant could create a connective tissue with longitudinal layers, which would be a better medium for nerve regrowth compared with the normal whirled structure, which is the result of usual microsuturing [3]. In our study it could seem that the connective tissue structure shows a non-whirled structure. It has also been postulated that fibrin sealant gives sufficient support at the suture sites, so that microsuturing can be avoided [2, 3]. However, despite the use of the end-to-end technique or the cuff technique, as described by Kuderna [2], we saw complete rupture of all suture sites with gaps of 4–8 mm in our studies. Apparently gluing did not support the field in our series. It must be emphasized that we have not measured the tension at the suture sites.

Following this we have redesigned our study and will continue to use microsuturing, as proposed by Millesi [4, 5], but combined with fibrin sealant in different concentrations, especially regarding thrombin and aprotinin, in an attempt to establish a histological description of the suture sites, which may be of help in determining whether fibrin sealant should be used in peripheral nerve surgery at all. It must be emphasized that none of the clinical results and experimental studies so far published have demonstrated any superiority of nerve gluing over microsuturing according to Millesi's technique and we must therefore advocate that fibrin sealant is not to be used in human cases at present.

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Prevention of Cerebrospinal Fluid Leak in Translabyrinthine Surgery

O. BYNKE, and L. EKVALL

Key words: Acoustic tumours, translabyrinthine surgery, postoperative cerebrospinal fluid leak, preventive management

Abstract

In translabyrinthine surgery leakage of cerebrospinal fluid (CSF) is a common complication which always involves a risk of postoperative meningitis. The mechanisms of leakage have been analysed in a series of 50 consecutively operated patients. The following preventive measures proved effective:

1. Dural closure by suturing as far as possible
2. Blocking the eustachian tube, the middle ear cleft, the recesses medially to the facial nerve, and the hypotympanic cell tracts with pieces of periosteum
3. Closure of the dural defect with a fascial graft
4. Filling the mastoid cavity with strips of fat
5. Glueing the different transplants with fibrin sealant
6. Firm mastoid dressing for the first 5 days

By these procedures the frequency of posterior CSF leak decreased from 36% in the first 25 patients to 8% in the next 25 patients. No liquorrhoea through the eustachian tube has occurred in the latter series, nor has any revision surgery been necessary.

Introduction

Leakage of cerebrospinal fluid (CSF) is a common and serious complication in translabyrinthine surgery, always involving a risk of infection. The reported incidence has varied between 25% and 9,5% but the definition of CSF leak was not uniform [1, 3, 5, 8]. The leaking CSF may be drained through the eustachian tube

Table 1. Incidence of postoperative CSF leak and CSF leak revisions in large series of translabyrinthine surgery for acoustic tumours

Authors	No. of operations	CSF leak (%)	Revisions (%)
Glasscock et al. (1978)	180	25	?
House et al. (1979)	251	20	8.5
King and Morrison (1980)	150	21	14
Tos and Thomsen (1985)	200	9.5	3.0

Fibrin Sealant in Operative Medicine
Ophthalmology – Neurosurgery Vol. 2
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and in that case it may be present as a rhinorrhoea. The CSF may also find its way out through the skin incision and in that case it may be called wound leakage. Meningitis following CSF leak occurred in 6% of the patients in Glasscock's series [3]. Many patients with CSF leak require surgical closure of the fistula (Table 1).

In the present study we have analysed the pathways of the leaking CSF and the preventive management to avoid a CSF fistula.

Methods

The CSF leak after translabyrinthine surgery has been studied in 50 consecutively operated patients. Different preventive measures were worked out mainly in the first 25 patients and the experienced surgical procedures were performed in the following 25 patients. The series were comparable regarding age, sex, tumour size, and histological diagnosis (Table 2). The classification of tumour size was based on the results of computed tomography (CT) and the intraoperative findings. The tumours were arranged in three groups:

Small The tumour did not involve the brain stem and its cisternal diameter did not exceed 15 mm.

Medium The tumour impinged moderately on the brain stem and its intracranial diameter did not exceed 25–30 mm.

Large The tumour impinged severely on the brain stem and its intracranial diameter exceeded 25–30 mm.

Table 2. Summary of data in 50 consecutive patients with acoustic tumours

	Series 1 Cases 1–25	Series 2 Cases 26–50
Mean age (years)	52.9	54.4
Sex		
Female	15	17
Male	10	8
Tumour size		
Small	7	3
Medium	7	12
Large	11	10
Histological diagnosis		
Neuroma	23	22
Meningioma	2	3

Results

Cerebrospinal fluid rhinorrhoea was present in three patients of series 1 (Fig. 1). The onset was noted during the second postoperative day in one patient and after hospital discharge in the other two. The first patient was treated with continuous lumbar drainage without complication, and the other two needed surgical closure.

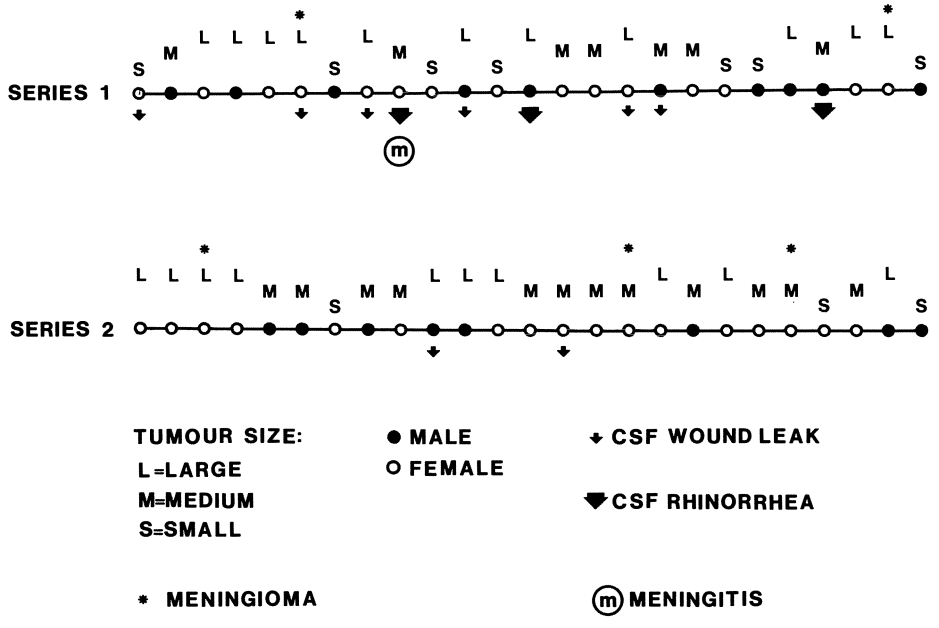

Fig. 1. Cerebrospinal fluid leak after translabyrinthine surgery for acoustic tumours in 50 consecutive patients. The described preventive procedure was developed in patients of series 1 and used in series 2

The leakage stopped after revision in both patients but one of them had meningitis 6 months later. No CSF rhinorrhoea was present at that time. CSF wound leakge occurred in six patients of series 1. The onset was within 48 h after surgery in five patients and after 7 days in one patient. The leakage stopped promptly after treatment with lumbar drainage.

No CSF rhinorrhoea was noticed in the patients of series 2 but two patients presented wound leakage in the early postoperative course.

Analysing the correlation between postoperative CSF leak and tumour size indicates that the incidence of leakage increased with increasing tumour size (Table 3).

Table 3. The correlation between postoperative CSF leak and tumour size

Tumour size	CSF leakage	
	(n)	(%)
Small (n = 10)	1	10
Medium (n = 19)	4	21
Large (n = 21)	6	29
Total (n = 50)	11	22

Careful preparation of the translabyrinthine approach will often disclose feasible pathways of the cerebrospinal fluid. Some of them are easily visualized as the aditus and the two isthmi tympani. The deep recesses medially to the facial nerve, the sinus tympani and the posterior tympanic sinus may be opened unnotedly. In patients of series 2 we paid especial attention to these pathways and the deep hypotympanic cell tracts which encircle the inferior aspect of the internal meatus.

Several methods were used to avoid CSF leak. The following procedures proved to be effective in patients of series 2:

1. *Dural closure by suturing.* The dura is opened with a "T"-shaped incision to facilitate closure by suturing after the tumour removal. One incision is performed in front of and parallel to the sigmoid sinus and the other runs to the internal auditory canal. The flaps are easily closed by suturing but a defect corresponding to the meatus will always remain. Its size depends largely on the size of the intrameatal tumour extension. In patients with meningiomas parts of the dura also have to be excised.
2. *Blocking the eustachian tube and the middle ear.* The incus and the head of the malleus are removed, the tendon of the tensor muscle cut and the epitympanum and the protympanum drilled out with diamond burs. Then the middle ear is completely packed with pieces of periosteum. The recesses medially to the facial nerve must be carefully blocked. In cases with an extensive pneumatization of these areas the ear canal and the middle ear cleft are exenterated and the external canal orifice sutured.
3. *Closure of the dural defect with fascia.* A large piece of temporalis fascia is used to cover the openings to the middle ear cavity, the dural defect and the bony remnants of the internal meatus. The graft is glued to this bed with a fibrin sealant.
4. *Filling the mastoid cavity with strips of fat.* Strips of abdominal fat are placed in the mastoid cavity and glued to the fascial graft with a fibrin sealant.
5. *Firm mastoid dressing for the first 5 days.*

Discussion

Several methods have been described to avoid postoperative CSF leak in translabyrinthine surgery [2–8]. The procedures proposed by us have partly been used by others and the series of 25 patients is too small to be conclusive regarding the preventive effect on CSF leak. Our primary goal has been to seal the dural opening as watertightly as possible. It is therefore our opinion that the dural closure by suturing and by glueing a large piece of fascia to the bed is an important step. However, blocks of the way out of the surgical cavity must also be raised if the first closure fails. We have observed that the recesses medially to the facial nerve and the hypotympanic cell system are important pathways for the leaking CSF and have to be blocked when present. A firm dressing, which has been practiced and proved to be an effective preventive method in neurosurgery for a long time, has been proposed by Tos and Thomsen [8] as one of the most important factors. The pressure from the dressing exerted via the strips of fat on the fascial graft will presumably secure a firm healing.

Possibly a routine removal of the posterior bony canal and an exenteration of the middle ear with direct plugging of the eustachian tube orifice will be the most effective measurement in preventing CSF rhinorrhoea. Such a procedure will also widen the access to the cerebellopontine angle.

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Subject Index