G. Schlag H. Redl (Eds.)

Fibrin Sealant in Operative Medicine

Volume 6

General Surgery and Abdominal Surgery

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo

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ISBN-13: 978-3-540-17280-2 e-ISBN-13: 978-3-642-95513-6
DOI: 10.1007/978-3-642-95513-6

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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
H. Redl

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I. Principles of Fibrin Sealing

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).

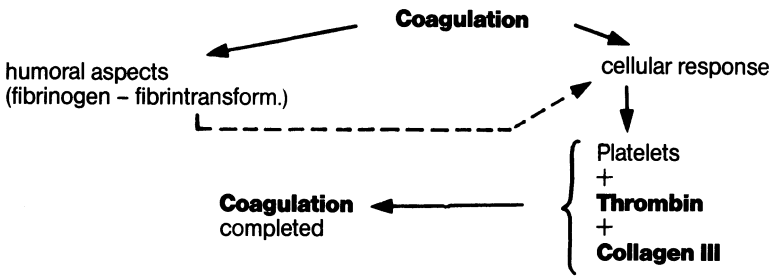

Fig. 1. Primary events following injury

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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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

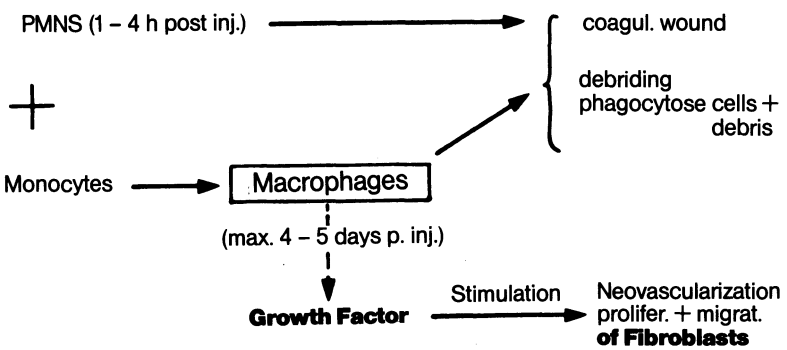

Fig. 2. Inflammatory phase of wound healing

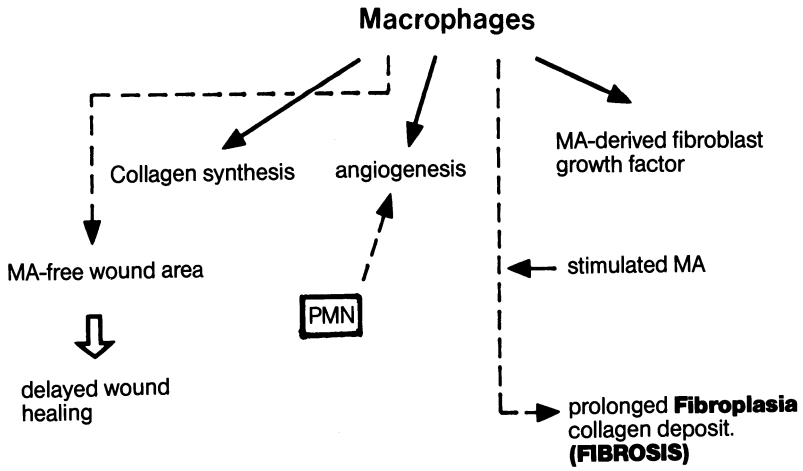

Fig. 3. The role of macrophages during the inflammatory phase of wound healing

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-

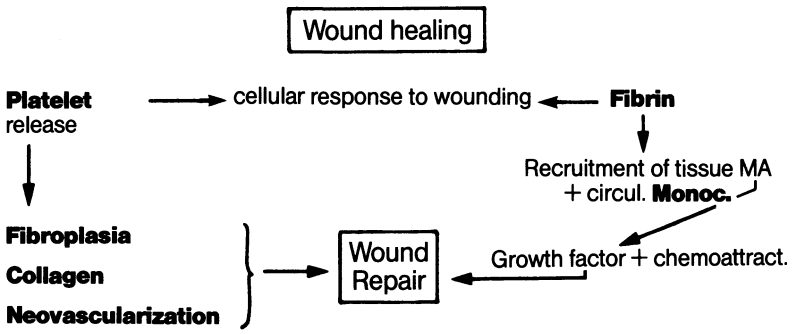

Fig. 4. The concert action of fibrin and platelets

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-

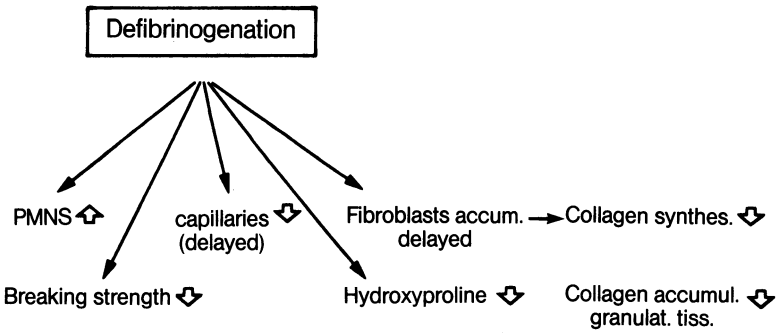

Fig. 5. Effect of defibrinogenation on the different aspects of wound healing

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

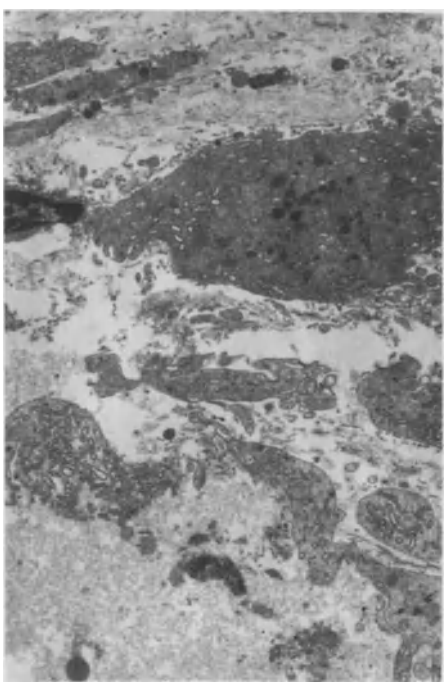

Fig. 6. Immature (undifferentiated) cells in granulation tissue of adriamycin-treated rats (7 days after implantation of spongiosa blocks). EM, x5 000

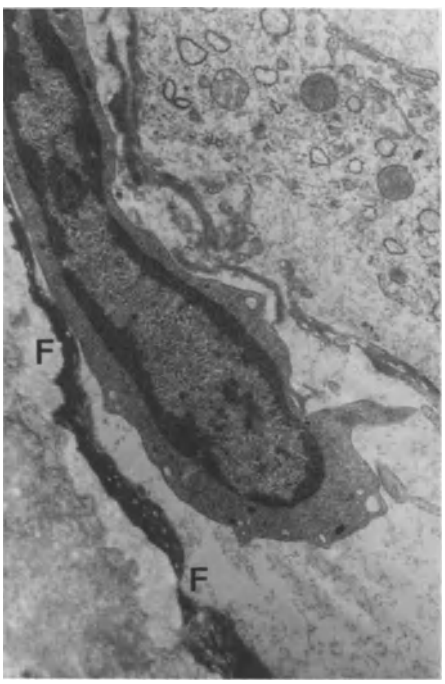

Fig. 7. Seven days after implantation – fibrin strands (*F*) of applied sealant are still visible in the adriamycin group

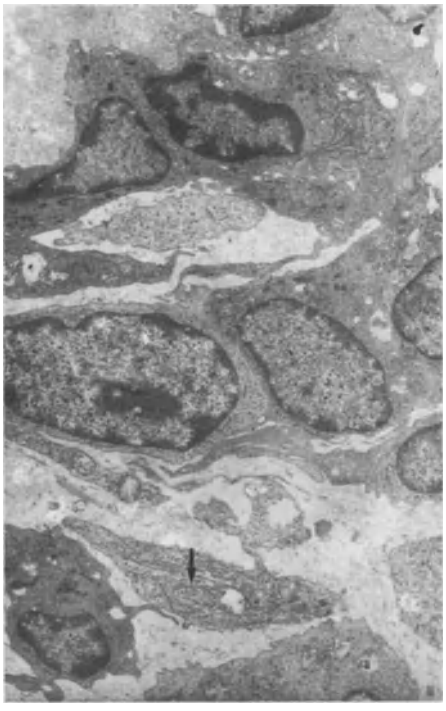

Fig. 8. Without adriamycin application fibrin is completely degraded after 7 days; mature cells are seen in which rough endoplasmic reticulum is already visible (*arrow*)

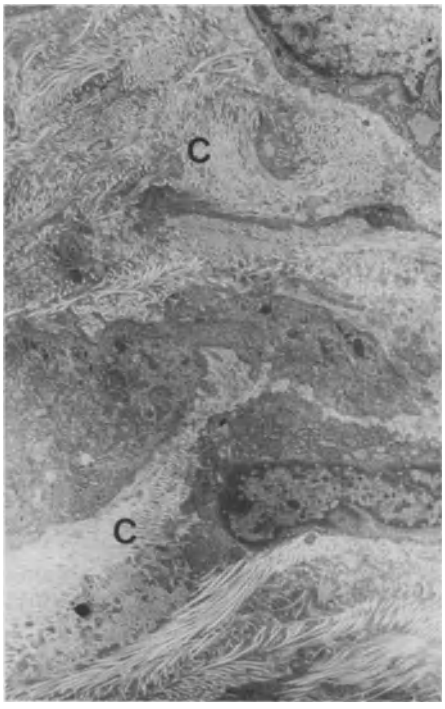

Fig. 9. Two weeks after implantation a marked collagen (*C*) structure is visible in the Tissucol group, which cannot be observed after adriamycin application

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

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
© Springer-Verlag Berlin Heidelberg 1986

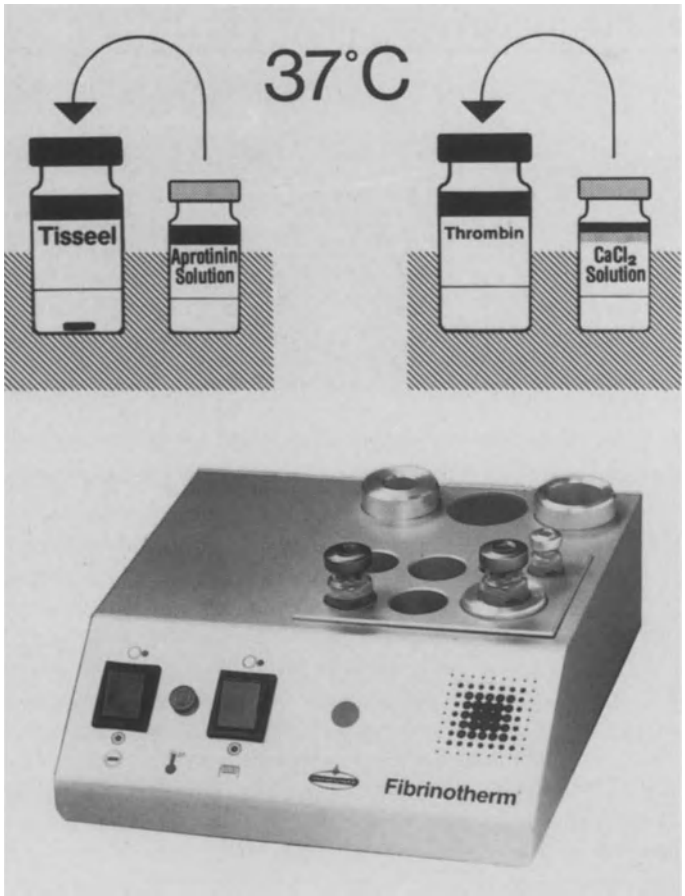


Fig. 1. Component preparation – Fibrinotherm

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

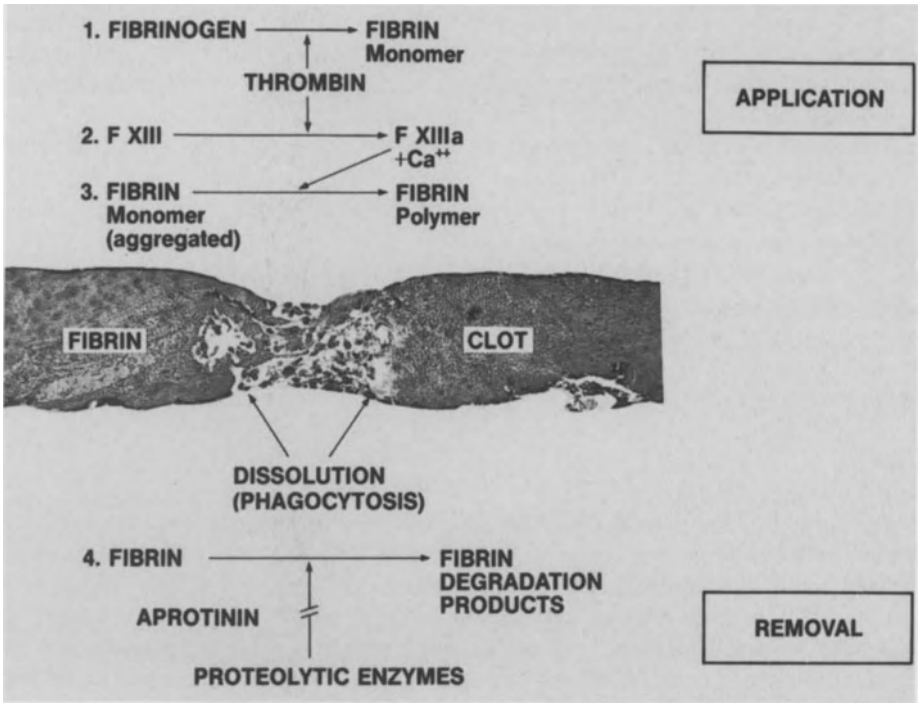


Fig. 2. Fibrin clot formation and removal

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.,

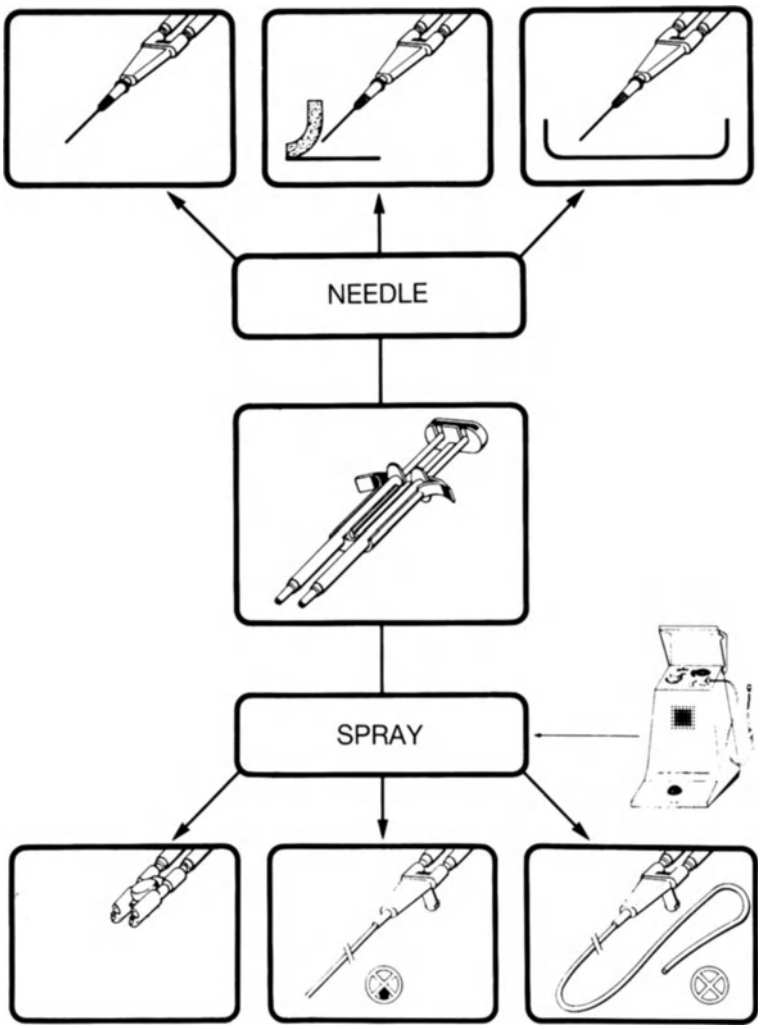
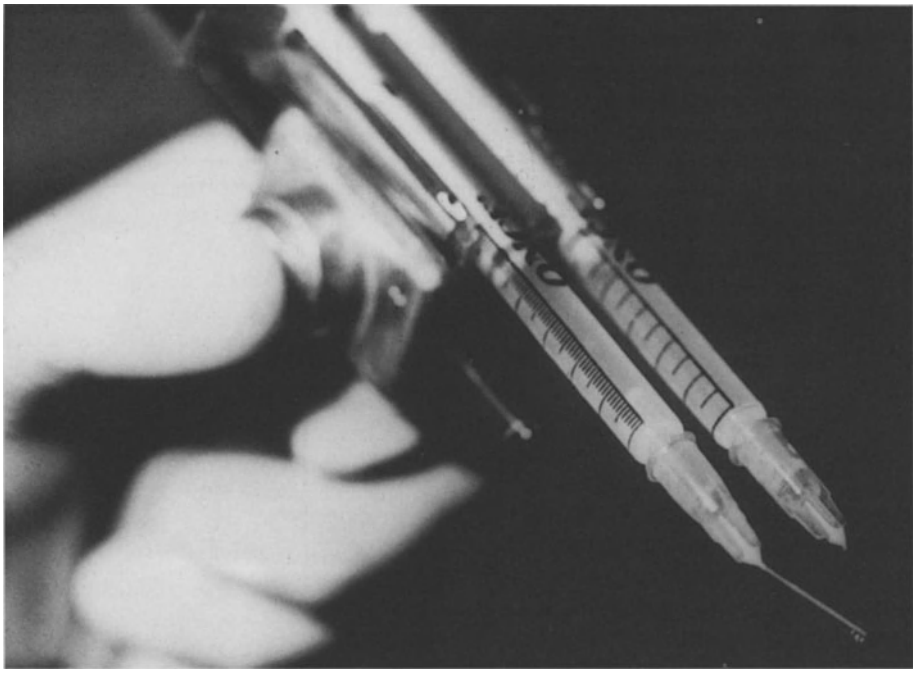


Fig. 3. Duploject system

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.



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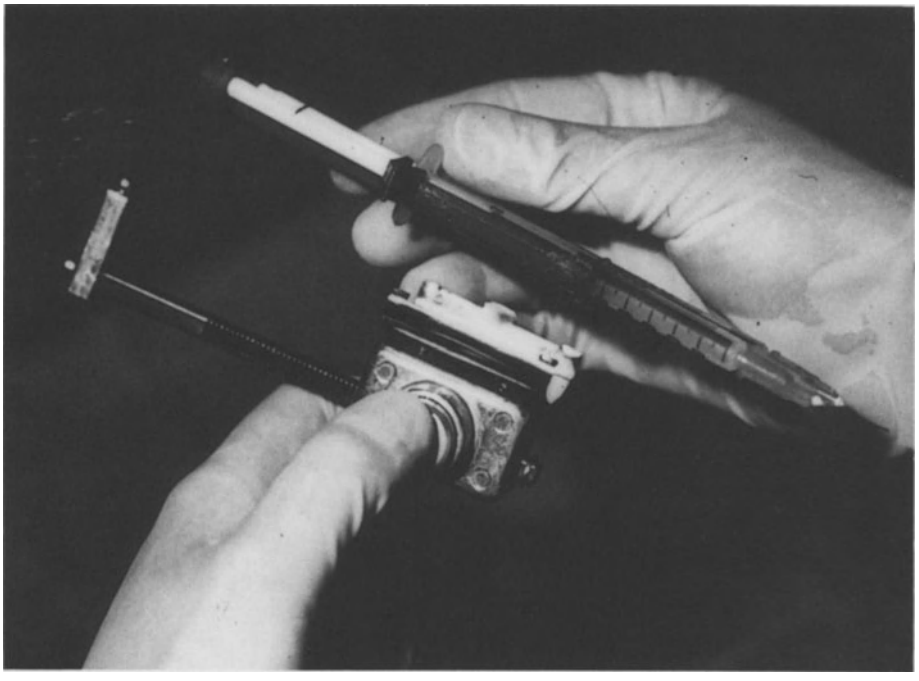


b

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.

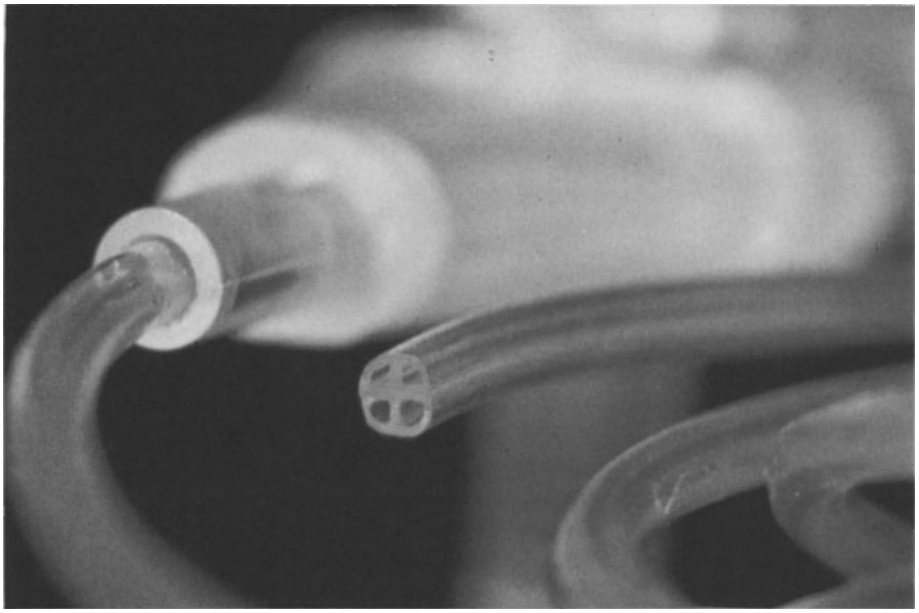


Fig. 5. Spray catheter with characteristic four-lumen design

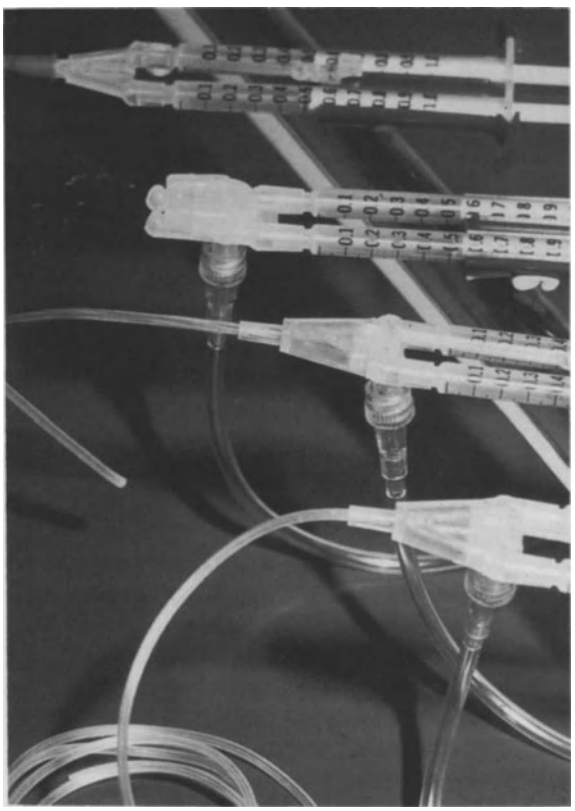
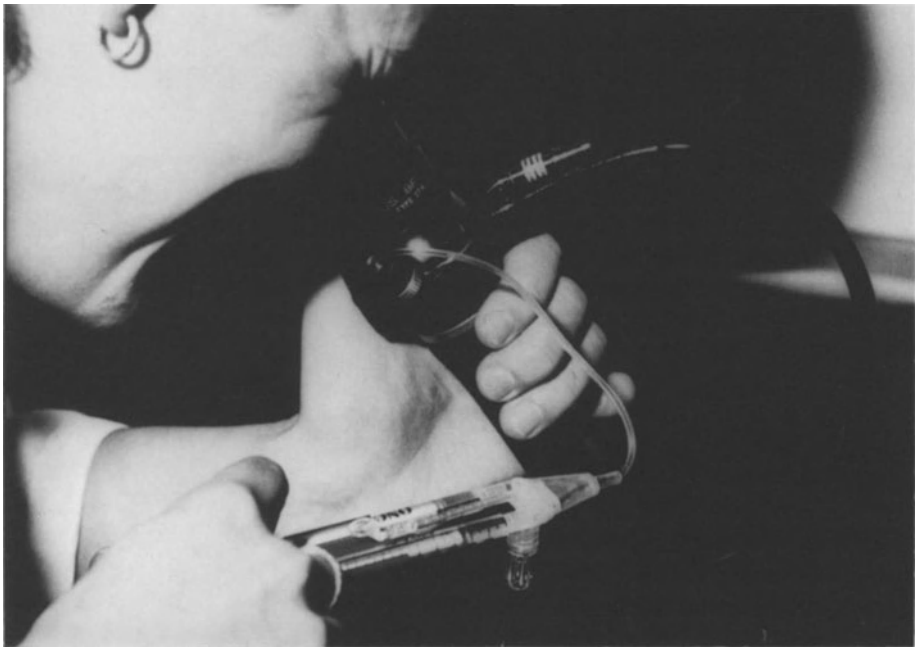


Fig. 6. Spray adaptors of the Duploject system



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

H. REDL, and G. SCHLAG

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
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
© Springer-Verlag Berlin Heidelberg 1986

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	198 45* (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

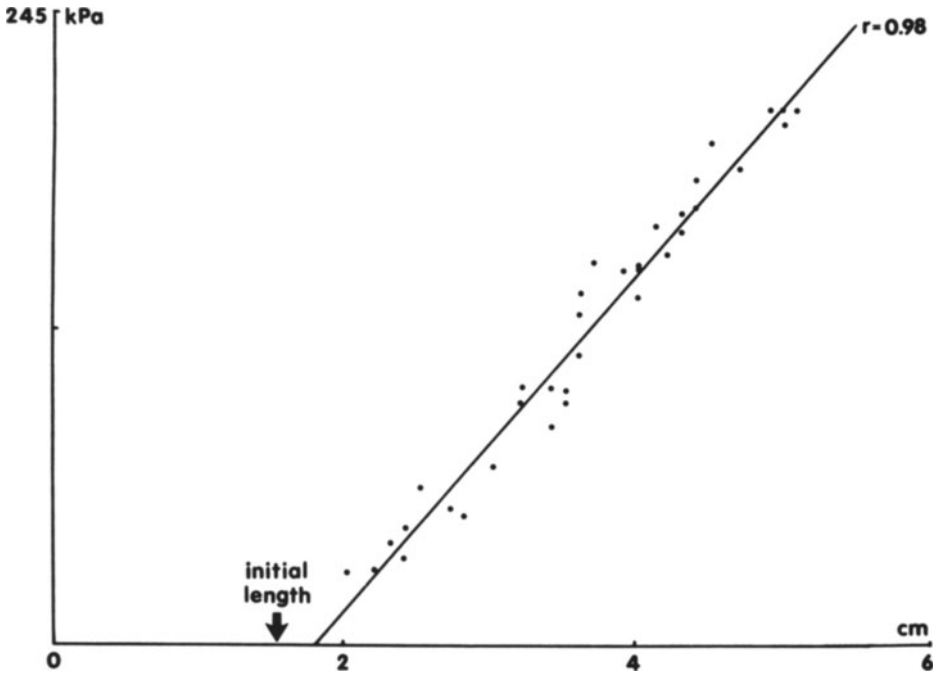

Fig. 1. Stress-strain diagram of PS coarse clot

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

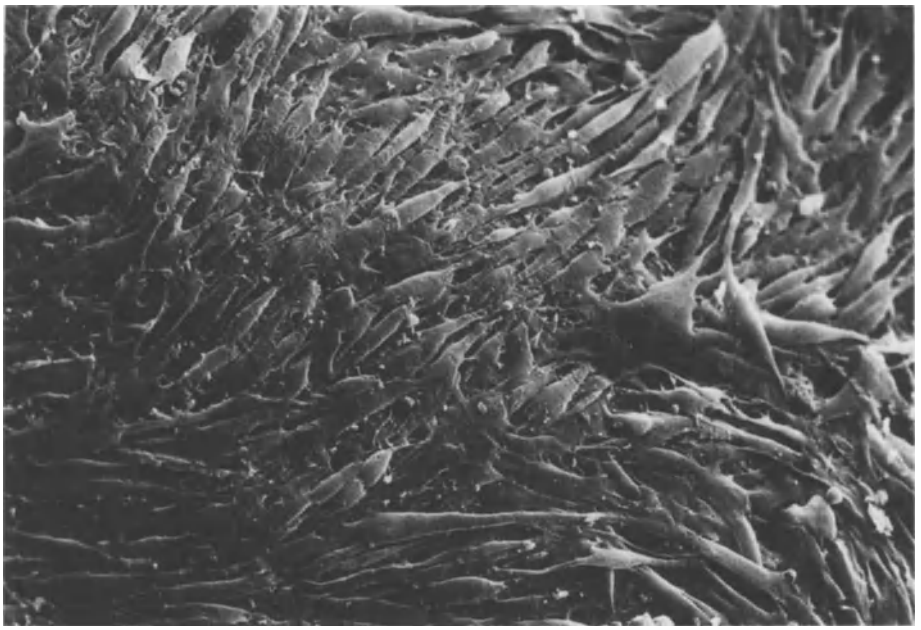

Fig. 2. Rich proliferation of fibroblasts on a cut PS clot. SEM, after critical point drying, x 1000

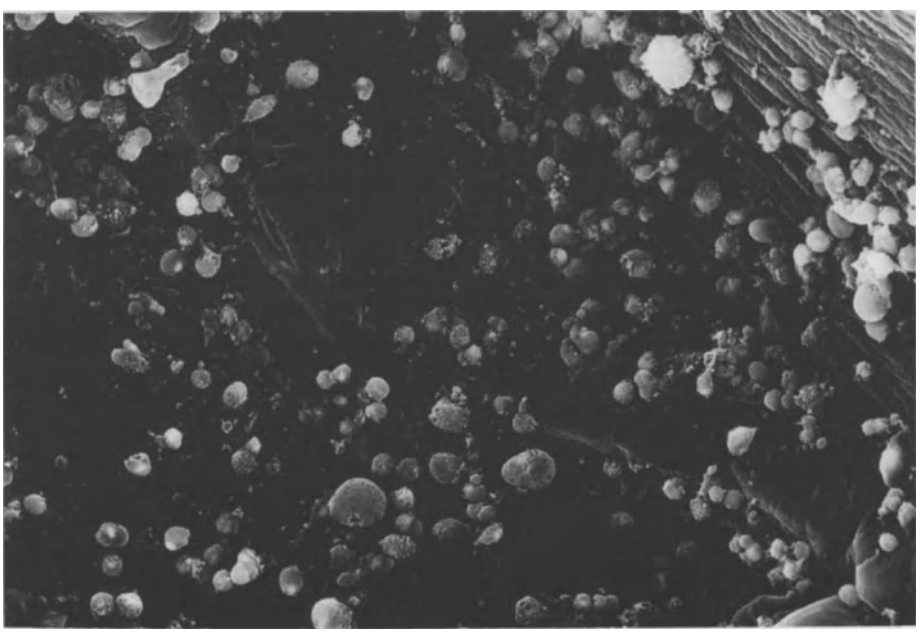

Fig. 3. Spheroidally deformed (damaged) fibroblasts on a HS fine clot after identical treatment as in Fig. 2

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 –
	PS	HS	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

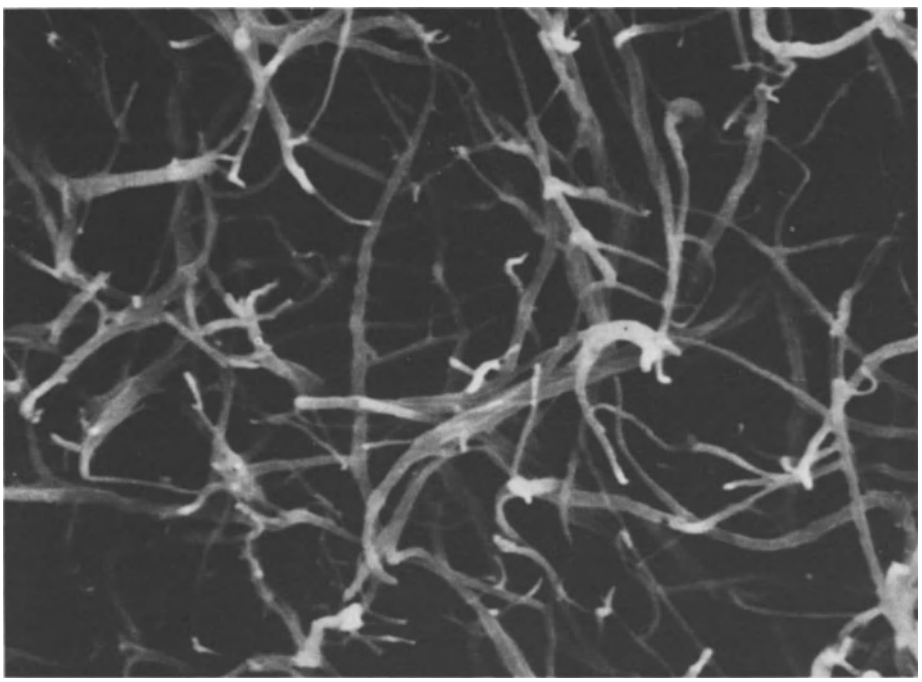

Fig. 4. Fibrin network in PS coarse clot very similar to plasma clot fibrin network. Scanning electron micrograph (SEM) after critical point drying.

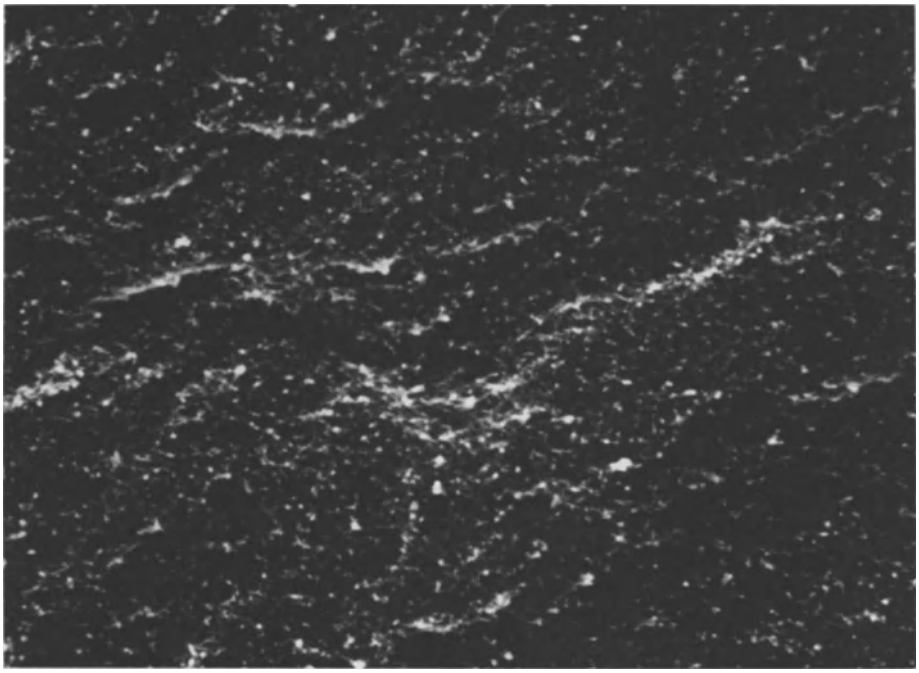

Fig. 5. Hardly detectable fibrin strands in a HS fine clot, conditions as in Fig. 4

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

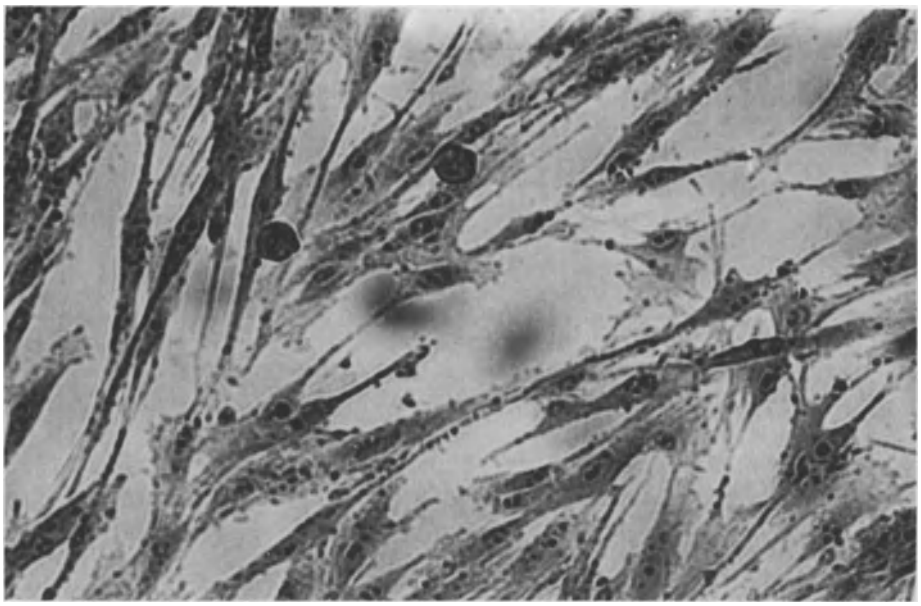

Fig. 6. Layer of fibroblasts 30 minutes after covering with PS, diluted 1 + 1. No detectable differences to controls. LM, carbol fuchsin staining, x 125

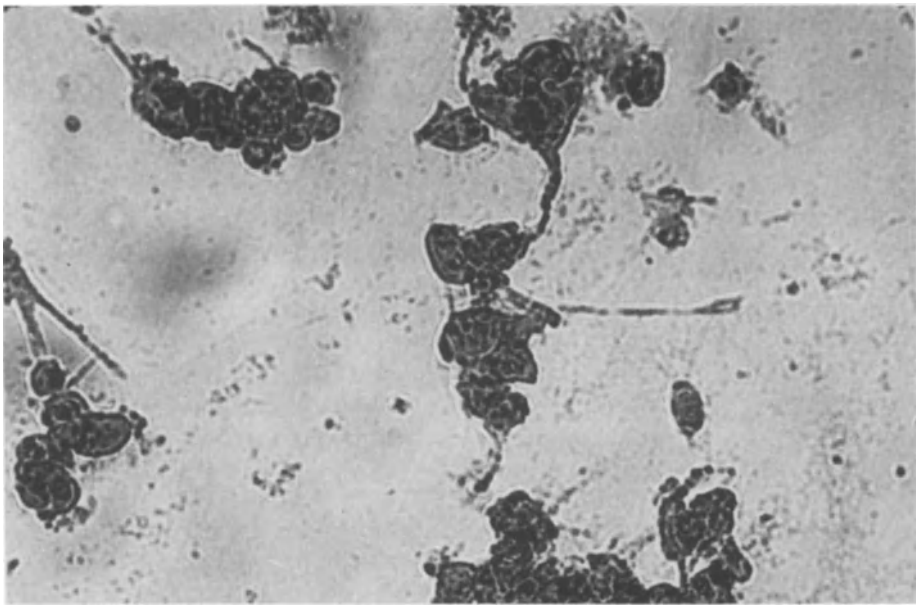

Fig. 7. Layer of fibroblasts 4 (!) minutes after covering with high salt concentration diluted 1 + 1. Damage to cell structure is clearly visible. Staining and enlargement as in Fig. 6

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.

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
© Springer-Verlag Berlin Heidelberg 1986

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.

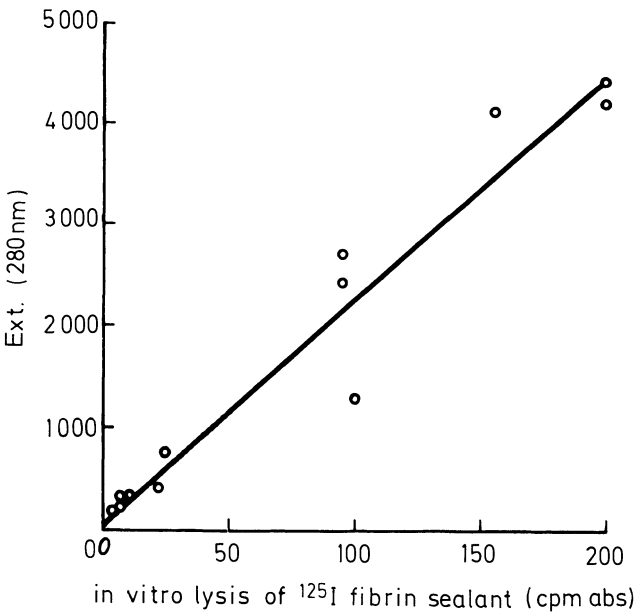

Fig. 1. Correlation of protein concentration and counted radioactivity. Measurement every 12 hours after lysis of a ^{125}I. Fibrin sealant clot with urokinase – plasminogen

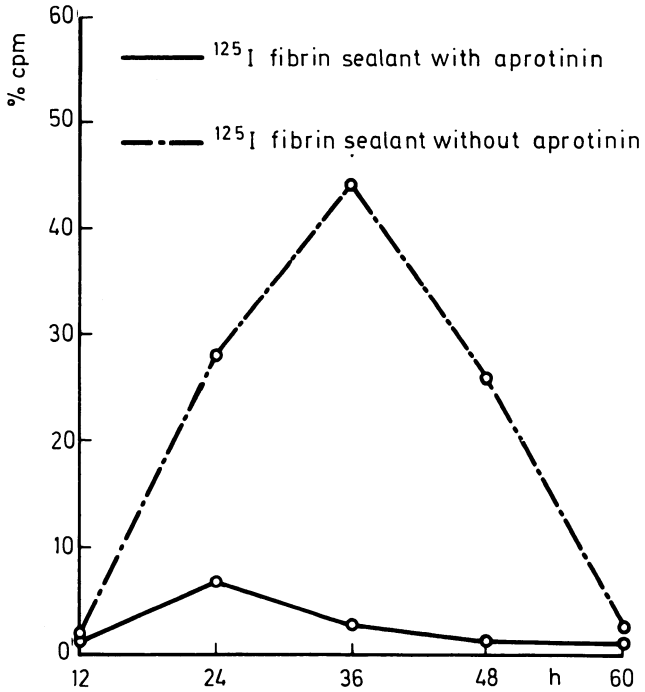

Fig. 2. Fibrinolysis expressed in radioactivity counted in 24h urin samples of rats

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

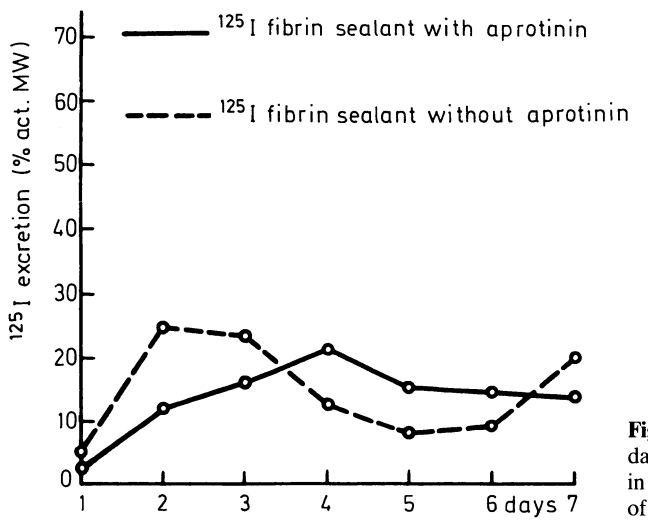

Fig. 3. Mean values and standard deviation of ^{125}I.-excretion in 24h total urin, indicated in % of total excretion per 10 days

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.

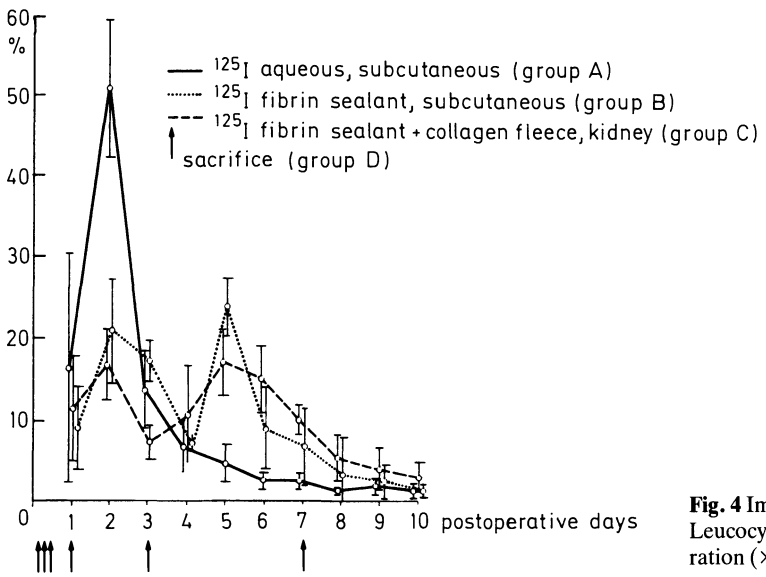

Fig. 4 Imigration of Leucocytes 24^{h} after operation (× 4.500)

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,

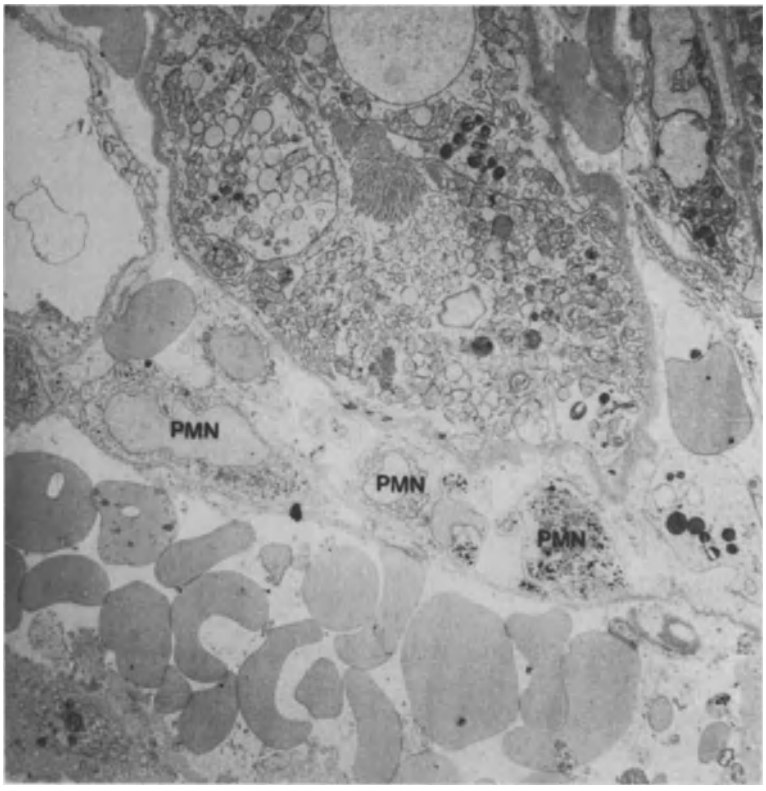

Fig. 5. Cell rich granulation tissue and Leucocytes 3 days after Operation (× 32)

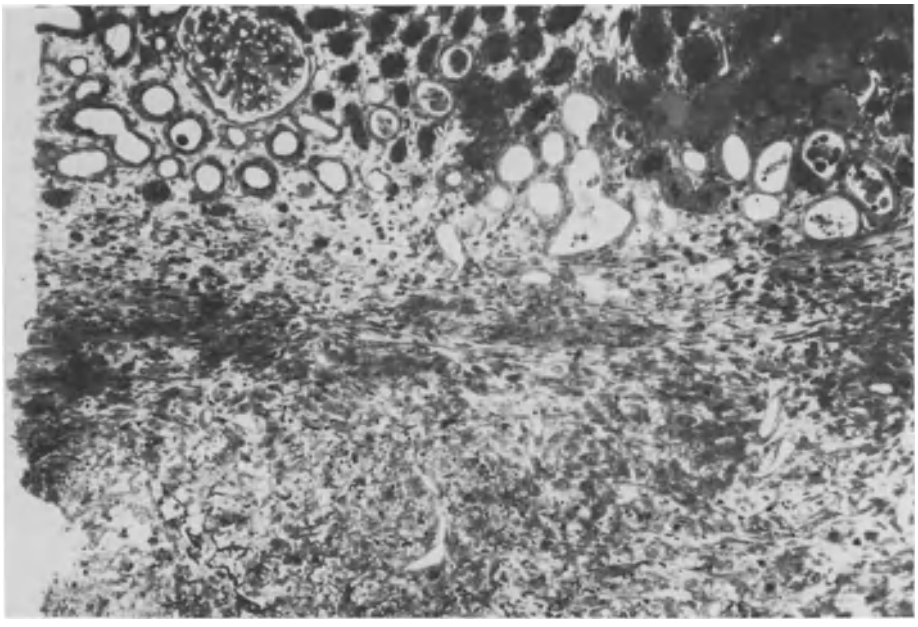

Fig. 6. FTAS-resorption by macrophages 3rd postoperative days (c 5.700)

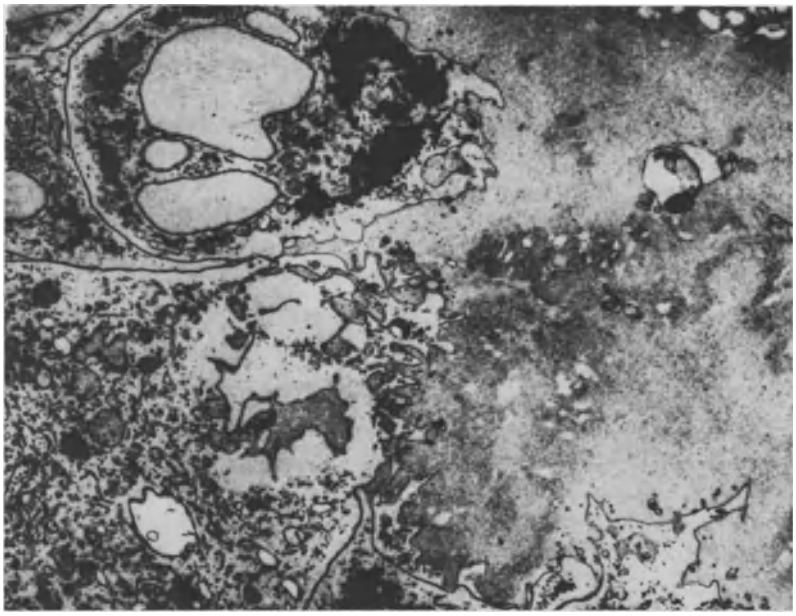

Fig. 7. Partial enlargement of Fig 6 (× 27.000)

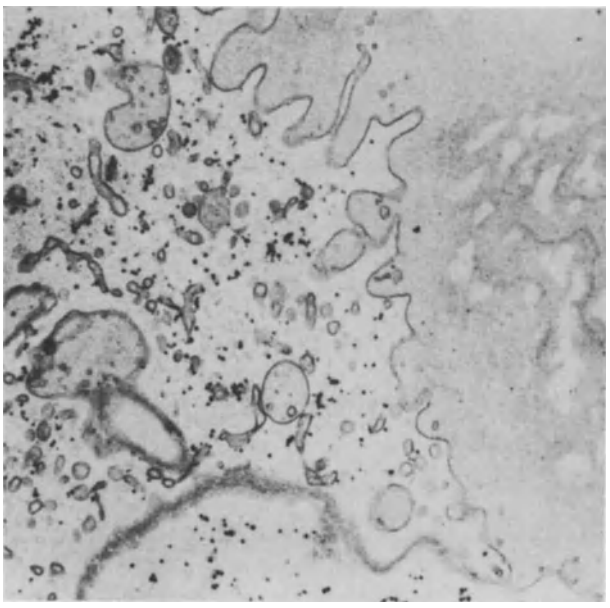

Fig. 8. Macrophages with stored labeled FTAS-degradation products 14^{rh} post-operative day (×320)

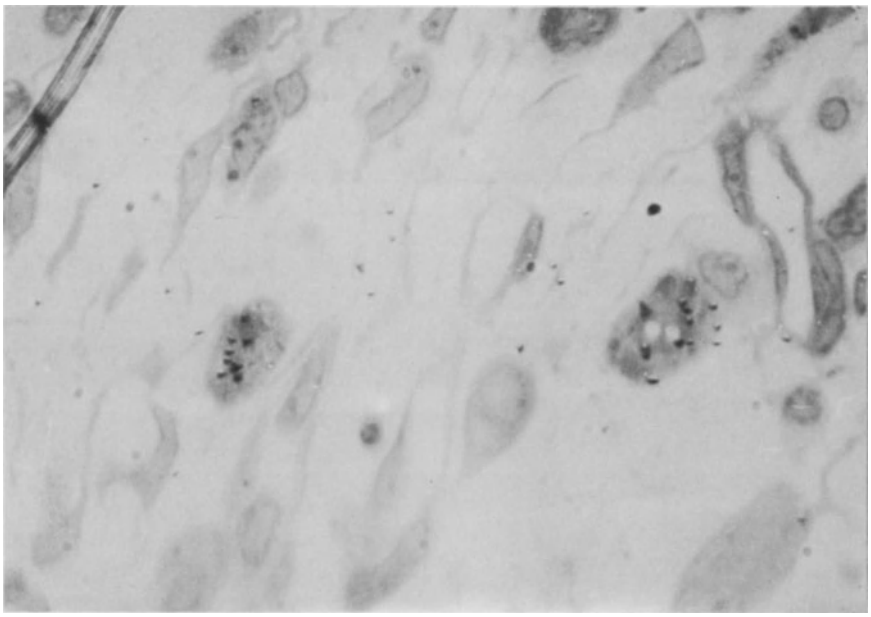

Fig. 9. Macrophages with incorporated degradation products of ^{125}I-labeled fibrin sealant

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)

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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.

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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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

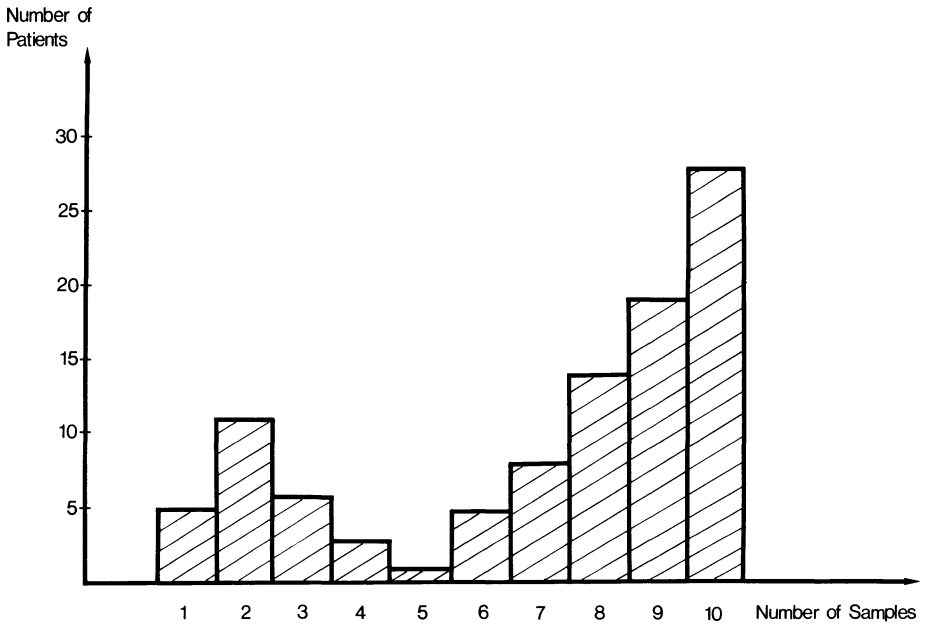

Fig. 1. Frequency distribution of patients and blood samples

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 $\geq$ 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. General Surgery and Abdominal Surgery

Clinical Experience with Fibrin Sealing in General and Thoracic Surgery

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Key words: Fibrin sealing, general and thoracic surgery, clinical experience, postoperative complications

Abstract

Between January 1978 and June 1985 a total of 574 fibrin sealings was carried out on patients of the Surgery I Department of the County Hospital, Salzburg. The main indications for fibrin glueing were additional sealings of sutures and anastomoses ($n = 328$), hemostasis and wound dressing on parenchymatous organs ($n = 122$) and glueing of skin grafts ($n = 97$).

In eight cases (6.4%) postoperative complications arose after hemostasis on parenchymatous organs (lethality 1.6%); a relaparatomy was necessary in one case, however. The rate of fistulations after additional sealings of sutures and anastomoses on the digestive tract amounted to 4.9% (lethality 0.6%). In 11% the healing of skin grafts was not satisfactory. We saw no side effects (for example, hepatitis) of the fibrin sealant.

Introduction

The idea of biological tissue adhesion with human fibrinogen has existed for many years (Grey 1915; Young 1940; Cronkite 1944). In the initial stages of its development, however, this idea could not be realized. This was due to the fact that a sufficient concentration of fibrinogen could not be prepared and that there was a lack of a fibrinolysis inhibitor.

Only after fundamental animal experimentation, clinical research [2, 7, 14, 15, 16] and the successful production of fibrinogen concentrate by the pharmaceutical industry [13] were the basic requirements established for the wide application of fibrin adhesion in operative medicine. This came about in the 1970s.

The promising results obtained led to the development of a fibrin sealant (Tissucol; Immuno, Vienna), which consists of two components. For application of the sealant these two components are mixed; the *then* rubber-like fibrin polymer adheres well to the tissue or bone surface to which the mixture of the two components is applied.

A great number of application possibilities with regard to this new technique are known. The use of the fibrin sealant in general surgery has been especially successful for hemostasis or wound treatment on parenchymatous organs [6, 12]. It has also been very successful for adhesion of skin transplants [18] and for anastomotic sealing of the digestive tract [1, 11].

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Based upon our practical knowledge [1, 19] and upon our studies in animal experiments [10, 20], we report the experiences we have had and the results we have obtained with the use of fibrin sealing.

Indications

During the time between January 1978 and June 1985 a total of 574 fibrin sealings was carried out on patients of the I Surgical Department of the County Hospital in Salzburg. The freeze-dried fibrin sealant Tissucol [13] was used in all these cases.

The main indications for the glue were additional suture and anastomosis sealings especially on the digestive tract in 328 cases, 122 times the hemostasis or wound treatments on parenchymatous organs such as lung, liver, spleen and pancreas, and 97 times the sealings of skin grafts. Fibrin sealing was carried out on an additional 27 patients with sutured nerve and tendon anastomoses.

Methods and Results

Digestive Tract

Suture or rather anastomosis insufficiency is a dreaded complication following resection operations, especially on the digestive tract. This complication also accounts for a high mortality rate among patients. There are many reasons for the cause of this insufficiency. In addition to the suture technique, anemia, hypoproteinemia, tumor disease and infections are important factors. The supplementary fibrin sealing on sutured anastomoses has proved to be a reliable method in reducing the suture insufficiency rate [11]. It is carried out either by sealing the sewn anastomosis or as mechanically stressed sealings, in which the sealant is applied before knotting the sutures on the anastomosis contact surfaces. Suture insufficiencies were clearly diagnosed by clinical criteria, such as stool or bile fistulae, or by radiological controls of the anastomoses [1].

Results

1. Insufficiencies occurred in 8 of 204 cases, that is 3.9%, in which additional fibrin sealings of sutured anastomoses were carried out. One patient died due to a subphrenic abscess following anastomosis insufficiency after gastrectomy; in another case a colostomy was performed following an insufficient colon resection. The other seven insufficiencies could be treated conservatively. It is worth mentioning that in 79 gastroduodenostomies (Billroth I) no suture insufficiency could be found (Table 1).
2. In 125 suture sealings with fibrin glue eight of these operations, that is 6.3%, involved complications caused by the suture. Here the choledochotomy sutures were the most insufficient; one patient died of a diffuse bilious peritonitis. Twice dehiscences of sutures following enterotomies made a relaparatomy necessary. On the other hand, postoperative progress was free of complications in 67 duodenotomies within the scope of transduodenal papillae plastics (Table 2).

Table 1. Additional fibrin sealings of anastomoses ($n = 204$), 1978–1985

Indications	n	Complications	Therapy
Esophagus resection	4	1	Conservative
Esophagojejunostomy	16	2 (1*)	1 × conservative 1 × relaparotomy
Cardia resection	2	0	
Gastroduodenostomy (BI)	79	0	
Ileum resection	19	0	
Colon resection	59	4	3 × conservative 1 × colostomy
Bypass anastomoses			
Biliodigestive	12	1	Conservative
Intestinal	11	0	
Total	204	8 (1*) = 3,9%	

* Number of patients who died

Table 2. Additional fibrin sealings of sutures ($n = 125$), 1978–1985

Indications	n	Complications	Therapy
Gastrotomy	10	0	
Duodenotomy	67	0	
Choledochotomy	37	6 (1*)	Conservative
Entero-, colotomy	11	2	Relaparotomies
Total	125	8 (1*) = 6,3%	

* Number of patients who died

Naturally, we claim no statistical significance for these numbers since a controlled study does not exist. And also, a multitude of factors are jointly responsible for the healing of anastomoses. However, the additional anastomosis or rather suture sealing seem to be especially indicated by dangerous anastomoses and by operation groups, which are generally prone to a high insufficiency risk [11].

Parenchymatous Organs

Bleedings and wound defects of the parenchymatous organs following traumas, resections and intraoperative lesions often posed a difficult task for the surgeon. This has been confirmed by the numerous techniques documented in the medical literature. Single sutures enlarge the primary defect and can lead to postoperative ischemic necroses as well as to fistulations. The sole use of diathermy is an unsafe procedure and is only suitable for small capsule defects. Fibrin sealing, if correctly applied, has been shown to be an effective and protecting tissue method [12]. Its application is independent of the systemic clotting situation.

Fig. 1. Wound dressing and hemostasis with fibrin sealant following a liver rupture

Liver

Following resections and injuries of the liver parenchyma, fibrin sealing effects hemostasis and prevents postoperative bile fistulae. Furthermore, it makes the dressing of the wound lesions following resections combined with diathermy and collagen fleece easier [6, 17].

We used this method on 27 patients with traumas, resections and bleedings following cholecystectomies. Fibrin sealant was applied directly onto the tissue in the case of traumatic liver lesions; in addition we used collagen bands for compression (Fig. 1). Collagen fleece proved to be a good carrier for the glue in the case of liver resections.

RESULTS: In one case bleeding following the fibrin sealing of a liver rupture occurred; therefore a relaparotomy was required for another glueing of this lesion. In another case a capsule hematoma was identified by postoperative computer tomography and could be treated conservatively. Two patients died of liver dystrophy and traumatic shock, respectively; the autopsies showed no bleeding out of the liver parenchyma (Fig. 2).

Spleen

For a long time splenectomy was viewed as the best method for treating traumatic and intraoperative injuries of the spleen. This was due to the fact that this organ was not regarded as vital to the patient's life. Animal experiments and clinical research

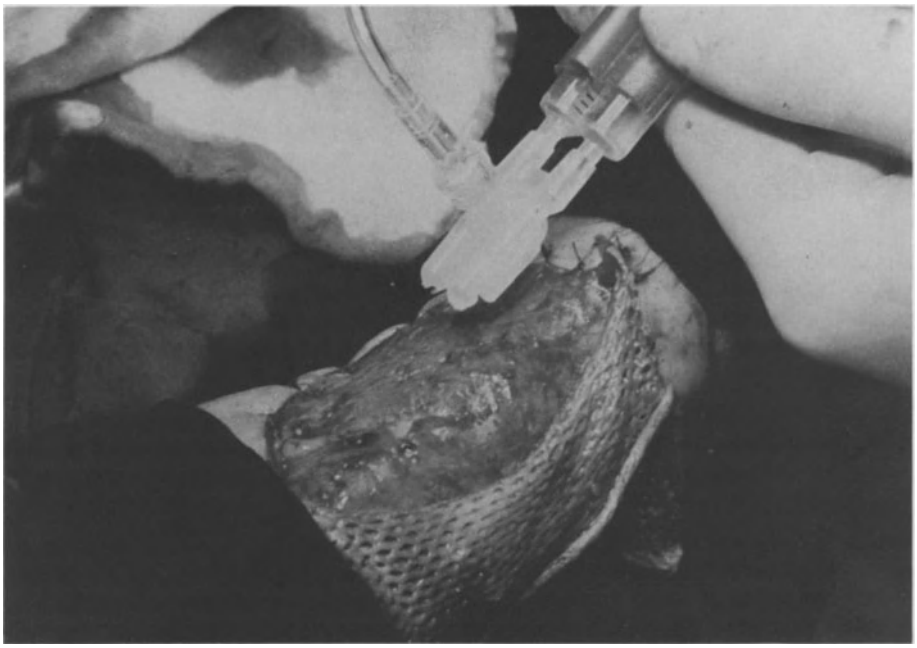

Fig. 2. Fibrin sealing of a skin graft

proved, however, that splenectomy caused considerable disorder by way of immunity resistance. This supports favorable conditions for the formation of sepsis [5, 8, 9]. The frequency of local complications, such as subphrenic abscesses, increases following splenectomies [12]. The preservation of the spleen is made possible by the fibrin glue. Fibrin sealant was used in 14 intraoperative lesions in the scope of abdominal operations and in 8 cases of traumatic injuries. Each time the two components of the fibrin sealant were first applied into the wound fissure. After about 5 min of digital compression, the ruptured organ capsule was plugged by the application of a collagen fleece soaked with fibrin sealant (Fig. 3).

RESULTS: Since 1981 we always took the fibrin sealing into account for hemostasis following traumas and intraoperative lesions of the spleen. In 22 of 42 cases it was performed (Table 3). If it was not successful ($n = 2$) or the ruptures were too deep and combined with lacerations of the hilar vessels ($n = 20$), splenectomies had to be performed. In four of these cases an autotransplantation of specially prepared splenic tissue was successfully employed. With this method we were able to preserve the spleen in 55% of all cases ($n = 42$).

Pancreas

The tissue-specific attributes of the pancreas parenchyma, especially its low tear resistance and its high tryptic activity, cause many complications (fistulations, bleedings, etc.) following resections and traumas [5]. By using fibrin sealant on

Fig. 3. Fibrin sealing of a rupture of the spleen

pancreaticodigestive anastomoses ($n = 13$), resections ($n = 5$), traumas ($n = 2$) and biopsies ($n = 10$) we have definitely decreased this complication rate in the past several years. The fibrin glue was applied either directly on the sutured anastomoses or a soaked collagen fleece was fixed on the parenchymal lesions.

RESULTS: Two of 13 patients with pancreaticodigestive anastomoses following Whipple operations died of anastomosis insufficiencies. In all other cases we saw no fistulae or infections (Table 3).

Lung / Bronchus

Postoperatively persistent parenchymatous leaks following pneumoresections can be the focus of serious complications, such as pneumothorax or pleuraempyema. Therefore a tight closure of these leaks is necessary; this is mostly successful with a continuous suture or a sewing clamp. Nevertheless, should air escape from tissue leaks, fibrin sealing has proved of advantage in these cases [4].

RESULTS: Fibrin sealings were applied 21 times for parenchymatous leaks following pneumoresections. The tightness was always checked intraoperatively by filling the chest cavity with water. Postoperatively there were no complications. In a further 22 cases we carried out additional fibrin sealings on the mechanically occluded bronchus stumps after lung resections. One bronchus stump insufficiency following pneumonectomy was treated successfully with a fibrin clot by an endobronchial endoscopic passage on the fifth postoperative day [20] (Table 3).

Table 3. Fibrin sealings (FS) on parenchymatous organs ($n = 125$), 1978–1985

Organ	Indication	n	FS application	Complications	n	Therapy
1. Liver ($n = 27$)	Trauma	14	FS + collagen bands	Capsule hematoma rebleeding traumatic shock	1 1	Conservative relaparaotomy + FS
	Resection	10	FS + collagen fleece	liver distrophy	1*	
	Bleeding	3	FS + collagen fleece		1*	
					0	
2. Spleen ($n = 22$)	Trauma	8	FS + collagen fleece		0	
	Intraoperative injuries	14	FS + collagen fleece	Subphrenic abscess Intraoperative bleeding	1 2	Conservative splenectomy
3. Pancreas ($n = 30$)	Trauma	2			0	
	Resection	5	FS + collagen fleece		0	
	Biopsy	10			0	
	Pancreatico-digestive Anastomosis	13	FS	Dehiscences	2*	
4. Lung + bronchus ($n = 43$)	Parenchyma Lesion	21	FS + collagen fleece		0	
	Bronchus Stump	22	FS		1	Endoscopic treatment
5. Kidney ($n = 3$)	Trauma	3	FS + collagen fleece		0	
Total		125			8 (2*) = 6,4%	

* Number of patients who died

Skin

Fibrin adhesion makes skin transplantation much easier. This is especially true for malignant melanomas, where for radical oncological reasons wide skin excisions are necessary. Therefore large skin lesions exist on regions of the body which make the healing of skin grafting unfavorable. An example of this are the joints. In these areas fibrin glueing has proved to be of great help – the skin graft can be fixed to the uneven wound surface. In this way a removal of the transplant due to a hematoma can be avoided. Furthermore, we see important advantages which are of special significance to elderly patients: the shortened operating time and the possibility of earlier postoperative mobilization [18, 19].

Skin transplantations were required in 76 cases after excisions of malignant and benign skin tumors, in 11 cases after traumas and in 4 cases within the scope of an ablatio mammae. A further six vascular and actinic skin lesions underwent transplantations. The sealant was usually applied with a double syringe and a combined piston in the form of a Duploject system, whereby the two components of the fibrin

Table 4. Fibrin sealings of skin grafts (n = 97), 1978–1985

Indications	n	Healing pp	ps
Melanomas	58	53	5
Other skin tumors	18	17	1
Traumas	11	10	1
Ablatio mammae	4	4	0
Vascular/actinic lesions	6	3	3
Total	97	87	10 (11%)

pp: per primen
ps: per secundem

glue are mixed in equal parts. In the past 2 years, however, this application system was frequently combined with the use of a spraying head Tissomat (Immuno, Vienna) (Fig. 3). In this connection, both components are dispersed in an airstream whereby a thin fibrin film is momentarily produced [13]. With this method we were able to achieve a better utilization of the fibrin sealant (1 ml for 80 cm^2) [13].

Results

In the light of the healing process of skin grafts the utilization of this procedure can be viewed favorably. In 87 cases we achieved good postoperative results while in 10 patients, that is 11%, the skin transplant was partly or fully repelled (Table 4).

These failures resulted from a number of factors: from initial difficulties in the application of the fibrin glue, from lack of cooperation among the patients as well as from operative and technical problems, such as insufficient preparation of the transplant and wound surface. In cases of malignant melanoma, where excisions on the back area were followed by skin grafting, the rate of healing disturbances among our patients was high. For this reason subsequent back skin transplantations took place in two phases; in the first session the skin tumor was excised and a skin graft was removed; a few days later the skin graft was fixed onto the wound surface with fibrin sealant [3]. Our results with fibrin glueing on vascular and actinic skin lesions were not as satisfactory either as we would have liked. This was due to the fact that the blood circulation on the wound surface was naturally impaired.

Discussion

In the past 6 years we have gained much experience with fibrin sealing. A controlled study does not exist. The patients in whom the fibrin sealant was employed represent a risk group: when the surgeon wanted to have every technical possibility besides suturing, fibrin sealant always was used. In analyzing the results of this technique we have taken into consideration the technical difficulties, which arose at the beginning. These were mostly due to the fact that not every surgeon ($n = 6$) was equally familiar with the use of the sealant. In view of these circumstances we may

be satisfied with our results. We saw no side effects with the fibrin glue. Due to the easy handling of fibrin sealant and the positive results attained, it must be said that this technique has gained its place in the operative and technical repertoire of general and thoracic surgery. Finally it should be pointed out, however, that the question of cost must also be taken into account when using fibrin sealant.

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The Use of Infrared Sapphire Contact Coagulation and Fibrinogen Adhesive for Hemostasis After Partial Hepatectomy: A Comparative Study

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Key words: Infrared sapphire contact coagulation, fibrinogen adhesive, hemostasis, partial hepatectomy

Abstract

With conventional surgical technique, bleeding, biliary fistulae, parenchymal necrosis and secondary subphrenic abscesses represent major complications in the postoperative course following liver resection. Promising adjuvant methods for improvement of surgical hemostasis include a biological two-component fibrin sealant (FS) and, recently, the so-called infrared sapphire coagulation (ISC).

The purpose of the present study was to evaluate intra- and postoperative characteristics of both methods and to compare them with each other. Using a controlled animal model in 20 miniature pigs a standardized left-side hepatectomy was performed. Intraoperative control of bleeding was achieved either by FS or ISC. On postoperative day 12 hepatobiliary scanning (HBS) was done followed by second-look laparotomy including removal of the liver remnant for pathohistologic examination and for nuclear magnetic resonance spectroscopy (NMR).

Intraoperative comparison demonstrated that, in order to achieve preliminary hemostasis, necessary clamping time of the hepatoduodenal ligament (FS, 22.1 ± 1.0 min; ISC, 29.1 ± 2.2 min) as well as application time of each method during clamping (FS, 5.8 ± 0.6 min; ISC, 13.5 ± 1.2 min) was significantly less when using FS ($P < 0.05$). Time needed to achieve complete hemostasis after unclamping was comparable (FS, 6.4 ± 1.2 min; ISC, 6.2 ± 1.4 min). Concomitantly, intraoperative blood loss was less in the FS group (FS, 210 ± 20 ml; ISC, 270 ± 20 ml; $P < 0.05$). In all animals, bleeding could be controlled sufficiently, and the postoperative course was uneventful. HBS excluded extravasation of bile in every case. On second-look laparotomy, a search for biliary fistula or formation of hematoma turned out to be negative. However, compared with FS, the ISC-treated group showed markedly more pronounced adhesions in the resection areas. Parenchymal necrosis was two to three times deeper when using ISC. On NMR, in all animals at a tissue depth of more than 2 cm, no more alterations than with normal tissue were detectable.

Fibrin sealant and ISC proved to be highly effective methods for control of hemostasis and for preventing biliary leakage in liver surgery. Compared with ISC, FS is faster and less aggressive towards tissue, thus significantly reducing ischemia and hemorrhage. However, since application of FS is still compromised by its high costs, combined use of both methods may be preferable.

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Introduction

Remarkable progress has been made in hepatic surgery during the past decade. Thus, even following severe hepatic injury mortality rates below 10% were reported [1, 2]. This was mainly due to standardized general principles of traumatic liver surgery including adequate exposure, meticulous and complete control of hemorrhage, adequate debridement of devitalized tissue, control of biliary ductal leakage and adequate evacuation of blood, bile and parenchymal slough in the postoperative period [3, 4]. Concomitantly, death after elective hepatic surgery for benign or malignant disease also decreased to 4%–9% [5, 6, 7].

However, associated with a high mortality rate, incidence of serious postoperative hepatic complications like bleeding, biliary fistulae and subphrenic abscesses or bilious peritonitis still varies between 5% and 15%, insufficient control of hemorrhage or biliary leaks being the major cause [1, 2, 5, 6, 7, 8, 9]. In order to reduce mortality and complication rates further, numerous alternative methods of hemostasis in addition to conventional surgical technique have been developed.

An important improvement in this field was reported almost 10 years ago by Spängler [10], who used a fibrin adhesive for sealing parenchymal organs. After widespread experimental work this approach appeared to be most promising for control of bleeding after injury or operation on parenchymal organs in man [11, 12, 13, 14, 15, 16].

However, broad use of fibrin adhesive is still compromised by its high costs. Thus, a new technique was recently presented that seems to combine high efficiency for control of hepatic bleeding with only moderate cost. This so-called infrared coagulation technique was originally developed for endoscopic control of gastric or duodenal bleeding [17]. Its hemostatic effect is based upon transformation of light to heat energy via a sapphire crystal being adjacent to the bleeding tissue. Because of the special physical properties of this single crystal, coagulation is not restricted to the surface of the tissue but also reaches deeper structures, thus improving control of hemorrhage. Now the first clinical experiences with this new device are available that support the favorable results of experimental surgery [18, 19, 20, 21, 22].

The goal of the present study was to determine and compare the beneficial effects of both methods for hemostasis in a controlled animal study and to find out if one of these techniques is superior to the other. This was done by evaluation of the intraoperative characteristics as well as of the postoperative rate of complications including biliary leakage, rebleeding and adhesions, and by histological studies of tissue changes.

Materials and Methods

Experimental Protocol

Miniature pigs were selected as the experimental model because of the similiarity to man for liver size as well as histology. Two groups of 10 animals each were fasted over 2 days prior to experimental surgery. Mean weight was 25 ± 3 kg. On the study day the animals were anesthetized (pentobarbital sodium, Abott Laboratories,

North Chicago, IL, administered intravenously, 20–30 mg/kg), intubated and artificially ventilated (BIRD respirator, Bird Co, Palm Springs, CF, room air). After insertion of a nasogastric tube, perioperative antibiotic prophylaxis was administered in all animals (Procain penicillin, Wyeth Laboratories, Philadelphia, PA, 600 000 U). Subsequently, the pigs were prepared for a sterile operative procedure and a transverse epigastric incision was performed. By the Pringle maneuver the portal triad was occluded, clamping the hepatoduodenal ligament [23]. Then, a partial left-sided hepatectomy was done through sharp dissection over clamps, using a standard-size resection area. The resection area should be as close to the hilus of the liver as possible. For better comparison the main vascular structures were sutured without employing the finger fracture technique for adequate exposure [24]. Major vessels or bile ducts were handled by simple ligatures. Concomitantly occurring blood loss was roughly corrected by infusion of Ringer's lactate.

Preliminary hemostasis was achieved in each group using either infrared sapphire coagulation or fibrin sealant, the use of Collagen fleece being optional with the latter. After applying the sealing procedure, warm ischemia was finished by unclamping the hepatoduodenal ligament. Correspondingly, hemostasis was completed, eventually by reclamping the portal triad. After definitive control of bleeding the laparotomy was closed in three layers. Postoperatively, the animals were allowed free access to water and food. A clinical evaluation was done daily. On postoperative day 12 a cholescintigraphy was performed, followed by a second-look laparotomy including removal of the liver remnant for pathologic-histologic examination and for NMR scanning.

Recordings

During the surgical procedure, total clamping time of the hepatoduodenal ligament, application time of fibrin sealant and infrared sapphire coagulation before and after removal of the clamp, total blood loss as well as incidence of arterial oozing hemorrhage after unclamping were documented. Evaluation of the postoperative course included duration of recovery, oral food intake and physical activity. Hepatobiliary scans were examined for signs of biliary insufficiency. During the second-look operation gross findings were recorded. The parameters to be registered were manifest biliary leakage, hematoma formation, signs of peritonitis, adhesions and progressive granulation. Pathohistological examination as well as tissue NMR scans were used to document tissue changes and extent of parenchymal necrosis.

Procedures

Fibrin Sealant (FS)

The main ingredient of the two-component fibrin adhesive system (Immuno AG, Vienna, Austria) is lyophilized human fibrinogen (total of 120 mg/ml). The other component consists of dried thrombin (500 units/ml), which is dissoluble in calcium chloride solution. For practical application fibrinogen is mixed with antifibrinolytic

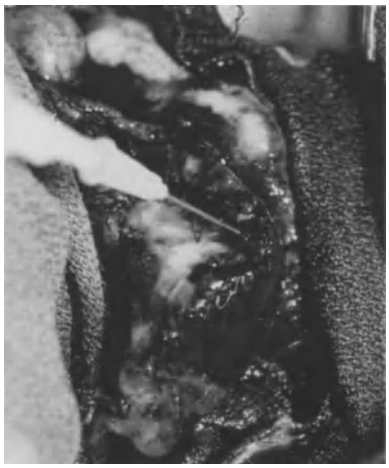

Fig. 1. Application of FS on the hepatic resection surface by means of the syringe

agents, usually aprotinin (3000 KIU/ml). This solution contains besides fibrinogen additional clotting factors (2–7 mg/ml fibronectin, 20 U/ml factor XIII, 65 mg/liter plasminogen). In order to dissolve all the particles the solution has to be warmed up to about 37°C. Simultaneous mixture of the two components is achieved by means of a double-barreled syringe when administering FS [25] (Fig. 1). After application of the mixture to the resection surface, FS begins to solidify within seconds, achieving 70% of its maximum tensile strength within 10 min [26].

For complete control of hemorrhage several layers of FS may be necessary. Thus, the total resection area will be covered consecutively, by a white, rubber-like mass (Fig. 2). In order to accelerate achievement of hemostasis a collagen fleece

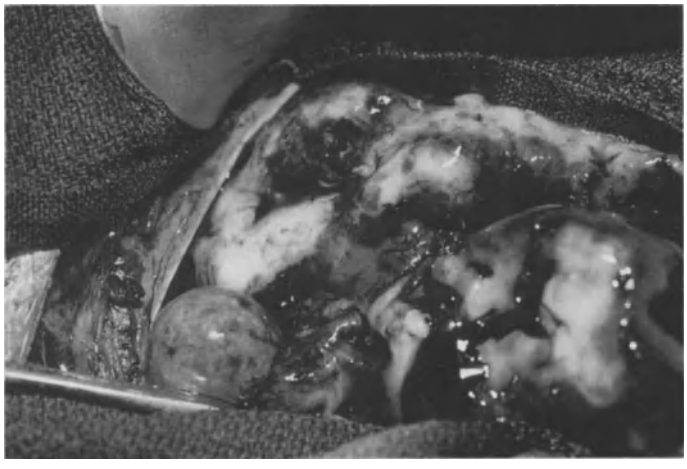

Fig. 2. Aspect of the resection surface after finishing the sealing procedure

(Tachotop, Hormonchemie, Munich) was used in five pigs, which may prevent washing away of the adhesive before polymerization, especially in large surface areas with excessive bleeding [12]. For immediate availability FS was prepared before clamping the hepatoduodenal ligament to avoid additional time loss.

The theoretical base of fibrin sealing includes splitting of the applied fibrinogen into fibrin monomeres by thrombin action. Polymerization of the monomers is mediated by factor XIIIa [27, 28]. Addition of aprotinin prevents local rapid fibrinolysis [26]. Thus, a cohesive elastic mass will be formed, being tightly bound to the adjacent structures. Subsequently, during wound healing FS is absorbed completely by lysis and phagocytosis [29].

Infrared Sapphire Coagulation (ISC)

For coagulation an infrared contact coagulator (ISK 250, NK-Optik, Munich) was used (Fig. 3). The basic technical principles were summarized recently [19, 20]. Light originating from a Wolfram halogen lamp is focused over a gold-coated reflector into an emission area consisting of a sapphire crystal. During coagulation the non-adherent sapphire is pressed upon the tissue for dispelling blood from the tissue surface and for compression of bleeding vessels. The transmitted light energy is absorbed and transformed into warmth thus causing coagulation. The sapphire crystal is indestructible within the range of clinical application and it is chemically inert. Because of its good heat conductivity, the immediate surface heat of the tissue is rapidly conducted away as it is applied to the tissue. Thus, the detrimental adhesive effect through generation of glue is delayed and infrared radiation can become more effective within the tissue, leading to coagulation there. Depending on the various bleeding sites of parenchymatous organs, differently shaped probes have been developed (flat, 90° angled, wedge shaped). The application cycle can be varied between 1 and 6 s, thus increasing depth of coagulation. For practical purposes, the application time used, never exceeded 3 s in order to minimize tissue necrosis. After the probe has been applied to the tissue surface to be coagulated, it is turned on by pressing a foot switch. Development of steam can be observed (Fig. 4).

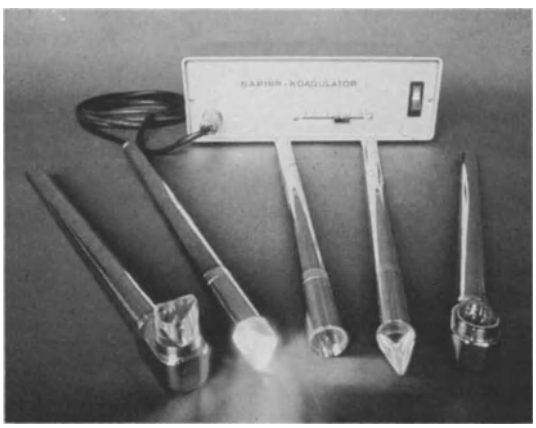

Fig. 3. ISC device with the differently shaped probes

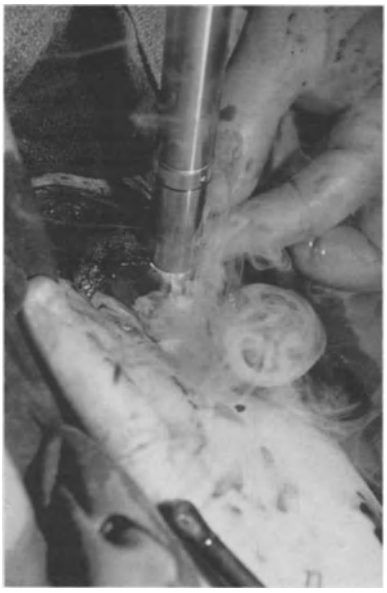

Fig. 4. Application of the ISC device to the hepatic resection surface. Development of steam indicates tissue coagulation

After completing the preset application time, radiation stops automatically. As many single coagulations were performed as necessary for complete surface sealing (Fig. 5). In between several coagulation procedures, a careful cleaning of the crystal surface is necessary to avoid disturbing adhesions following repeated application. When using the device over longer periods the probes must be cooled down in a bowl of cold isotonic salt solution between applications.

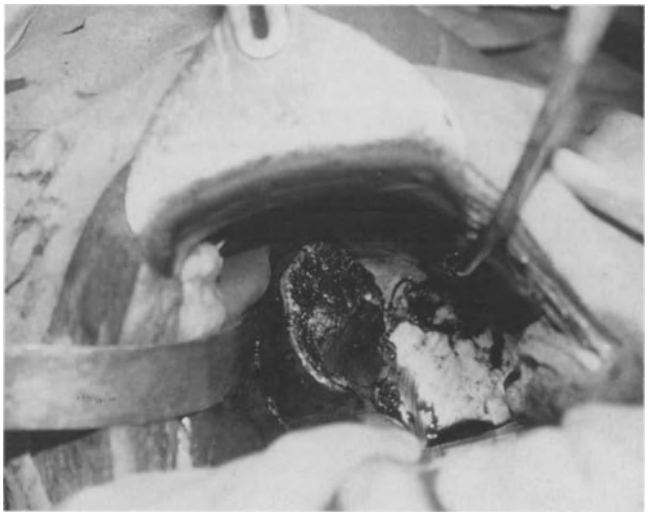

Fig. 5. Aspect of the resection surface when complete hemostasis is achieved by several application cycles of ISC

Hepatobiliary Scan (HBS)

After injection of 1 mCu 99m-technetium-Disofenin (New England Nuclear) by an ear vein, serial scans were recorded every 15 min across the upper abdominal region using a crystal collimator.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Samples of liver tissue (~ 2 g) were taken from the resection area (= 0 cm) as well as at a distance of 1, 2, 8 and 20 cm from it. Tissue samples of 20 cm served as reference probes. For NMR imaging a Bruker PC 20 spectrometer (20 MHz/37°C) was used. T1 values were obtained by inverse recovery.

Statistical Analysis

Data were compared by means of Student's *t*-test for unpaired samples. A statistical significance was assumed at $P < 0.05$. Data are expressed as mean values ± SEMs.

Results

In no animals did major intraoperative complications occur. Clamping time of the hepatoduodenal ligament for parenchymal sealing amounted to 22.1 ± 1.0 min in the FS group compared with 29.1 ± 2.2 min when using ISC (Fig. 6; $P < 0.05$). According to the procedure described above a comparable resection area (FS, 29.2 ± 2.1 cm^2; ISC, 31.5 ± 1.1 cm^2) was obtained in all animals by continuous sharp dissection of parenchymal tissue. Major vessels and bile ducts seen grossly on the resection area were ligated meticulously. Bleeding from the resection surface was controlled effectively by FS as well as ISC. With both devices several application cycles were necessary, FS was administered twice (two syringe fillings) in each procedure, and, when using ISC, up to ten coagulations were performed. With the latter, however, in four cases ligatures already placed previously, were destroyed by heat display, so that time-consuming religation of the affected vessels had to be carried out. Thus, total application time during clamping that was necessary to achieve a preliminary hemostasis was significantly lower using FS (FS, 5.8 ± 0.6 min; ISC, 13.5 ± 1.2 min; $P < 0.001$; Fig. 6). On the other hand the time that was needed to achieve complete hemostasis of the resection area after unclamping the hepatoduodenal ligament, was comparable (FS, 6.4 ± 1.2 min; ISC, 6.2 ± 1.4 min). At this point, manifest arterial oozing hemorrhage was observed in seven out of ten animals using FS, whereas with ISC this did not occur (Table 1). In the first group, this resulted in partial "sub-bleeding" of the applied adhesive. In our study additional application of collagen fleece did not lead to faster control of hemorrhage. Finally, in all animals complete hemostasis could be achieved. Total intraoperative blood loss was significantly lower in the FS group (210 ± 20 ml) compared with 270 ± 20 ml with ISC ($P < 0.05$; Table 1).

The postoperative course was uneventful in all animals. After a recovery period of 2 days the pigs showed normal physical activity and adequate food intake. No animal developed signs of local or systemic infection. Intravenous cholescintigraphy on

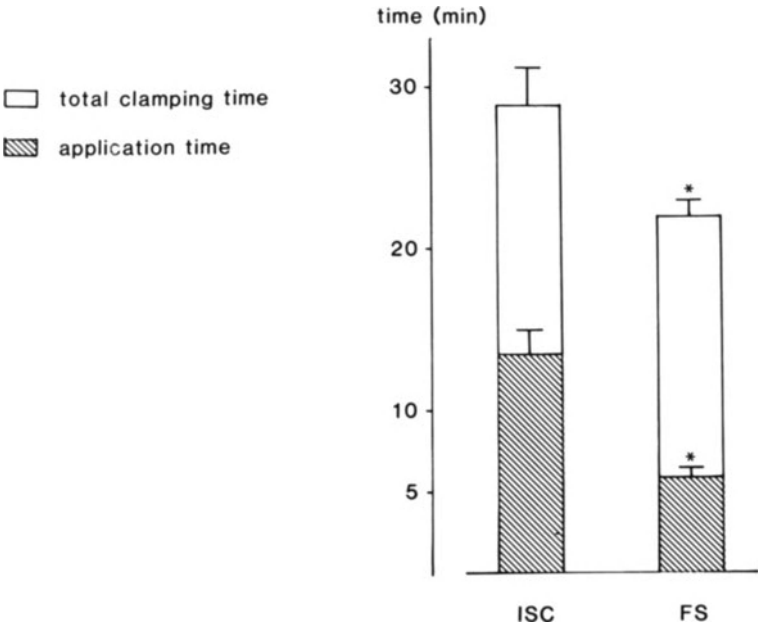

Fig. 6. Duration of clamping of the hepatoduodenal ligament (*open bars*) and duration of application during clamping (*hatched bars*) when using ISC ($n = 10$) or FS ($n = 10$). *, significant difference at $P < 0.05$, ISC vs. FS (unpaired t-test)

Table 1. Intraoperative blood loss and incidence of oozing hemorrhage and of complete hemostasis when using ISC ($n = 10$) or FS ($n = 10$)

	ISC	FS
Intraoperative blood loss (ml)	270 ± 20	210 ± 20[a]
Arterial oozing hemorrhage	0/10	7/10
Complete hemostasis	10/10	10/10

a Significant difference at $P < 0.05$, ISC vs. FS (unpaired t-test)

postoperative day 12 was without pathological findings in all animals, resulting in an immediate hepatic phase without signs of biliary leakage. Extravasation or increased radioactivity near the resection area could be excluded in each case.

At second-look laparotomy there was no evidence for biliary fistula, formation of hematoma as well as signs of peritonitis. Upper abdominal adhesions were present in all animals. The ISC group, however, showed markedly more pronounced adhesions at the site of the resection area. In three pigs small suture abscesses of the abdominal wall were found.

Histological examination revealed parenchymal necrosis of different depth (0.1–0.4 cm following FS, 0.3–1.3 cm following ISC) that was sharply demarcated

from the surrounding normal liver. As an interface between the necrosis and the subjacent normal liver a band of fibroplasia (cellular fibrosis with lymphoid cells and macrophages) was evident in both groups, indicating resorptive inflammation at the base of the necrosis. Lobar parenchymal configuration was uninjured without destruction of epithelial or mesenchymal tissues. Thus, in all animals regenerative power of liver tissue was well preserved. In none of the examined samples could signs of cholestasis or cholangitis be seen. In the FS group the adhesive was already partly absorbed.

Nuclear magnetic resonance spectroscopy demonstrated in both groups comparable significant tissue changes at a depth of 1 cm compared with control samples. At a depth of more than 2 cm no more changes compared with normal were detectable.

Discussion

Adequate use of conventional surgical technique with meticulously set ligatures and sutures represents the major precondition for control of hemorrhage and biliary leakage following hepatic trauma or partial liver resection. However, in a significant number of cases arterial oozing hemorrhage and extravasation of bile will persist, thus increasing postoperative complication rate. Further aggressive attempts to complete hemostasis such as the use of deep ligatures or thick non-absorbable strands will compromise additional vessels and lead to large tissue compartments that are hypoperfused and, consequently, threatened by necrosis [30].

In the past, different adjuvant methods for control of hemorrhage have been developed. They include coagulation by cautery, laser or hot air, surgical procedures like ligation of hepatic artery or permanent tamponade by flapped greater omentum, temporary tamponade by gelatin sponges, oxidized regenerated cellulose or collagen fleece and, finally, application of gelatin foam and cyanoacrylate adhesive [for literature see 4, 13, 19, 20, 31, 32]. However, so far definitive superiority of one of these methods has to be proved. Complications like toxicity, lack of adhesiveness or promotion of abscess formation and disadvantages like lack of compression or sophisticated technique were reported [4, 19, 33, 34, 35].

Thus, the ongoing search for alternative methods in order to control hepatic hemorrhage is not surprising. As emerges from recent clinical studies, application of fibrin sealant, a two-component adhesive consisting of fibrinogen and thrombin, seems to be a reliable new method for achievement of hemostasis in liver surgery [11, 12, 16]. Together with other experimental work [35, 36, 37, 38] our results confirm these findings. Relaparotomy in the FS group did not reveal signs of major complications and only minor adhesions were detectable. Pathohistological evaluation demonstrated only minimal tissue reactivity to FS. However, as can be seen by the intraoperative characteristics, in spite of adequate compression of the fresh fibrin sealing in 70% of the cases plane arterial oozing hemorrhage developed after unclamping the hepatoduodenal ligament. This resulted in several reapplications of FS. Thus, partially some "sub-bleeding" of the applied adhesive occurred. The major disadvantage of this method, however, is still its high costs, amounting to up to $ 150 per surgical procedure.

After all application of FS appears to be an appropriate adjuvant method for control of hemorrhage. Application by means of gas pressure may further improve anatomical adherence of the adhesive mixture, thus lowering incidence of sub-bleeding as well as reducing costs by decreased consumption of the adhesion [39]. In contrast to earlier reports [37], in the present study it could not be confirmed that FS in combination with collagen fleece was superior to the sole use of FS in terms of reducing application time during clamping the hepatoduodenal ligament.

In the face of the expensive FS, alternative methods of hemostasis are still under investigation. In 1975, a new device was presented for endoscopic control of gastrointestinal bleeding: the infrared-contact coagulation [17]. Since 1979 the first serial models were available for control of hemorrhage in gynecological procedures and tonsillectomies and for sclerosing of hemorrhoids [19]. However, the initial procedure of infrared-contact coagulation with its vulnerable Teflon cap did not always withstand concentrated use in extensive parenchymatous bleedings. Therefore, in 1980 the improved contact sapphire coagulator was developed [40]. When pressing the nonadherent sapphire upon the bleeding tissue for coagulation, the transmitted light energy is absorbed and transformed into warmth, thus causing coagulation. By special properties of the sapphire crystal this method is superior to laser and electrocoagulation applications, since tissue can be coagulated as deep as 5 mm. This is far more than the superficial carbonization achieved with previous methods. Thus, danger of delayed rebleeding by lysis of the superficial wound scab is minimized.

So far infrared sapphire coagulation was tested clinically and in experimental work for control of parenchymal bleeding in liver, spleen and lung tissue [19, 20, 21, 31, 41]. Our findings underline the positive results of these studies. The fact that, following partial hepatectomy, no animal exhibited signs of postoperative bleeding, biliary leakage or abscess formation during HBS and at relaparotomy, supports the use of ISC as a new adjuvant method for control of hemorrhage. This use is further potentiated by the reasonable costs of the device, of which the major part is due to the initial cost ($ 7 000), the costs for the single application cycles being negligible. Thus, after using the device in less than 50 patients its cost will be equal to that of FS.

In summary, FS as well as ISC turned out to be a safe and reliable method for control of hemorrhage in liver surgery. Nevertheless, direct comparison of both methods revealed significant intra- and postoperative differences. Thus, total clamping time of the hepatoduodenal ligament for temporary control of hemostasis and application time during clamping was markedly less when using FS. This may be explained in part by the fact that ISC leads to destruction of piercing ligatures already placed previously by heat display. Therefore, time-consuming re-ligations of the affected vessels and additional coagulations had to be done. Re-opening of ligated vessels by ISC might have also been responsible for the higher intraoperative blood loss seen in the ISC group. At re-exploration the different extent of formation of adhesions was striking. According to others [23, 38], in the FS group gross tissue reaction was minimal, being limited to small omental adhesions to the injured liver surfaces. Use of ISC, however, resulted in markedly more pronounced adhesions at the site of the resection area. Correspondingly, histological studies also demonstrated only minimal tissue reactivity to FS. Due to the coagulation procedure depth

of parenchymal necrosis was two to three times larger following ISC. However, clinically these changes were not relevant and cellular regenerative power of the liver remnant remained unaffected.

In part, our findings are in contrast to a previous report that compared the use of FS and ISC following liver resection in a rat model [42]. The authors reported lower application times and less adhesions when administering ISC. This discrepancy may be explained by the different animal models. Rat liver is notably smaller than liver of miniature pigs, resulting in smaller size of resection area as well as vessel diameter. Thus, compared with our experiments, the absolute amount of bleeding presumably might have been markedly lower. This, in turn, could favor the use of ISC.

In conclusion, both FS and ISC provide adequate control of hemorrhage in liver resection. Compatibility of FS with hepatic tissue is excellent, but its broad use is still compromised by its high costs. On the other hand, use of ISC is cheaper in the long run and the device can be applied not only for prophylaxis of rebleeding after liver resection but also for therapeutic purposes when compression is necessary in management of severe hepatic trauma. However, ISC is more aggressive to the tissue and may need more time in order to achieve complete hemostasis. Successful use of both methods requires familiarity of the surgeon with the device and expertise with technical details. For practical purposes, we feel that combined use of both methods might be superior to the use of one alone. Consecutively, initial rough control of major bleeding should be done by ISC, whereas FS is then applied for control of oozing hemorrhage.

After all, it should be emphasized that both methods do not substitute a surgical technique but are adjuvant methods to optimize results.

Acknowledgements. For technical assistance thanks are due to Marie Longo. FS and the ISC device were donations of Immuno AG, Vienna, Austria, and of Lumatec Co., Munich, FRG, respectively.

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Survey of Liver Regeneration Following Liver Resection by Application of Fibrin Sealant

S. KARÁCSONYI, G. FARKAS, M. KARÁCSONYI, H. BAJUSZ, and T. OLÁH

Key words: Fibrin sealant, liver regeneration, liver resection

Abstract

The resected surface was sealed by fibrin sealant in ten cases of human liver resection. In the postoperative period the liver regeneration was traced by 99 Tc and it was found that regeneration in these patients started faster and was marked. Experiments were carried out on rats to set up the model of this observation.

Liver resections were performed in 42 CFY female rats. The animals were killed 72 h, 1 week, and 2 weeks following resection. At the three points of time the resected livers of seven animals were sealed by fibrin sealant, while seven other animals were not treated in this way; they were used as controls each time. It was found that the rat liver regeneration showed the most marked morphological picture 72 h later, and it was very apparent in the livers covered by fibrin sealant. In these the necrotic zone was remarkably smaller than in those not treated with fibrin sealant.

These findings suggest that in the case of liver resection liver regeneration is favorably influenced by fibrin sealant, the postresective necrosis being reduced and the regenerative activity being accelerated in time and volume.

Introduction

At the Department of Surgery, University Medical School, Szeged, 37 human liver resections have been carried out as a result of focal diseases of the liver during the past 3 years. In the course of the operations the resected liver surface was covered in different ways. Epiploplasty was performed and the margins of Glisson's capsule were sewn up, respectively, and in ten cases a two-component fibrin sealant was used. Of the above-mentioned clinical cases the liver regeneration was studied in 17 patients. To measure the regeneration we used the gamma camera method with 99 Tc by means of planimetric determination of the area with a scintigraphic picture taken from two directions. Our method is based on the studies by Lin and coworkers (7). In the postoperative period the regenerative rate and the changes in the functioning mass of the liver were examined. Regeneration could be detected 14 days following the operation and it was usually completed 1 year later, depending on the extent of resection.

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
© Springer-Verlag Berlin Heidelberg 1986

The comparative measurements were carried out by using self-controls taking the preoperative state as a starting point. Regeneration was compared with that and the increase was given as a percentage. These measurements showed that the regenerative rate was about 20%– 30% higher following the operations where fibrin sealant was applied, and the functioning liver mass grew in accordance with this. Since clinical cases were rather heterogeneous according to age, primary disease, and the extent of the resection, we intended to check our observations in an experimental way.

Material and Methods

Experiments were made on 42 CFY female rats weighing between 200 and 230 g. Independently of the present experiment the liver weight of the rats of this sex and weight was determined and was found to be 13 g on average.

Six groups were formed for the examinations. Each consisted of seven animals. In the rats the atypical resection of the two largest lobes was performed, and in this way 30% of the total liver mass was removed. In half of the animals the resection surface was covered by fibrin sealant while in the other half it was left unsealed (Fig. 1). The latter were used as controls. The animals were killed 72 h, 1 week, and 2 weeks later.

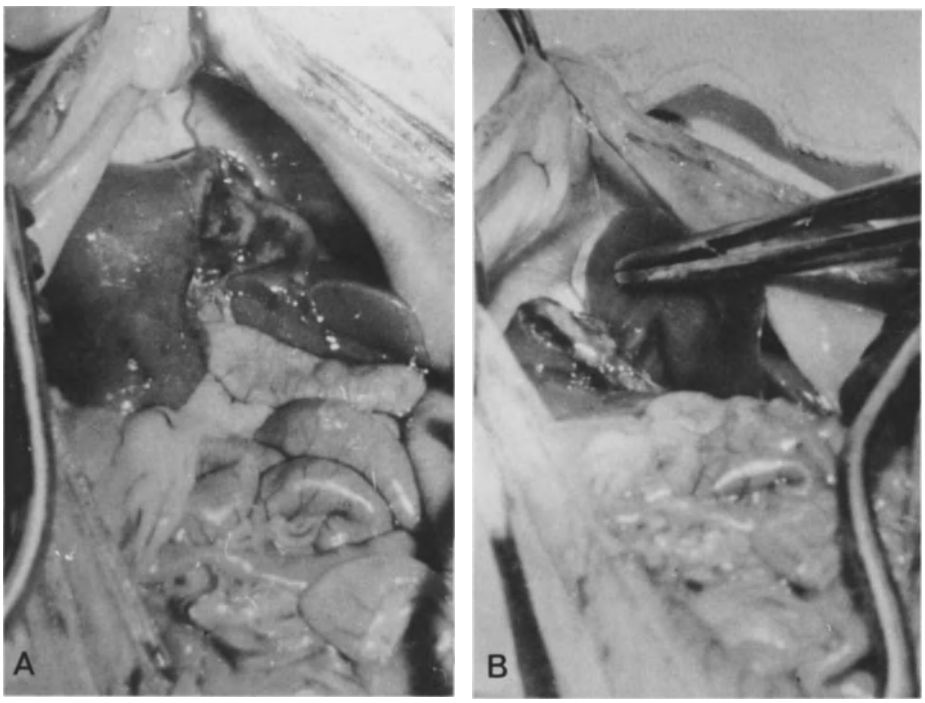

Fig. 1a, b. Method of liver resection, **a** Control group; **b** the resection surface covered by fibrin sealant

Thus, at each point of time the livers of seven animals were available whose surface was sealed by fibrin sealant, and we had seven as controls. The mass of the livers sealed by fibrin sealant and that of the controls removed on each occasion was weighed and the changes in values were compared on curves. Samples were taken from the removed liver and the histological findings of livers treated with fibrin sealant and those untreated were compared from the viewpoint of liver regeneration.

Results

Weight measurements (Fig. 2) showed that liver regeneration started earlier in the case of liver resection where fibrin sealant was applied, and a significant increase was observed in the liver mass. The histological findings obtained 72 h after the resection demonstrated disintegration of the structure as well as degenerative changes both in the resected livers treated with fibrin sealant and in the controls. Cell division was frequent both in the control group and in that treated with fibrin sealant (Fig. 3). The difference between the two groups was indicated only by the higher incidence of necrosis in the control group (Fig. 4). One week after resection it became evident in both groups that cell division had decreased, the nuclear polymorphism was marked, and the cytoplasm was swollen and subtly vacuolized. After freezing and staining with Oil-Red-O stain, neutral fat appeared in the vacuoles. One nucleolus could be found in most of the nuclei. The Kupffer cells had accumulated focally. In the group treated with fibrin sealant a greater number of fibroblasts could be found around the necrotic site and the proliferation of the bile duct was also of greater size. Two weeks after resection there was no apparent difference in the histological samples between the group treated with fibrin sealant and the control. Regeneration seemed to have been completed. Cell division could

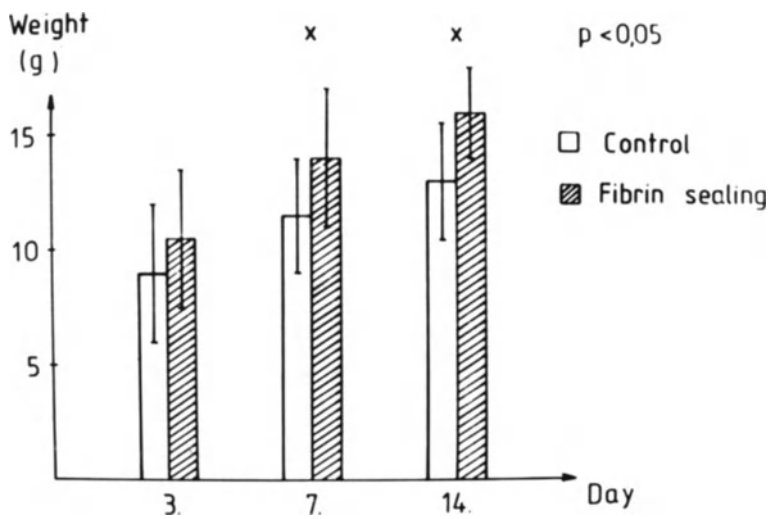

Fig. 2. The weight of liver during the regeneration ($n = 7$, average ± SD)

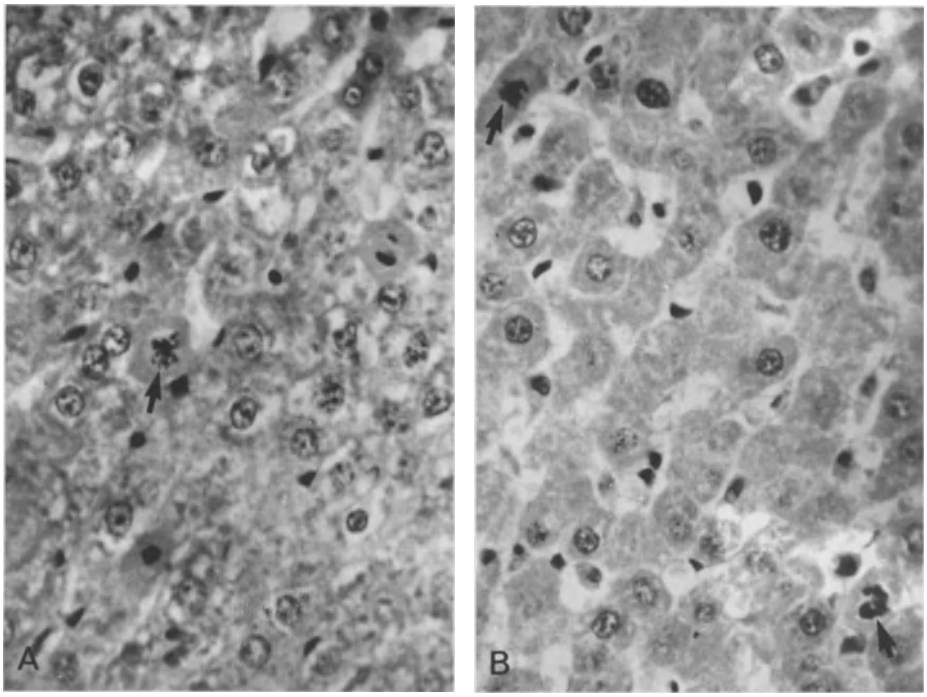

Fig. 3a, b. Cell division (*arrow*) in the control group **a** and in the treated group **b.** × 560

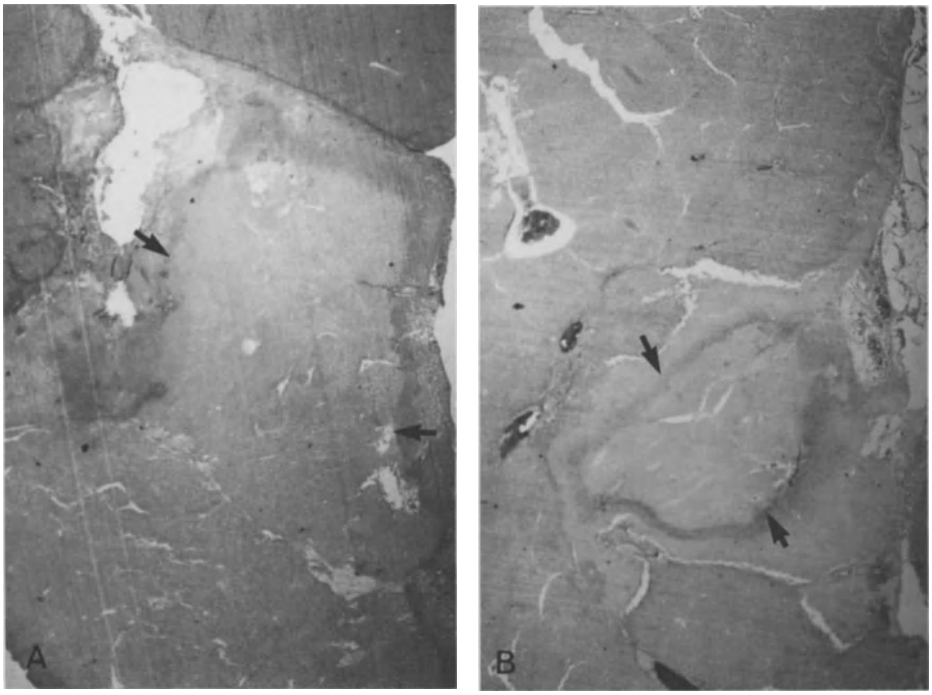

Fig. 4a, b. Liver necrosis (*arrow*) in the control **a** and in the treated group **b.** × 14

rarely be seen, nuclear polymorphism was not significant, the cytoplasm was the same size as that of the healthy liver cell, and vacuolization could hardly be demonstrated. The sinusoids were moderately wide.

Discussion

In the past 25 years liver resections of different extents have become routine procedures for treating the space-reducing processes of the liver. Check-ups following resections have proved that the resected human liver is also able to regenerate [8].

The regenerative ability of the human liver is regarded as a new observation, since, in animal experiments, this regenerative activity has been disclosed before. It has been proved by extensive experimental studies that the liver regeneration can be influenced and determined by several factors. For example, the liver of the rat is able to regenerate remarkably well. From 48 to 72 h later the animal can reproduce the removed liver tissue almost totally. In animal experiments Starzl and coworkers [10] demonstrated the so-called hepatotrope factor in the blood of the vena portae. The relationship between the volume of portal flow and liver regeneration was proved by Kotohito [4]. The connection between liver regeneration and the intraperitoneal injection of liver homogenizate in rats was proved by Blomqvist [1]. Grisler and coworkers [3] revealed a factor in the small intestine affecting liver regeneration. In the same field certain results were observed by Toshio Chiba [12] from the intake of amino acids. Terblanche and coworkers [11] carried out experiments to study the effects of the incision of cytosol extract on liver regeneration, which proved to be quite positive. Another factor affecting this had been found earlier by Lavigne [5] in the supernatant of the isolated hepatocytes of rat liver. Observations made on both experimental and human liver resections seem to clarify that the regenerative rate is considerably influenced by surgical technique as well. In cases where the resection is difficult to carry out, e.g., in pig or man, the start and progress of regeneration is slow, while in species where the resective technique is easier, e.g., in rat and chicken, the regeneration can take place faster. The favorable effects of fibrin sealant have already been reported by Giakoustidis [2] as a treatment for liver injuries. The medical application of fibrin sealant has been reported in several fields [9]. The pathological background of these favorable effects, however, has not been revealed yet. It appears to be obvious that fibrin sealant has no direct effect on the metabolism of liver regeneration. According to our studies the local effects of fibrin sealant can be accounted for by the fact that sealing the resected liver surface is effective when the necrosis caused by surgery is minimal. The extent of necrosis seems to play an important part in the start and rate of liver regeneration. Prior to the application of fibrin sealant the problem of sealing the remaining liver surface and drainage following resection was unsolved. The application of fibrin sealant seems to exert a favorable influence on that problem by minimalizing injury to the resected surface with sutures and there is no need for complicated drainage.

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Experience with the Use of Fibrin Sealant in Surgical Therapy of Liver Tumours

H. LIPPERT and H. WOLFF

Key words: Fibrin Sealing, liver tumours

Abstract

The diagnosis of liver tumour used to be frustrating in the past, and many medical centres were more than reluctant to use aggressive surgical therapy. However, a 3-year survival rate of something between 58% and 66% has been reported in more recent publications for patients who had undergone hemihepatectomy for malignant tumours [1, 3].

This report has been prepared for the purpose of presenting results obtained at the authors' hospital, using fibrin glue.

Introduction

A total of 411 patients received surgical treatment for liver tumours at the Surgical Hospital of Charité between 1979 and 1984 (Table 1). A diagnostic diagram (Table 2) was used to establish severity, expansion, and operability of the tumours. Exploratory laparotomy was considered to be indicated in cases in which no definite information was obtainable from such diagnostic check.

Indication for Hemihepatectomy

Surgical therapy was decided on for the authors' patients on the established assumption that hepatectomy was the only possible curative therapy of liver car-

Table 1. Number of patients with liver tumours and forms of therapy

	Benign	*Malignant*
Resection	86	58
Resection drainage	22	–
Exploratory laparotomy	21	119
No surgical therapy	39	66
	168	243
	411	

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Table 2. Diagnostic strategy

Case history	PTC	Liver arteriography
Clinical examination	PTC-D	Reflux portography
Laboratory examination	Computer tomography	Liver scintigraphy
Sonography	Bolus injection (dynamic CT)	Functional scintigraphy
Tentative diagnosis	Fine-needle puncture (cytology)	Infusion cholangiography
Icterus	Selective angiography	
ERCP		

cinoma, but with the condition that the tumour was operable. For patients with benign tumours of the liver, indications depended on tumour size and complaints.

Indications for hemihepatectomy were limited by impaired liver function, general tumour-related cachexia, and accompanying diseases of high severity.

Tumour growth in either lobe, walling-in of central vessels, advanced liver cirrhosis, and metabolic decompensation were counterindicative to hemihepatectomy.

Surgical Therapy

Incision of the costal margin on the right with possible expansion was chosen as the surgical access route. One to three liver segments usually had to be removed. Hemihepatectomy proved to be the only possible approach to larger tumours limited to one lobe but infiltrating several segments.

So-called atypical hemihepatectomy is used in cases with smaller tumours in peripheral positions, in metastasis or benign processes, without paying attention to segment boundaries. Efforts were always made to remove the smallest possible amount of intact liver tissue and not to leave the patient with ischaemic liver areas.

As a particular surgical problem, tumour removal had to be followed by sealing of the resection surface with the effect that biliary effusion or postoperative bleeding was avoided. Mattress suturing, electrocoagulation, infrared coagulation, and fibrin sealing were the means available to that end. The authors have been using these methods in combination for about 3 years. When active bleeding had stopped from the resection surface, the liver parenchyma was manually compressed before fibrin glue was applied together with collagen fleece. Omentofixation to the liver was avoided, with the view to prevent additional provocation of necroses. A few adapting sutures were occasionally made, when the parenchymal bridge was not too wide. Drainage was applied for 3–8 days.

Table 3. Types and frequency of hepatectomy for benign and malignant liver tumours

	Malignant liver tumours	*Benign liver tumours*
Hemihepatectomy, right	18	17
Hemihepatectomy, left	12	11
Atypical hemihepatectomy	28	58
	58	86

Table 4. Diagnosis of 21 patients with malignant ailments of the liver

Diagnosis	Number
Liver cirrhosis	4
Crigler-Najjar syndrome	2
Biliary atresia	2
Budd-Chiari syndrome	3
Hepatocellular carcinoma	7
Cholangiocellular carcinoma	9
Liver metastasation	4
Liver sarcoma	1
Total	32
One-year survival	30%

Results

Only 58 in 243 malignant liver tumours could be radically removed by hepatectomy. In another 21 patients, the tumorous livers were replaced by liver transplantation (Tables 3, 4).

Resection was also performed on 86 of 168 benign liver tumours (Table 3).

Seven of 144 patients with hemihepatectomy died within 30 days from surgery, which raised lethality to 5%. Liver failure associated with pneumonia was the most common cause of death (five patients). One patient died of septic shock and another of pulmonary embolism. The 3-year survival rate of patients with malignant liver tumour and therapeutic hepatectomy amounted to 50%. Only one in 185 patients with inoperable malignant tumours survived for 2 years.

Rates of complications were studied in the context of 135 patients, with particular consideration of fibrin glue on the site of resection.

Tissucol (Tissucol Kit, Duploject) was used for fibrin sealing. It was primarily applied to patients who were known to have impaired coagulation. Complications of

Table 5. Complications of a general nature in the wake of hemihepatectomy with and without fibrin sealing

	With fibrin glue (Human Immunokit) Duploject	Without fibrin glue
Number of patients	$n = 61$ (%)	$n = 74$ (%)
Wound healing	12 (20)	15 (20)
Impaired protein synthesis	10 (16)	12 (15)
Impaired coagulation	9 (15)	8 (10)
Pneumonia	6 (10)	8 (10)
Pleural effusion	2 (3)	3 (4)

Table 6. Locally delimited complications in the wake of hemihepatectomy with and without fibrin sealing

	With fibrin glue (Human Immunokit)	Without fibrin glue
Number of patients	$n = 61$ (%)	$n = 74$ (%)
Subphrenic effusion	2 (3)	9 (12)
Haematoma	1 (3)	6 (8)
Bile	1 (3)	8 (10)
Abscess	1 (3)	5 (7)
Resection area		
Biliary fistulation	2 (3)	7 (10)
Postoperative bleeding	1 (3)	3 (4)

a general nature, following hemihepatectomy, which were primarily attributable to inadequate immune defence or to disorders in liver and protein metabolism, were not even controllable by the use of fibrin glue for local haemostasis (Table 5).

Locally delimited complications, such as haematoma, biliary fistulation, and liver abscess, were substantially reduced (1%–3%) by means of fibrin sealing (Table 6). Locally delimited complications were recorded from 4%–12% of an equally large group of patients to whom no fibrin glue had been applied.

Discussion

Every single liver tumour needs to be accurately elucidated for severity and operability. Hepatectomy was found to be applicable to all types of operable malignant and benign liver tumours (adenoma, haemangioma) [6].

Hepatectomy so far has been relatively rarely applied to cases of primary liver carcinoma, with reported figures being between 10% and 31% [2, 3, 4, 7]. Our own rate has been 24%.

Surgical lethality, according to data in the literature, has ranged from 2% to 25% [3, 5], while our own rate was 5%.

It is felt by the authors that by using fibrin sealing surgical lethality can be favourably influenced via unambiguous reduction of complications on the resection surface. For example, following fibrin sealing of 61 patients, postoperative bleeding was recorded only in one single case and biliary fistulation twice. Seventy-four patients without fibrin sealing, on the other hand, exhibited biliary fistulation in seven instances and postoperative bleeding in three.

Fibrin sealing has proved to compare favourably to other haemostatic techniques, including electrocoagulation, infrared coagulation, and mattress sutures, in that no additional necrosis is provoked, an aspect of great relevance to the prevention of infection.

The authors also feel that fibrin sealing alone is not sufficient, when it comes to large-lumen vessels and bile ducts in the region of hepatectomy.

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Splenic Salvage by the Use of Fibrin Tissue Adhesive

J. SCHEELE

Key words: Splenic salvage, splenic resection, fibrin tissue adhesive

Abstract

A biologic tissue sealant has been used for repair of traumatic and incidental splenic lesions in 188 patients, and for elective splenic resection in 10 cases. The technical details of fibrin adhesive application, including the combination with a collagen fleece, are described. Complete hemostasis was achieved in 183 patients. In five of them, rebleeding required delayed splenectomy. However, in incidental splenic lesions the success rate of the method was 92%, and the overall salvage rate 84%. The corresponding figures for traumatic injury were 83% and 55%, and for elective splenic resection 100%. It can be concluded that the use of fibrin tissue adhesive is a safe and reliable method for repair of the majority of incidental and traumatic splenic injuries and facilitates elective splenic resection.

Introduction

An increasing awareness of the hazards associated with splenectomy [1, 3, 5, 10, 18, 20, 25, 27] has encouraged surgeons to preserve splenic function for various indications. Though incidental and traumatic lesions of the spleen represent the vast majority, they also include partial splenic preservation in benign tumors [18, 21], staging procedures [12, 18, 21], and functional reduction for hypersplenism [13].

The techniques used to control bleeding can be classified into different categories:

1. Conservative treatment with close observation by ultrasound, CT scan, or radioisotope liver-spleen imaging [17, 25]. Other authors [17] have recommended this as the routine management for children with stable vital signs. We consider it justified, however, only in very selected cases, since concomitant injuries may be missed.
2. Conventional control of surface arterial or venous bleeders by isolated ligation, suture ligation, or clipping [4, 6, 18] and of diffuse oozing by compression of wound edges, using simple sutures, a circularly placed "ladder" of threads made by the surgeon [3], or a commercially manufactured resorbable woven mesh [8].
3. Interruption of the blood inflow by ligation of the splenic artery [4] or its major branches [3, 18].
4. Induction of a superficial coagulatory necrosis at the bleeding area by cautery [6], laser [9], or infrared light [12].

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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5. Activation of the patient's own hemostatic potential using gelatin sponges, oxidized cellulose, or microfibrillar collagen [4, 6, 9, 18].
6. Application of synthetic [6] or biologic tissue adhesives [21, 22, 23], the latter representing in addition a substitution of coagulatory factors.
7. Reimplantation of primarily removed splenic tissue [7, 14, 24].

All these techniques are aimed at preventing the substantial risk of postsplenectomy septicemia, which results in a 2%–3% late mortality from overwhelming infection despite aggressive antibiotic therapy [10, 19, 25].

A critical evaluation of different techniques has to consider both the feasibility of the method or – in other words – the remaining percentage of splenectomies necessary despite a routine effort of splenic salvage, and the rate of complications or even mortality related to the salvage procedure.

Theoretical and Technical Aspects of Fibrin Tissue Adhesive

The major advantages of this method include the avoidance of any additional tissue damage and the direct application of concentrated components of the coagulation system, in particular fibrinogen and thrombin, to the wound surface. This results in reliable hemostasis even in the event of severe systemic coagulatory failure [23].

The sealant is, however, unable to control major arterial bleeding. Therefore, in about 20% of the cases conventional debridement and sometimes splenic resection represents the first step of the operation. As far as arterial bleeding is controlled, even moderate oozing will be stopped completely by the sealant.

A specific problem is the time needed for this hemostatic process; it takes usually 3–5 min until the tissue adhesive has reached sufficient internal stabilization and anchoring to collagen fibers of the wound surface. This is of less importance in the case of only minimal residual bleeding prior to application. If, however, considerable oozing persists, the sealant could be washed away before polymerization is complete. To avoid this and to guarantee a 5-min contact between the clotting factors and the bleeding surface, the adhesive has to be pressed to the wound area.

Technique of Complete Splenic Salvage

In nongaping fissures complete splenic salvage is realized by simultaneous injection of the two components directly into the wound cleft, followed by immediate manual compression for 3–5 min (Fig. 1a). For this particular technique, the use of a mixing syringe (Duploject) is recommended, since it results in the two components being more homogeneously dispensed, with a faster coagulation process and higher definite strength. In exceptionally deep ruptures a few sutures should be placed while compression is maintained, in order to reduce tension (Fig. 2).

In wide gaping parenchymal defects, superficial capsular tears or a raw surface following resection, temporary compression of oozing and thereby close contact of the sealant to the wound necessitates the use of a carrier plate, preferably a collagen fleece. This is resorbable itself and permits reliable evaluation of hemostasis (Fig. 1b, 3).

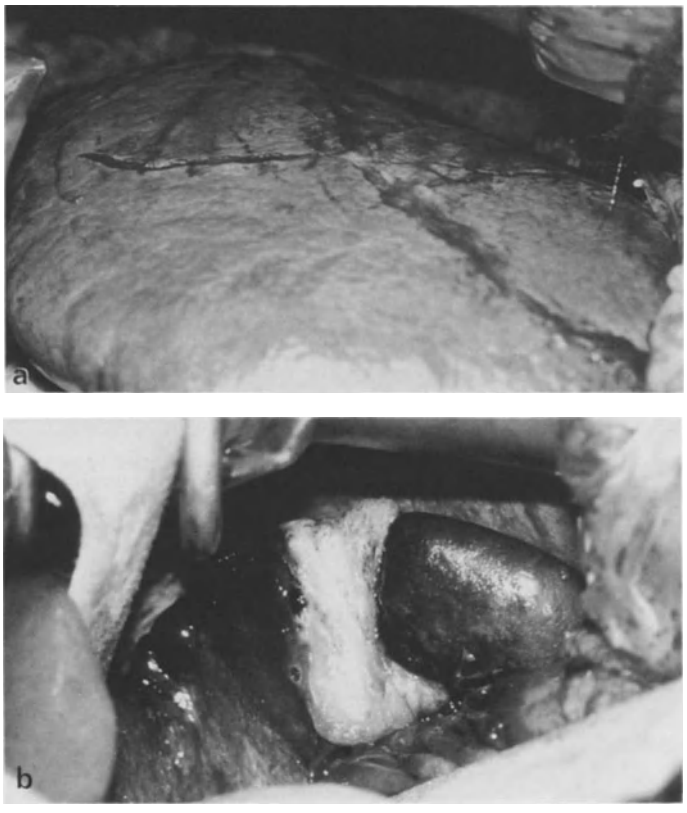

Fig. 1a, b. Repair of traumatic injury of the spleen.
a Bursting injury of the splenic convexity with multiple fissures, treated by application of fibrin sealant and compression. **b** Deep rupture of the anterior edge, covered by adhesive and collagen fleece. (Scheele et al. 1984)

The fleece is prepared by superficially wetting both sides with a small amount of thrombin solution. Subsequently, fibrinogen (approx. 0.5 ml/50 cm^2) is evenly spread over one side and distributed by the finger. Then the fleece is pressed over the lesion for 3–5 min by hand. Larger defects should be covered stepwise by patches with a maximum size of 5×5 cm, to avoid any dead space between the wound area and the collagen fleece (Figs. 4, 5).

Wetting of the outside of the fleece with thrombin solution or any other fluid is recommended in order to prevent it from adhering to the surgical gloves.

During the first 1 or 2 min, breakthrough bleeding may occur and the effort to induce hemostasis may seem ineffective. The author's personal experience showed, however, that even in severe initial oozing, persistent maintenance of compression always resulted in a quick decrease of bleeding intensity, since the thrombin content of the sealant induces occlusion of minor vessels by clotting the marginal endogenous fibrinogen. After 5 min, complete hemostasis is usually achieved. If some

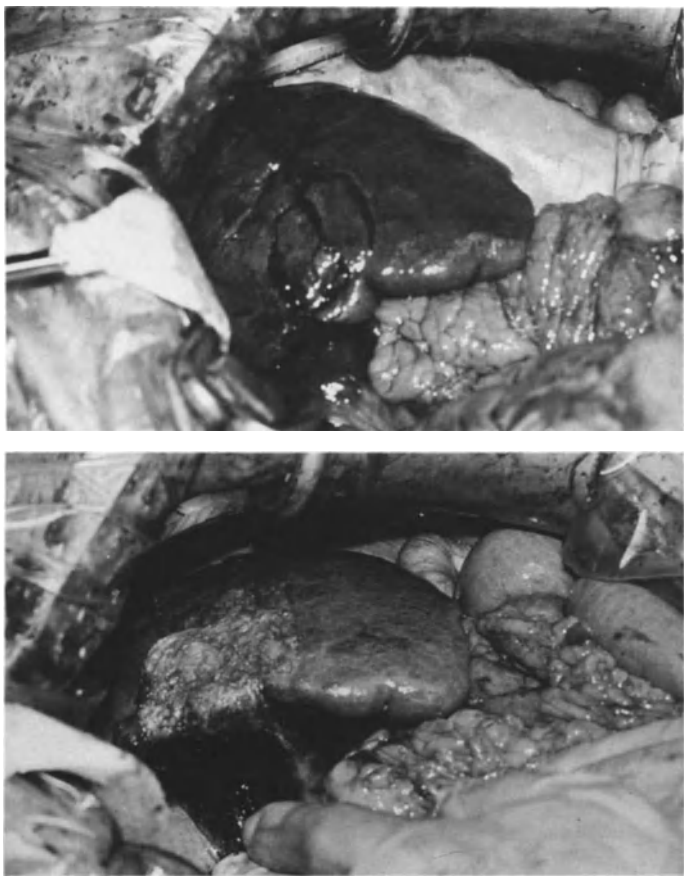

Fig. 2. Deep transverse splenic rupture, managed by a combination of injecting fibrin tissue adhesive into the wound cleft, sutures to reduce tension, and additional covering with collagen fleece. (Scheele et al 1984)

bleeding persists, the collagen fleece should gently be opened by scissors incision at the suggested area. Depending on the size of the still bleeding vessel, a short coagulation by cautery, a thin suture ligation, or only a second attempt at sealing will be performed.

The spray technique may be advantageous for other indications; in control of such moderate or severe bleeding mentioned above it is not effective. Therefore, it can not be recommended for splenic salvage.

Indications for Partial Splenic Salvage

In patients operated on for severe injury to one part of the spleen, despite complete intraoperative hemostasis parenchymal contusion may result in formation of intrasplenic hematomas and delayed rupture. In these cases as well as in lesions involving

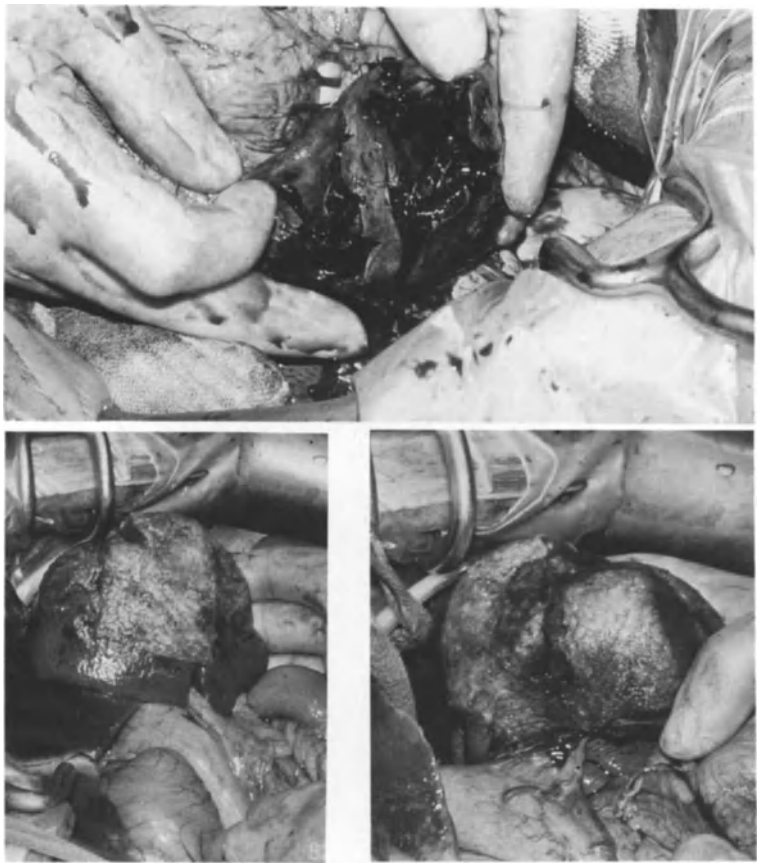

Fig. 3. Crushing injury of the spleen, treated by debridement, adhesive injection, a few sutures, and collagen fleece

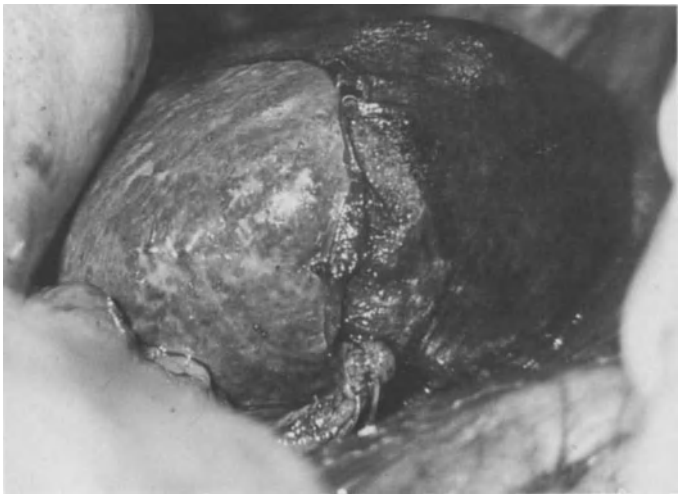

Fig. 4. Capsular defect at the lower pole, covered with collagen fleece. (Scheele et al 1984)

Fig. 5. Incidental large capsular defect of splenic convexity during vagotomy, stepwise covered with collagen patches after complete mobilization. (Scheele et al 1984)

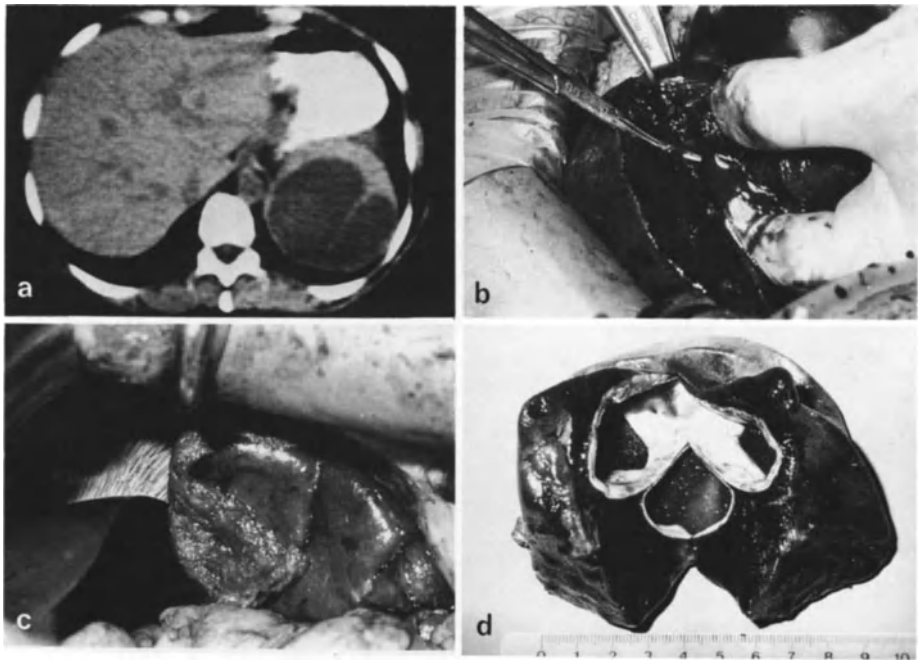

Fig. 6a–d. Resection of a cystic tumor-like lesion of the upper pole. **a** Preoperative CT scan. **b** Sharp dissection along the hypovascular intersegmental plane with clamping of a few vessels near the hilus. **c** Splenic remnant after covering of the raw surface with collagen fleece. **d** Removed part of the spleen

the hilus or major branches of the splenic artery, resection of severely damaged tissue provides the only safe method of (partial) splenic salvage [4, 9, 18, 23, 25]. Since, as a rule, the blood supply of the remnant of the spleen is not compromised, blood filtration and clearance function (15) remain intact. Therefore, this method seems at least as effective as splenic autotransplantation, and has been proven superior in some animal experiments [7, 14]. Within 6–12 months, regeneration of the spleen to a normal volume can often be seen, if a major branch of the hilar vessels is preserved (28, Fig. 8).

Benign tumors, such as cysts or hamartomas, present a rare but clear indication for splenic resection, if confined to one pole [18, 21]. They are removed with a small margin of clearence; however, intraoperative histological investigation seems imperative. Since a compensatory enlargement of the other part has often developed, a sufficient amount of intact splenic tissue can probably always be retained (Fig. 6).

Diagnostic resection in selected patients undergoing staging laparotomy for Hodgkin's disease remains controversial [10, 14]. Selection criteria are not yet clearly defined and, moreover, depend on and vary with the specific therapeutic strategy, and especially the consequences of splenic involvement. A further argument against both total and partial splenectomy could arise with increasing accuracy

of noninvasive investigations such as ultrasound, CT scan, or — in the future — NMR tomography and scintigraphy using radioisotope-labeled monoclonal antibodies. On the other hand, routinely performed total diagnostic splenectomy resulted in a risk of acute septicemia of up to 10%, with a 50% mortality; death was especially frequent in children [1, 5, 10]. Furthermore, in a recent prospective study, mortality related to even selective splenectomy was higher than that caused by the disease itself [27].

Another procedure that is still controversial is subtotal splenic ablation in preparation for renal transplantation [13, 28]. It could become of special value in children with end-stage disease, complicated by hypersplenism, mild neutropenia, and absence of WBC increase after cortisol administration. In such patients, "intolerance" to immunosuppressive drugs, in particular azathioprine, often mandates dose reduction which may result in allograft rejection [13]. On the other hand, after total splenectomy in transplant recipients, long-term immunosuppression was at least in one recent study significantly related to a threefold rate of fatal infections [20].

Even though some of the details regarding the indication in an individual patient remain controversial, the decisive prerequisite from the surgical point of view consists in a safe method to avoid any postoperative bleeding complications.

Technique

Elective splenic resection is facilitated by the segmental blood supply [9, 18, 21], described in detail by GUPTA et al. [11]. After dissection of the hilus and ligation of one of the two (84%) or three (16%) major branches of the splenic artery, capsular color change indicates a line of segmental demarcation (Fig. 7). Sharp resection using electrocautery or a surgical knife is performed exactly along this nearly avascular intersegmental plane. Usually only at the hilar rim do small longitudinally crossing vessels have to be clamped and ligated.

In the case of severely bleeding traumatic injuries, hilar preparation and preliminary vascular ligation would sometimes consume too much time. In these patients, either the trunk of the splenic artery has to be clamped before managing the splenic injury itself or the division of the parenchyma is performed in the manner of atypical liver resection by clamping larger crossing vessels.

After both types of resection, the raw surface is covered with a collagen fleece, prepared as described before, in order to eliminate any oozing.

Adjuvant Measures

To minimize intraoperative blood loss as well as the risk of postoperative rebleeding, the systolic blood pressure during and following extensive splenic repair or elective resection is reduced below 120 mmHg. If necessary, continuous infusion of antihypertensive drugs is maintained for 12–24 h. If the spleen has been completely mobilized, strict bedrest is prescribed for 2 or 3 days, until a sufficient refixation of the splenic remnant may avoid dislocation and consecutive kinking of hilar vessels.

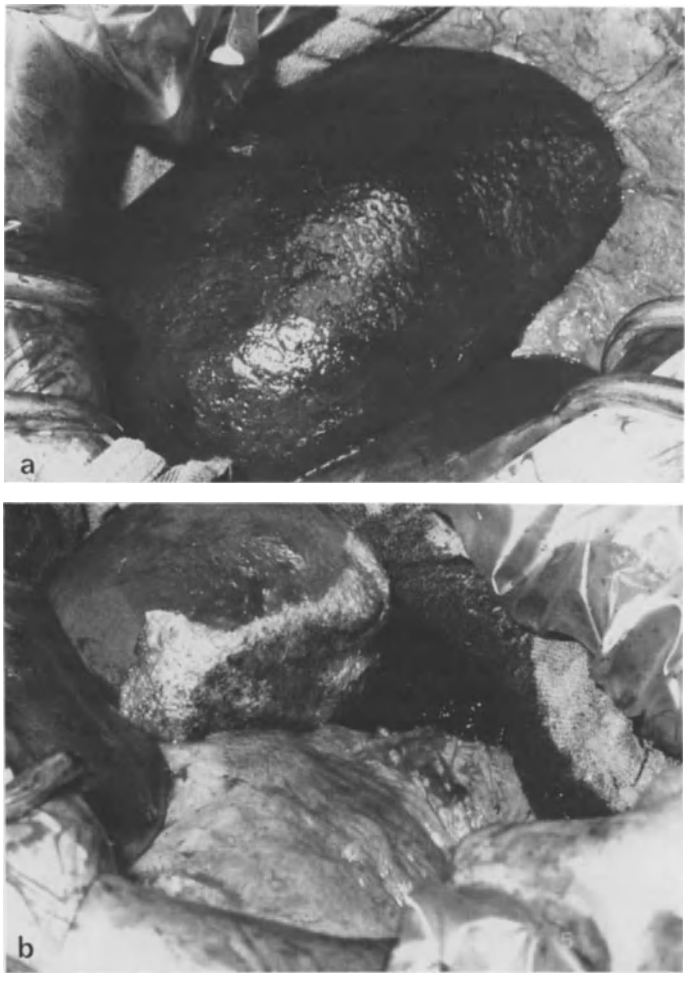

Fig. 7a, b. Hemisplenectomy in Hodgkin's disease. **a** Capsular color change indicating segmental blood supply. **b** Splenic remnant, sealed with fibrin tissue adhesive and collagen fleece

Clinical Experience

Patients

Between 1 November 1979 and 31 December 1984 a total of 188 incidental and traumatic splenic lesions were treated with fibrin tissue adhesive. In an additional nine patients, diagnostic splenic resection was performed during staging laparotomy, and in one female, a cystic tumorlike lesion of the upper pole was resected. The entire sample consisted of 126 men and 72 women aged 2–81 years (Table 1).

Table 1. Results of fibrin tissue adhesive application in traumatic and incidental splenic lesions (1 November 1979 – 31 December 1984)

	Incidental lesion	Traumatic injury	Elective resection
Fibrin tissue adhesive application	114	74	10
Splenectomy during the same operation	6	9	–
Splenectomy by relaparotomy	2	3	–
Definitive salvage	106	62	10
Postoperative death for other reasons	1	6	–

Results

Intraoperative hemostasis was not satisfactory in 9 of 74 traumatic injuries as well as in 6 of 114 incidental lesions, including one minor splenic resection, and the spleen was removed during the same operation. Of the remaining 183 patients with complete intraoperative hemostasis, five developed postoperative hemorrhage, originating from the splenic wound in four cases, which required relaparotomy and splenectomy 1–17 days after the initial procedure. Though the clinical course was protracted in one of these patients, all five did survive.

Of the 178 patients with definite splenic salvage, six died due to severe polytrauma, in particular concomitant head injury, one due to diffuse peritonitis following colon resection, and four had relaparotomy for other reasons. Splenic repair was proven intact in all of these cases at autopsy or surgical reintervention, respectively.

If we restrict our analysis to the 4-year period from 1 January 1981 to 31 December 1984, definite splenic salvage was achieved in 84% of incidental and 55% of traumatic splenic lesions (Table 2).

Despite this high effectivity with regard to splenic salvage, the method resulted in a 3% rate of severe postoperative failures. However, at least half of the 15 primarily unsatisfactory results and three of the five postoperative bleeding complications were related to an inadequate application technique. This is evident since in most of these cases the salvage attempt was performed for minor lesions.

Table 2. Effectivity and complications of splenic repair with fibrin tissue adhesive (1 January 1981 – 31 December 1984)

	Incidental lesions	Traumatic injury
All patients observed	109	110
Fibrin tissue adhesive application	100 = 92%	72 = 65%
Definitive splenic salvage[a]	92	60
Success rate	92%	83%
Overall salvage rate	84%	55%

[a] Seven patients with postoperative death from other causes are included

Table 3. Splenic resection: 29 September 1981 – 31 December 1984

	Minor resection, < 1/3 of the spleen		Major resection, > 1/3 of the spleen	
	n	ineffective	*n*	ineffective
Incidental lesion	4	1	–	–
Traumatic injury	8	–	6	–
Staging laparotomy	2	–	7	–
Tumor resection	–	–	1	–
Total	14	1	14	–

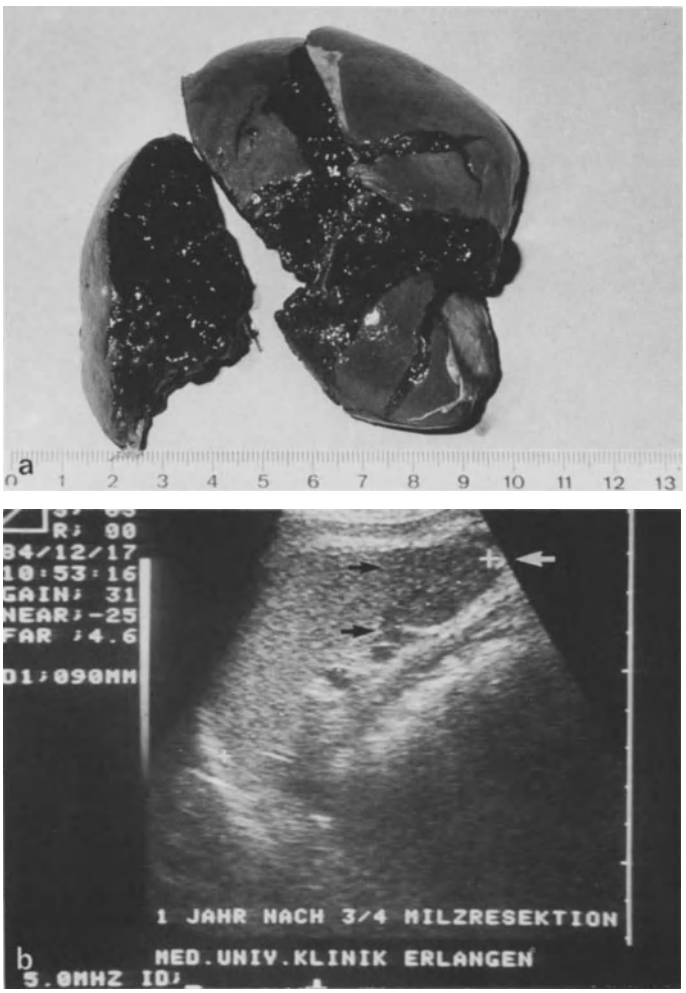

Fig. 8a, b. Splenic regeneration following subtotal resection. **a** Removed part of the spleen due to severe trauma. **b** 12 months postoperatively: regeneration has resulted in normal splenic volume. *Arrows* indicate the part of the spleen preserved at operation

Of the 28 patients undergoing splenic resection, there was only one intraoperative failure (Table 3) and two postoperative deaths related to cerebral injury. At autopsy, intraabdominal hemorrhage could be excluded in both cases.

Four patients were reinvestigated 6–12 months postoperatively by ultrasound, CT scan, or splenic scintigramm using ^{99m}Tc-labeled thermoaltered erythrocytes. Three of them showed a remarkable regeneration of the splenic remnant. This was most pronounced in a 29-year-old woman undergoing subtotal resection for severe but isolated splenic trauma. At laparotomy, only the lower pole of the spleen, measuring $3 \times 2 \times 1.5$ cm could be retained. Twelve months later, regeneration had resulted in a spleen of almost normal volume, measuring 9 cm in length (Fig. 8).

Conclusions

The use of fibrin tissue adhesive represents a feasible and safe method of splenic salvage. If a surgeon is familiar with this technique, particularly with details of the application modalities, the salvage rate should exceed 90% for incidental lesions and may reach 80% for traumatic injuries. Besides the preservation of the entire organ, splenic resection for severe trauma as well as for benign tumors or diagnostic or functional reasons is facilitated. The technique of preliminary vascular ligation at the hilus, sharp dissection along a hypovascular intersegmental plane, ligation of major crossing vessels, and, finally, covering the raw surface with fibrin tissue adhesive and collagen fleece has proven very reliable and safe in our experience; it avoids tissue damage as well as any postoperative oozing. Nevertheless, our 3% complication rate illustrates that this, like any other method of in situ splenic repair, requires a reliable surgical basis, a specific training, and a critical decision on whether

a) the entire organ can be preserved,
b) resection of severely damaged parts of the spleen is necessary, or
c) splenectomy with the option of subsequent reimplantation is preferable.

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The Use of Fibrin Sealant in Organ Preserving and Transplantation Surgery of the Spleen in Children

W. BRANDS

Key words: Splenic repair, direct gluing, sealant infiltration, orthotopic transplantation, graft fixation

Abstract

Treatment of splenic ruptures by means of fibrin sealant as well as optimal hemostasis by sealant infiltration of splenic tissue makes organ-preserving surgery generally possible. Partial splenic resection is facilitated by combined instillation of fibrin sealant along the line of rupture and infiltration of the parenchyma with sealant augmented by the application of a collagen fleece. In hopeless cases, tangential slices should be reimplanted orthotopically by fixation with fibrin sealant, which preserves functional splenic tissue through early vascularization. This can be demonstrated by sequestration-scintigram.

Introduction

Due to the hematological and immunological significance of the spleen, the preservation of the injured spleen is becoming increasingly important, especially among children. The introduction of fibrin sealant provided a breakthrough in clinical treatment. Numerous recent publications concerning the use of fibrin sealant on the spleen attest to this fact [2, 9, 10, 11].

Experiences and Results

We have previously pointed out the distinct advantages of the use of fibrin sealant with splenic injuries, whereby, apart from the excellent hemostatic effect, additional possible uses exist in splenic surgery [3, 4, 5]:

1. Direct sealing of ruptures
2. Sealing of capsule lesions and resection surfaces
   (e.g., with collagen fleece)
3. Infiltration of parenchyma
   (e.g., for more effective hemostasis)
4. "Wrapping" method with collagen fleece
   (e.g., in the case of total transverse ruptures in combination with fibrin sealant to decrease mechanical tension on the sealed tissue)

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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5. Graft fixation
6. (Temporary embolization
   tumors, metastases, hypersplenism)

We augmented the generally recognized instillation of fibrin sealant along the ruptured surface followed by 5 min compression with additional infiltration of fibrin sealant in the tissue adjacent to the rupture, which results in a considerably more effective hemostasis. There exists no danger of venous washout of the fibrin because, by virtue of its internal structure (e.g., the sinus endothelia), the spleen can actively eliminate coagulation products like fibrin and fibrin monomeres from the bloodstream or break them down [1].

We were able to confirm this through attempts to produce fibrin embolization in the spleens of hares using immune histochemical methods to trace the applied fibrin. No damage to the spleenic tissue resulting from temporary ischemia could be observed [6]. In the clinic we were only able to stop profuse bleeding from extensive splenic ruptures and thereby preserve the organ by infiltrating the parenchyma with sealant (see Fig. 1).

Following complete ruptures we reconstructed the spleen and, in order to reduce mechanical tension on the sealed particles, we wrapped a large collagen fleece around the sealed spleen after applying additional fibrin sealant instead of using the conventional but traumatic single sutures. This provides a more certain guarantee of unison of the splenic particles (see Fig. 2).

Preserving part of the spleen following extensive rupture is rarely possible but should be attempted, e.g., in the case of splenic cysts. The precise preparation and ligation of the appropriate blood vessels makes it possible to resect along the demarkation line without considerable loss of blood. Bleeding persisting along the resection surface can be controlled by infiltration of fibrin sealant. The wound is sealed by collagen fleece held in place by fibrin sealant.

Postoperativley the remaining splenic tissue is tested for function, size and localization by means of a sequestration scintigram using Tc 99^{m} labeled, heat-altered erythrocytes.

If the vessels are torn in the splenic hilus, no time should be wasted in attempting to preserve the organ, as they are generally doomed to failure. Better results are sure to be achieved by splenic autograft than by ligation of the splenic artery, which is occasionally attempted in hopeless cases but usually leads to secondary splenic necrosis [8].

Reimplantion of homogenized splenic tissue or of splenic particles in the greater omentum is not advisable among children because the fragility of the omentum majus at this age does not provide adequate fixation. The implanted tissue could torquate around the pendulous omentum chord [12]. The vulnerable location on the greater omentum is also disadvantageous.

We choose to cut tangential slices from intact splenic tissue and attach them orthotopically with fibrin sealant, i.e., under the relatively protected left diaphragm on the wound surface of the transsected gastrolienal ligament (see Fig. 3). This close spatial relationship between graft and graft site promotes unhindered and rapid capillary injection. The proliferating capillaries with their active endothelial cells quickly reach the graft and its internal vascular structures to form anastomoses

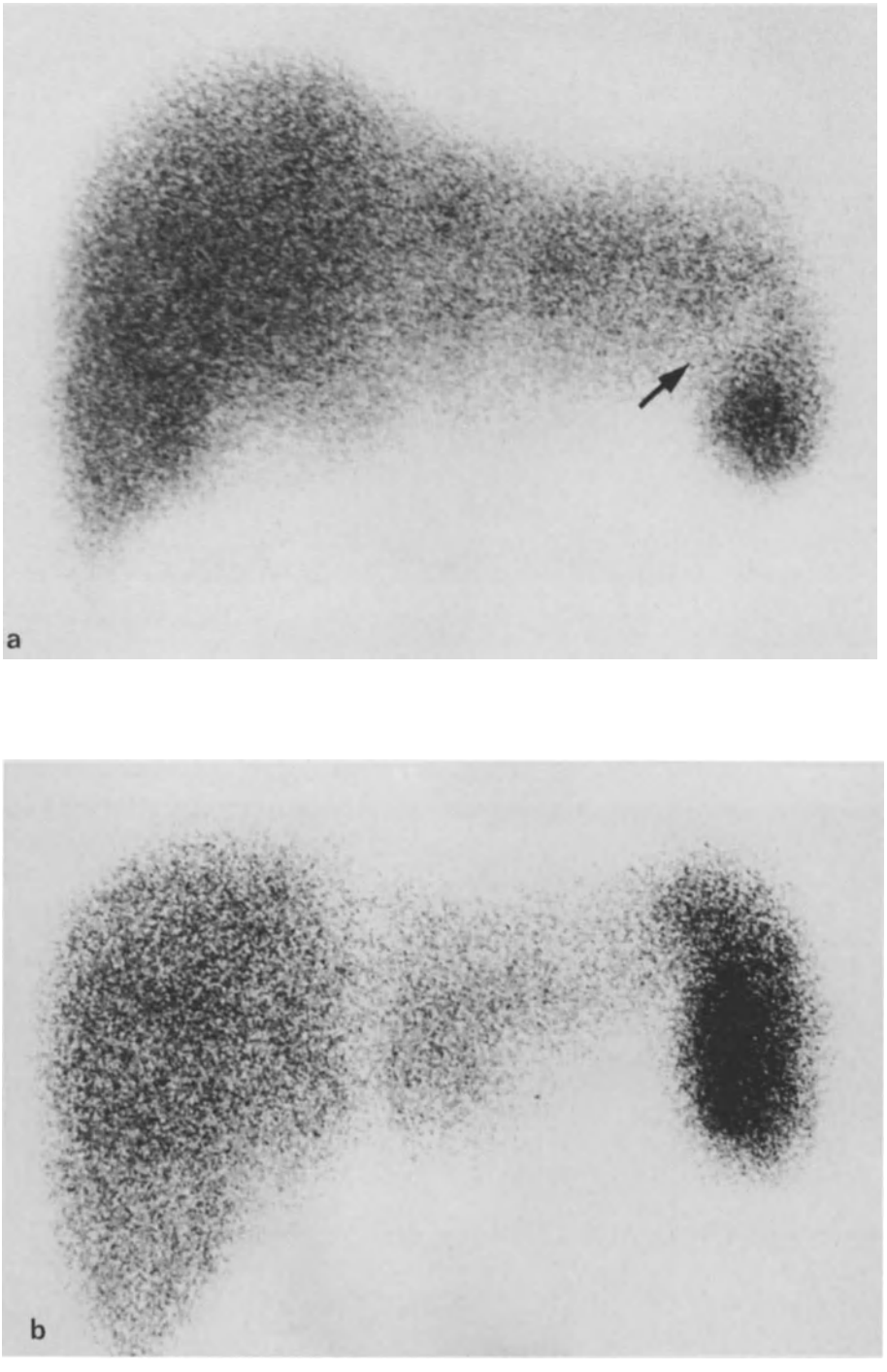


Fig. 1a, b. Scintigram of the spleen in posterior projection following surgical treatment of an extensive rupture with fibrin sealant infiltration (arrow). **a** 2 days postoperatively; **b** 2 weeks postoperatively

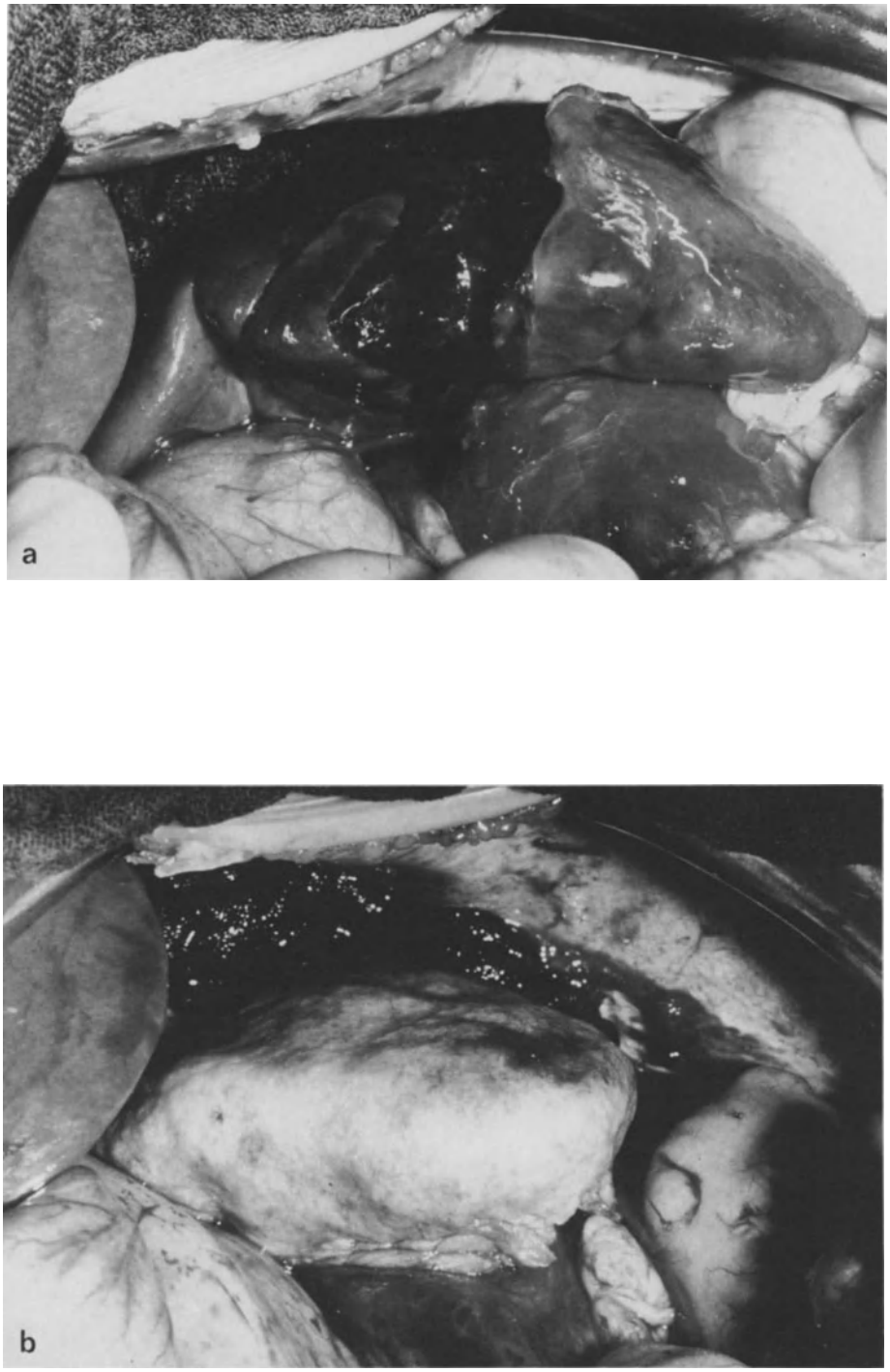


Fig. 2. a Complete transverse ruptures of the spleen. **b** The same spleen following "wrapping" with collagen fleece and fixation with fibrin sealant

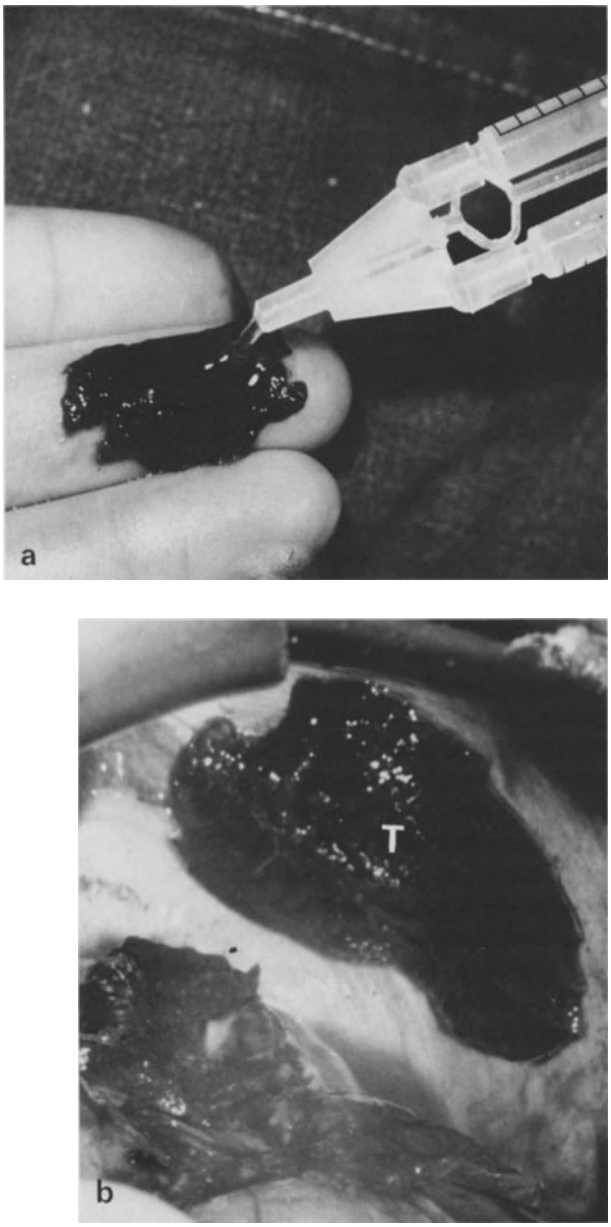
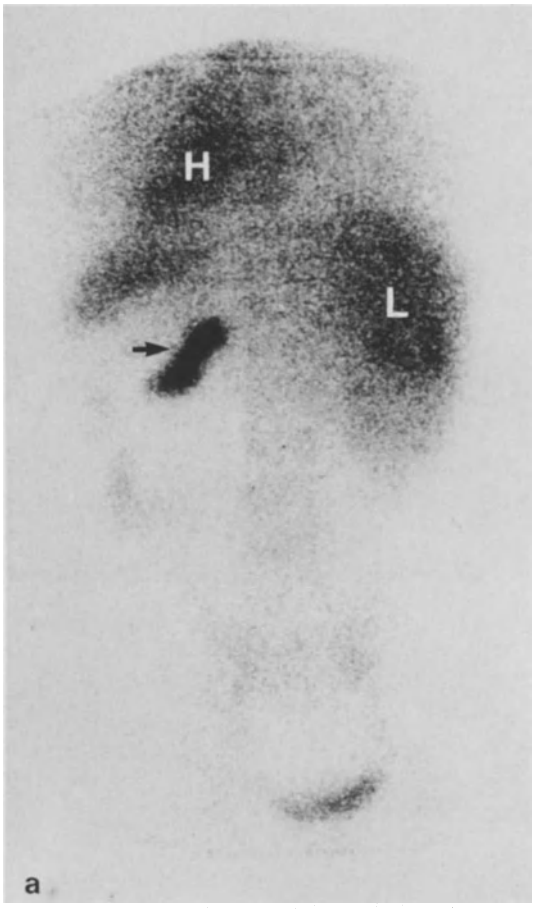


Fig. 3. a Tangential splenic slice covered with fibrin sealant prior to reimplantation. **b** Splenic graft fixed beneath the left diaphragm with fibrin sealant



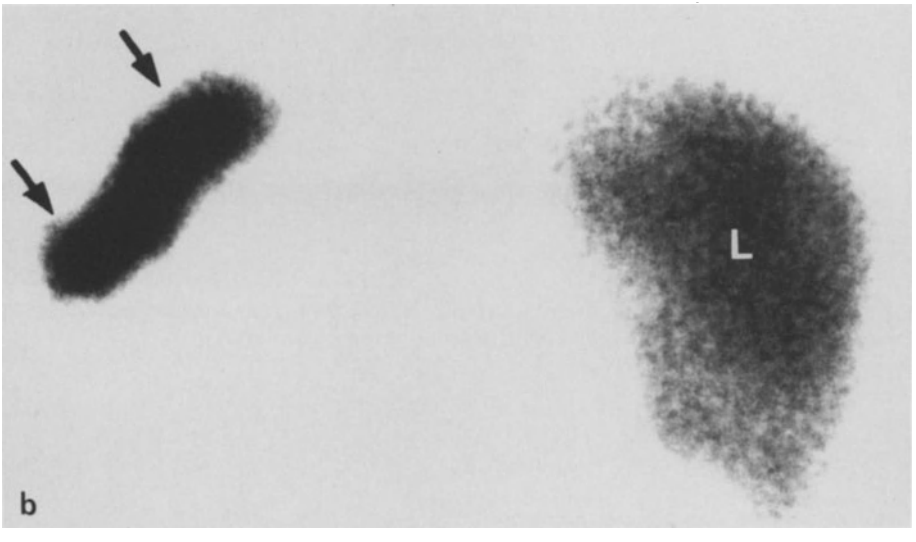


Fig. 4a, b. Sequestration scintigram in posterior projection following splenic transplantation. *H*, heart/blood pool; *L*, liver; ↑, splenic graft. After 2 weeks **a** and after 1 year **b**

through "kissing contact" as in the case of spongiosa transplants and thereby provide recanalization [7]. Functional, i.e., sequestered splenic tissue, can be identified 2 weeks following the operation (see Fig. 4a). After 6 months to 1 year the grafts will have increased in functional tissue mass according to the degree of patient exposition to certain pathogens (see Fig. 4b). Comparison between the filtration efficiency of the grafts (i.e., elimination of spherocytes per unit of time) and a normal spleen after a ½ year shows no significant difference. Clinical parameters, e.g., thrombocyte count and Howell-Jolly bodies, as well as immune parameters are normalized after 1 year.

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Experimental Splenic Injury Treated with Fibrin Sealant

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Key words: Spleen, splenic injury, splenic trauma, biological glue, fibrin sealant, splenic salvage

Abstract

Six adult mongrel dogs (two of them under heparin treatment) and 14 mice were submitted to midline laparotomy and their spleens were transversally cut into two halves with a scalpel, and then glued with fibrin sealant. After reoperation at intervals ranging from 10 min to 3 weeks, the animals were splenectomized and their spleens submitted to macroscopic and microscopic examination (by light and electron microscopy). On macroscopic examination, the dogs and all but two mice had their spleen integer, with some epiploic adhesion at the sealed site, and there was no evidence of recurrent bleeding. One mouse had some blood in the abdominal cavity (but was alive and well) and another had splenic disruption (without bleeding, proving the efficacy of the hemostasis). Histological examination has demonstrated progressive absorption of the adhesive with little inflammatory reaction, healing of the defect, and capsular scarring. On electron microscopy some details of fibrin phagocytosis were observed. The dogs on heparin had their spleen sealed without problems.

Introduction

Whenever possible, in the presence of a splenic trauma, preservation of the splenic function must be tried, on account of the small but definitive risk of overwhelming postsplenectomy infection (OPSI) [1, 2, 3].

Nonoperative treatment is possible in some selected cases, provided that a good emergency care unit is available. Even after laparotomy, the presence of a minor tear whose bleeding has stopped can be subjected to conservative management. However, if a bleeding spleen is to be preserved, one of the techniques of splenic salvage must generally be performed. These are splenorrhaphy, partial resection, splenic artery ligation or embolization [2, 3, 4, 5], splenic capping [6], splenectomy with autotransplantation [3, 7] and topical applications: gelatin sponge, cyanoacrylate adhesive, oxidized cellulose, thrombin, microfibrillar collagen [2, 8], laser and infrared irradiation [8], and, recently, fibrin adhesive [8, 9].

The outstanding reliability of fibrin adhesive to repair minor splenic tears [9] has led some authors to investigate the histological aspects of this kind of organ closure [8]. In this work, we have tried to assess the reliability of splenic sealing after major

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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splenic trauma (complete division of the spleen), in a reasonable number of experimental animals (6 dogs and 14 mice), to further the histological studies of the sealing area (with the use of both electronic microscopy and fibrin-specific techniques in optical microscopy, whenever necessary), and to confirm that the adhesive action is not altered by the presence of anticoagulation (heparin).

Material and Methods

DOGS. Six adult mongrel dogs weighing between 15 and 25 kg were anesthetized with sodium pentobarbital, and allowed to breath spontaneously. A midline laparotomy was performed, the spleen was mobilized and moved into the wound, and then it was sharply cut into two halves with a scalpel. Fibrin adhesive was then applied, and the two halves were pressed against each other for about 10 min. Some oozing points of bad coaptation generally needed some additional adhesive. After achieving hemostasis, the abdomen was closed. The animals were reexplored at intervals ranging from 10 min to 2 weeks, and their spleens were subjected to gross and microscopic examination.

HEPARIN. Two of the dogs were given a subcutaneous injection of calcium heparin (Calciparine, Laboratoires Choay, Paris) 250 μ/kg, about 1 h before the operation.

MICE. Sixteen white laboratory mice weighing about 20 g were anesthetized with ether and then subjected to an operation similar to that for the dogs. Reexploration was undertaken at intervals from 10 min to 3 weeks, and the spleens were subjected to macroscopic and microscopic examination. To assess the necessity of the adhesive, another group of 14 mice was operated on, but without sealing their spleens: no animal of this later group has survived.

FIBRIN ADHESIVE. The fibrin adhesive (Tissucol) consists of a two-component system of fibrinogen and thrombin. To obtain the first component, a freeze-dried mixture of human fibrinogen, fibronectin, factor XIII and plasminogen is dissolved in a solution of an antifibrinolytic agent (apronitin). The second component is obtained by the dissolution of freeze-dried bovine thrombin in a calcium chloride solution. After reheating both components at 37°C, they can be applied with a disposable double-barreled syringe (supplied with the kit).

OPTICAL MICROSCOPY. (Figs. 1–3) Histological studies were performed with H & E (hematoxylin-eosin) preparations to search for hematomas or abscesses, to analyze the inflammatory reaction, and to study the general aspects of the spleens and their capsules. Fibrin deposits were emphasized by Mallory-stained preparations.

ELECTRONIC MICROSCOPY (Fig. 4). This was performed with standard osmium tetroxide staining preparations, looking for the mechanisms of fibrin reabsorption.

Results

IMMEDIATE. Fibrin adhesive alone was enough to control bleeding in both mice and dogs. The application of a single layer was enough in the mice group, but dogs generally needed some additional applications at superficial bleeding points.

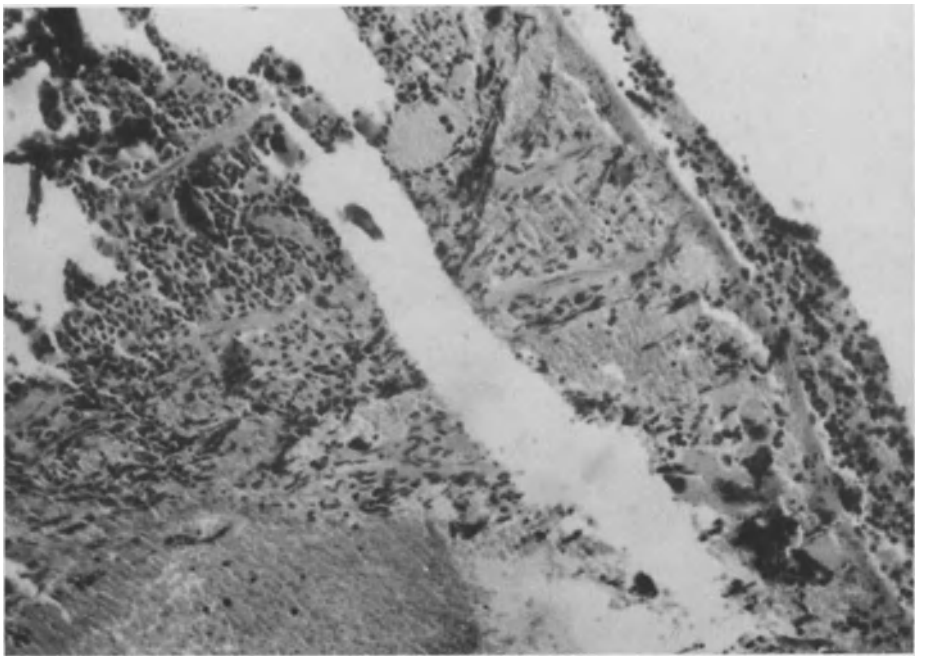

Fig. 1. Ten minutes after sealing, there were fibrin and hemorrhagic deposits. H & E, × 50

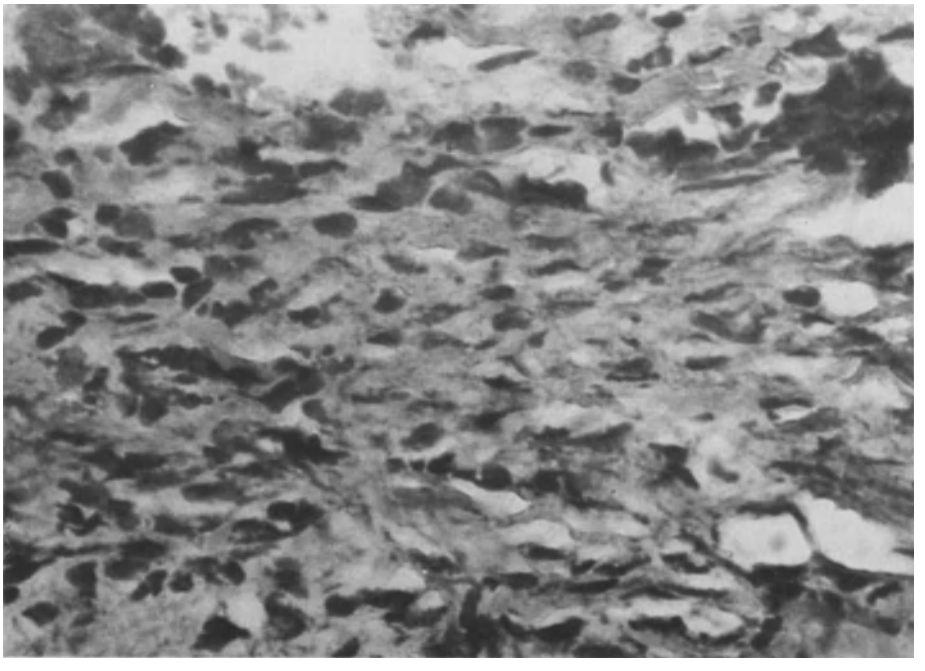

Fig. 2. After 1 week, aspect of progressive organization. Mallory, × 200

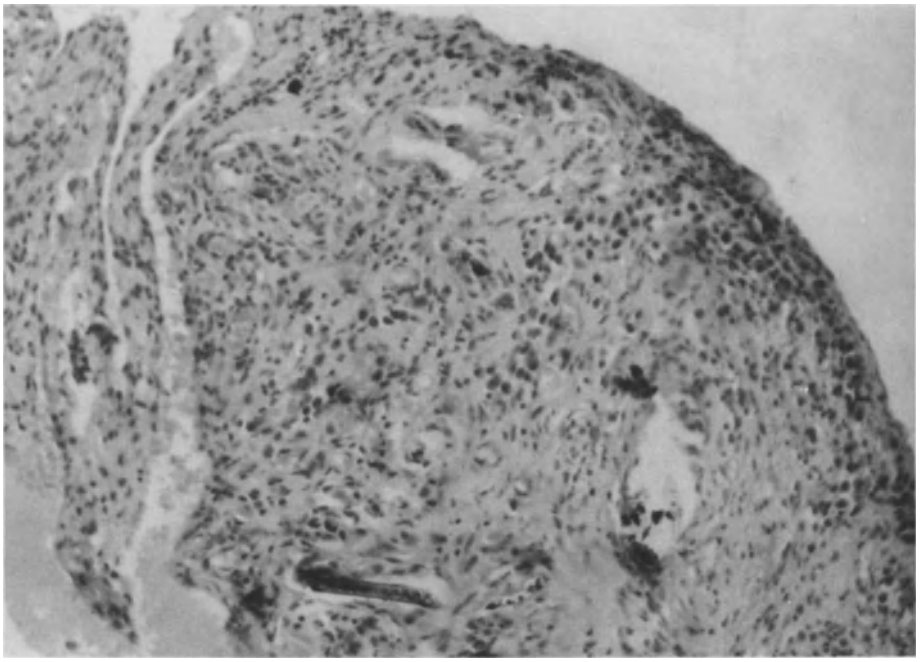

Fig. 3. In the 2nd week, organization and scarring. H & E, × 50

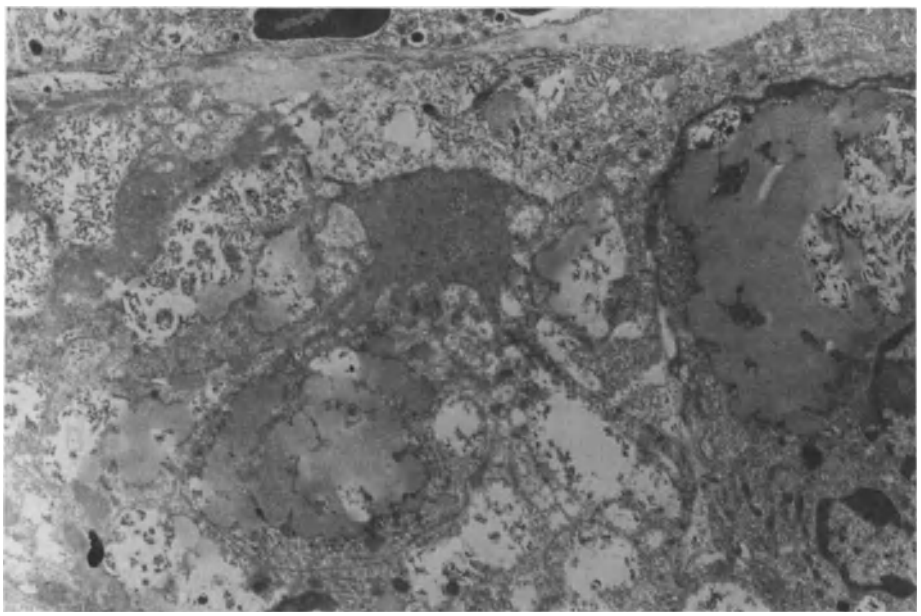

Fig. 4. Fibrin phagocytosis by macrophages, EM, 5000 × 2.5

Table 1. Group of mice

n	Reexploration	Comments
1	10 min	
2	10 min	
3	1 day	
4	1 day	Blood in the abdominal cavity
5	2 days	
6	2 days	Pregnant. Normal delivery (see text)
7	8 days	
8	8 days	Disjunction of the spleen, without bleeding
9	10 days	
10	10 days	
11	15 days	
12	15 days	
13	21 days	
14	21 days	

Table 2. Group of dogs

n	Reexploration	Heparin	Omental adhesions	Blood in the abdomen at reexploration
1	10 min	No	Yes	No
2	2 days	No	Yes	No
3	7 days	No	Yes	No
4	15 days	No	Yes	No
5	7 days	Yes	No	Small quantity
6	15 days	Yes	No	Small quantity

Although bleeding profusely through the abdominal wound, dogs on heparin had their spleen sealed fairly well in a slightly greater period. Histological examination at 10 min demonstrated a massive deposition of fibrin, limiting hemorrhagic areas. The splenic capsule was interrupted at the level of the wound, and general inflammation was minimal.

LATE. All animals survived (see Tables 1, 2). One mouse, reexplored at the 24th h, had a moderate quantity of blood in the abdominal cavity, but was alive and well. One female, pregnant when operated on and reexplored on the 2nd day, had a normal delivery in the meantime. One mouse reexplored on the 8th day had complete disjunction of the two splenic halves, but there were no signs of bleeding, assessing the value of the hemostasis afforded by the adhesive. All the other mice had their spleen glued, without bleeding. There were omental adhesions to the spleen in almost all mice.

The dogs that had not been submitted to heparin therapy had their spleen well sealed, with omental adhesions at the level of the wound. There were no signs of bleeding. The dogs on heparin had a slight quantity of blood in the abdomen, and they had no omental adhesions.

On microscopy, granulation tissue substituted for fibrin clot after the 1st week. The capsule has demonstrated progressive signs of healing, and progressive scarring

could be seen through the area of the wound. Fibrin phagocytosis could be well documented at electron microscopy.

Discussion

Topical applications have never been popular as methods of splenic salvage, because the reliability of hemostasis was far from satisfactory. To prove the efficacy of this new adhesive, we sharply cut the spleens into two halves, and the results were excellent. The rationale for the use of the fibrin sealant is that fibrin is the most physiological adhesive and, in the presence of thrombin, fibrinogen gives fibrin, even in the presence of a hemostatic deficit [8]. Indeed, we had no problems in sealing our dogs on heparin, although they were bleeding profusely through the wall wound. On reexploration, there was a complete splenic coaptation, with no more intrasplenic hematomas than in the other dogs. We can suppose that the small quantity of blood found in their abdominal cavity originated in the abdominal wall wound, because the splenic wound has no signs of having bled, and we were surprised by not having found omental adhesions to the splenic wound.

Until now, the most popular methods of splenic salvage have been partial splenectomy [2, 4, 11] and splenic suture [2, 10], both aided by application of topical agents [2, 10, 11] and/or mattress sutures or splenic capping [4, 6]. Topical applications available (gelatin, sponge, thrombin, and microfibrillar collagen being the most used), have never become popular alone as a method of splenic salvage, because they were reliable only in small splenic tears [8, 9].

With this work, we have tried to prove that this new fibrin adhesive can be used safely to treat major splenic injuries.

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Fibrin Sealing of Parenchymal Organs in Pediatric Surgery

H. ROTH and R. DAUM

Key words: Fibrin sealing, parenchymal organs, pediatric surgery

Abstract

Surgical operations on parenchymal organs in infancy and childhood cause technical problems because of the vulnerability of the tissue. Laceration of puncture channels and section of parenchymal tissue are the most feared complications of the surgical suture. Fibrin gluing as a supplement or even as an alternative to conventional suture techniques enables the pediatric surgeon to repair the organ or perform a tissue-saving operation.

Special attention is given in the paper to the preservation of splenic tissue in cases of traumatic rupture. According to the pattern of injury and the involvement of the splenic hilus a staging from I to IV is suggested. Each of the stages includes therapeutic consequences, from monitoring to subtotal resection. The mostly preferred adjuvant remedy in pediatric splenic surgery is fibrin glueing because of its safety in bleeding control and optimal preservation of orthotopic splenic tissue. Splenectomy and omental autotransplantation of splenic particles cannot be accepted as a standard therapeutic method in pediatric surgery.

Introduction

Surgical interventions on parenchymatous organs in children are always aimed at organ preservation, repair, or tissue-saving resection, except in malignant tumors. Due to the vulnerability of the infantile tissue, surgical sutures are not always sufficient, even if atraumatic suture material is used. Laceration of the puncture channels and intersection of the parenchyma sometimes cannot be avoided, even if the knots are carefully dosed.

During recent years surgical aids such as coagulative techniques with infrared or laser radiation have been applied in hemostatic parenchymal sealing. More or less severe tissue necroses have to be taken into account [5]. This side effect, unwanted in pediatric surgery, does not correspond to the requirement for optimal parenchymal preservation. The sole use of collagen fleece as a carrier for the blood coagulum does not guarantee safe hemostasis. Tissue sealants on the basis of cyanoacrylate induce foreign body reactions. The incomplete physiological degradation may induce unpredictable late sequelae [9]. Using homologous absorbable fibrin sealant, especially in combination with collagen fleece, the pediatric surgeon now has a

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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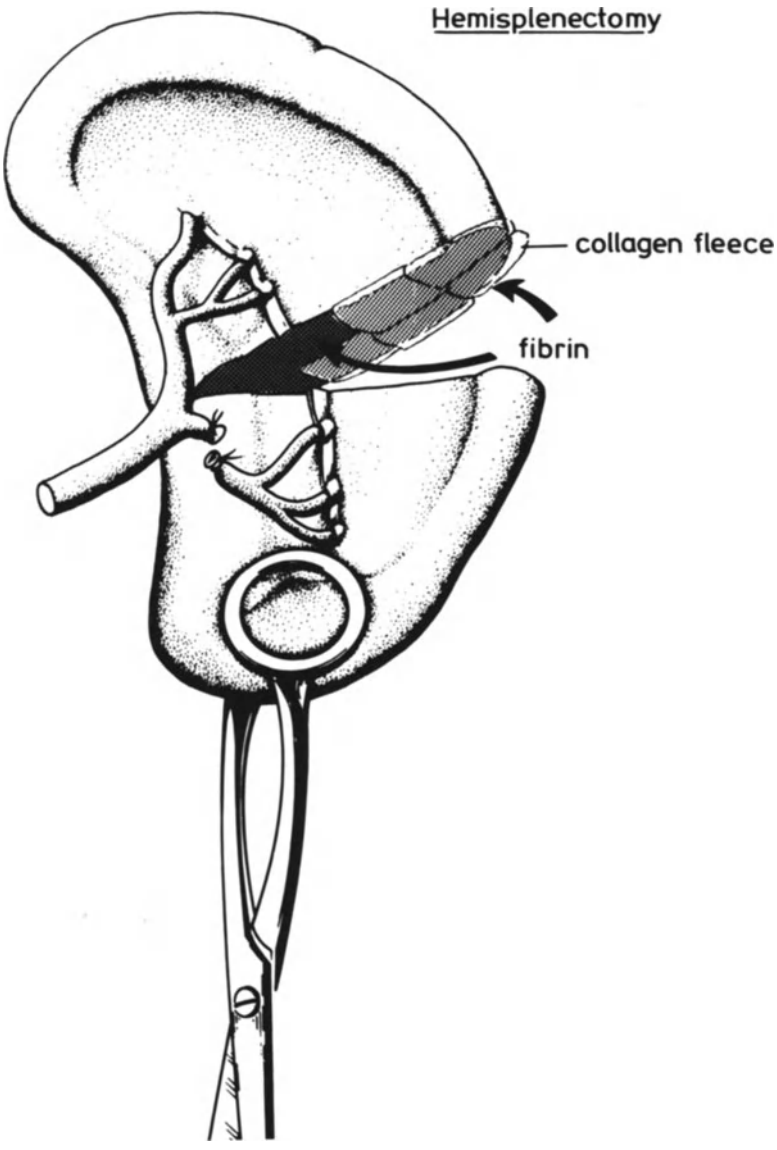

Fig. 1. Paving-stone-like fibrin-fixed patches of collagen fleece

in toto by repair or partially by tissue-saving resection, the fibrin adhesive system has to be preferred to other surgical aids such as infrared or laser radiation, as there is no additional loss of tissue by coagulation necroses. We recommend staging of the splenic rupture from I–IV, given the surgical consequences in childhood.

Extent of involvement of the splenic hilus is decisive for the surgical procedure (Fig. 3). The predominance of transverse ruptures is due to the horizontal segmental situation of the vessels. Rare longitudinal ruptures are mainly outside of the hilus area.

technique at his disposal that facilitates organ preservation and is a valuable complement or even alternative to sutures, partially avoiding additional loss of tissue [1, 4, 8, 11, 12, 14].

Indications and Technical Procedure

Since 1981 fibrin sealant has been used at the Heidelberg Department for Pediatric Surgery in operations on parenchymatous organs such as lung, liver, spleen, pancreas, and kidney. Principal indications are diffuse parenchymatous bleeding and parenchymal fistula that is not recognizable macroscopically.

The rapid clotting of the sealant that is desired in all cases is obtained by adding a high thrombin concentration of 500 INH-U per milliliter. A dose of 3000 KIU/ml of aprotinin is added to the calcium-thrombin solution as fibrinolysis inhibitor.

Larger vessels and parenchymal fistulae are primarily ligated before application of the fibrin sealant. The application of porous collagen fleece has proved a valuable replacement of the capsule. Handling of the fibrin sealant is thus considerably simplified and the sealant cannot be washed away in the event of diffuse bleeding of the tissue surface. The two components are simultaneously dripped onto the collagen fleece, which is immediately pressed on the parenchyma for 3–5 min.

As the collagen fleece becomes humid it may be modeled and sticks tightly to the tissue on top of the fibrin. The single collagen fleece patches should not be too large and should be applied like paving stones to prevent complete washing away in the event of a seeping hemorrhage. Special attention has to be paid to the sealing of the capsule edges to assure safe sealing. (Fig. 1). Tissue resection should be performed step by step to avoid large blood loss. In deep parenchymal ruptures or defects after resection the sealant is applied on the tissue fissure and the parenchymal surfaces are immediately pressed against each other (Fig. 2). It is very important to adhere to the time indicated for completion of the coagulation process, even if this strains the patience of the surgeon. Remaining bleedings may be stopped by another injection of both sealing components and another pressing of the tissue surfaces. The capsule defect is covered with collagen fleece in the way described above.

Although the collagen fleece has proved an excellent carrier for the fibrin sealant and an excellent capsule replacement, it is not recommended in interventions on the infantile lung parenchyma as it prevents an adequate dilation of the lung.

Sealing of the surgical suture with fibrin sealant after lobectomy, wedge resection, or tumor or cyst enucleation has proved particularly helpful in surgery of premature or newborn infants. Extremely small parenchymal fistulas, especially in the area of the puncture channels, can be closed safely with this method. It may even be possible to seal without sutures. According to our experience sufficient tissue sealing in premature infants may be obtained by adaptation of the parenchymal surfaces by fibrin sealant.

Surgical Peculiarities in Traumatic Rupture of the Spleen

Rupture of the spleen in childhood is no longer an indication for splenectomy – except if there is vital danger to the polytraumatized patient. For organ preservation

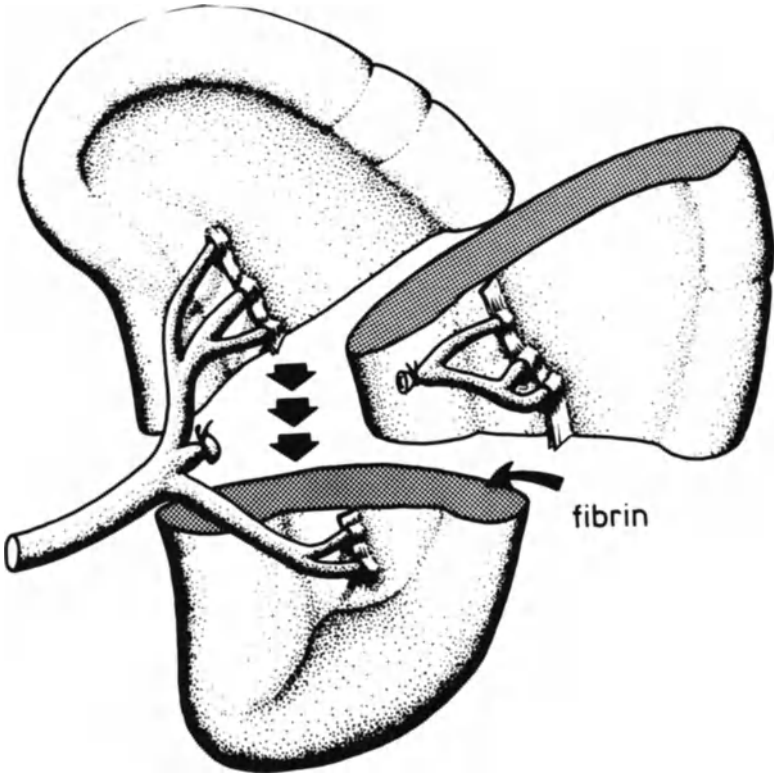

Fig. 2. Parenchymal sealing after resection of a central segment

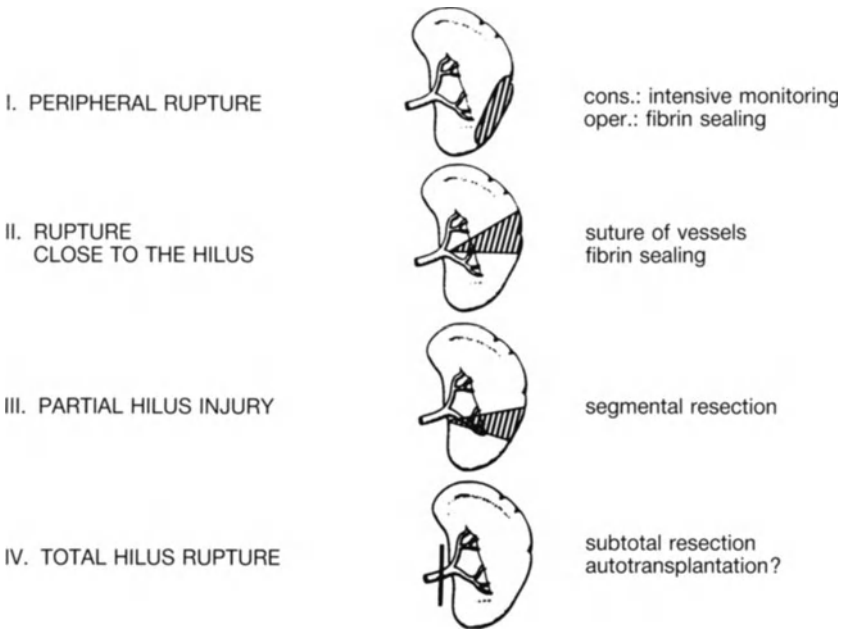

Fig. 3. Staging of splenic injury; surgical consequences in childhood

Stage I

By peripheral ruptures we mean subcapsular hematomas and small lacerations of the parenchyma outside of the hilus. We have two possible therapies at our disposal:
1. Conservative therapy with supervision in an intensive care unit
2. Operative treatment by hemostatic parenchyma sealing

Subcapsular hematomas are a classic example for conservative monitoring. In general they can be well recognized by ultrasonography. Peripheral ruptures with considerable perisplenic bleeding should in no case be supervised outside of a surgical unit if they are to be treated by conservative therapy. It is exclusively the decision of the surgeon whether he wants to perform a surgical intervention.

Stage II

Ruptures close to the hilus are to be treated primarily by surgery. To judge the extension of the injury it is necessary to mobilize the spleen from its ligamentous anchorage with ventral luxation. Deep parenchymal tears are managed with fibrin sealant and collagen fleece after removal of the clot and deep loop suturing of the vessel.

Stage III

In partial hilus injury segment resection is the therapy of choice. The extension of the resection depends on the involvement of the hilar vessel. Temporary hemostasis is usually possible by manual compression of the hilar vessels. Resection should not be performed by means of an electric scalpel to avoid coagulation necroses. Macroscopically detectable open vascular lumina are closed by interlocking deep loop sutures and the parenchymal surface is sealed with fibrin sealant and collagen fleece as capsula replacement.

Stage IV

There are two possible ways of managing a completely torn off hilus by surgery:
1. Subtotal resection
2. Autotransplantation of particles of splenic tissue into a rolled up omentum majus pocket, a method strongly recommended by several authors during recent years.

Orthotopic preservation of the remaining spleen has absolute priority from the pediatric surgeon's point of view. Observations of elective splenectomies have shown, that accessory vessels at the lower pole as well as a presence of the superior polar artery maintain vascularisation of the remaining parenchyma. According to reports in the literature, this fact may permit revascularization of the spleen with reestablishment of some of its functions after ligation or embolization of the splenic

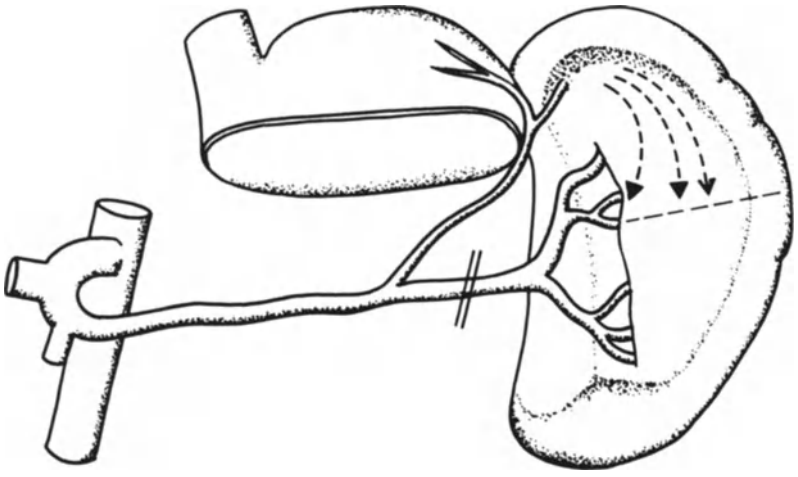

Fig. 4. Operative consequences in case of total hilus rupture

artery [6, 19]. Our own angiographic examinations of resected samples with isolated perfusion of the superior polar artery showed that subtotal vascularisation of the upper pole takes place by a few intersegmental vessels with increased pressure of primary exposure of a small upper segment. In the case of a torn off hilus vessel the preservation of the upper pole may be aimed at if the superior polar artery remained intact (Fig. 4). In this case regeneration of the parenchyma is to be expected, although during the operation a part of the upper pole seems to be less circulated. This assumption has been clinically confirmed by scintigraphic control of the development.

Splenic autotransplantation should be restricted to the few cases where preservation of the spleen at its physiological site is not successful [16]. In childhood splenic autotransplantation should not be performed into the entire omentum majus but into a left pedicled omentum flap that is rolled up and transferred to the splenic bed (Fig. 5). The fixation of the splenic particles into the omentum may be achieved by application of the fibrin spraying technique [13].

Discussion

Surgical interventions on parenchymatous organs carry less risk thanks to the application of the fibrin sealing method in addition to conventional operation procedures. Diffuse bleeding and parenchymal fistulas, especially feared in children, may be prevented to a large extent.

Fibrin sealing has brought about considerable progress in splenic surgery. Orthodoxly, splenectomy was the therapy of choice until 1980 in benign tumors or splenic injuries. Surgical procedures were determined by the apparent dispensability of the spleen and insufficient adequate surgical techniques. Thanks to increasing

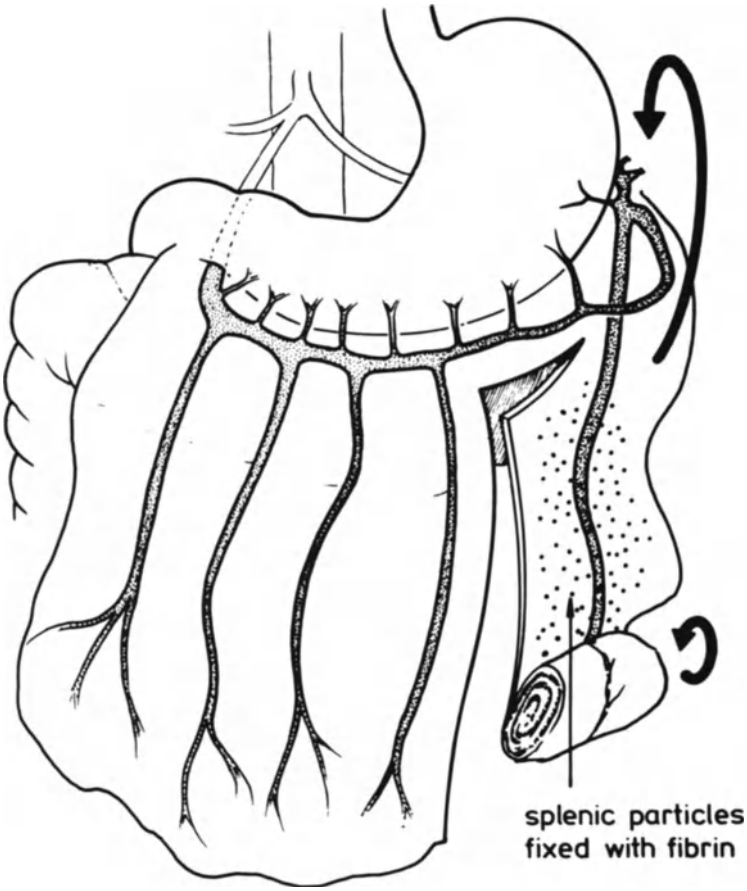

Fig. 5. Modified autotransplantation of splenic particles fixed with fibrin

knowledge on the immunological importance of the spleen, especially in childhood, a change has taken place in favour of preserving the functioning of the splenic tissue, not even excluding splenic autotransplantation.

Interventions to preserve the spleen after traumatic rupture in childhood are to be aimed at, given the decreased risk of sepsis or infection, but should not be forced if the patient's life is in danger [7, 17]. Staging according to involvement of the hilus is helpful in the choice of the surgical procedure.

Surgical methods aimed at organ preservation depend on the individual experience of the surgeon and the aids at his disposal. The fibrin adhesive system combined with collagen fleece is an optimal response to the request for maximum organ preservation in childhood. Theoretical basic knowledge of the sealing method [18] as well as practical possibilities of application are a prerequisite. Only repeated experience with this technique shows its advantage for surgical interventions not only in the spleen but in all parenchymatous organs.

Orthotopic preservation of the remaining spleen has absolute priority for the pediatric surgeon compared with the autotransplantation of particles of splenic tissue. The indiscriminate application of this method of application is a potential danger. The possibility of ectopic splenic affection [3] and ileus complications [2] as late sequelae in the growing patient have not yet received sufficient attention in the discussion on the validity of replantation. Perforating accompanying symptoms of the intestines are a contraindication for autotransplantation, but not for repair or partial resection of the spleen.

Replantation as such includes the risk of pseudo abscess formation, if the single particles conglomerate and do not find vascular connection [13]. A bacterial superinfection may have unpredictable consequences.

In conclusion it is worth stressing again: autotransplantation in splenic rupture in childhood should not be a matter of routine, as orthotopic splenic preservation should have priority.

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Use of Fibrin Sealant in Paediatric Surgery

H. ALESSANDRINI, A. PINESCHI, and A. GIANNOTTA

Key words: Fibrin glue, paediatric surgery

Abstract

Three years of experience in the application of fibrin sealant in paediatric surgery are summarized. Its adhesive, haemostatic and tissue-regenerating properties were clinically tested in 242 cases (including all branches of paediatric surgery such as maxillofacial, thoracic and abdominal surgery and urology) with excellent results. The ratio of the components of the sealant (aprotinin, lyophilized fibrin, thrombin) was chosen before any operation, according to the surgical target. We point out three main possibilities of use of fibrin glue: suture sealing permits a reduction of the number of stitches; haemostasis coupled to adhesion enables large amounts of parenchyma to be saved; and obliteration of blank spaces means direct healing and protection against sepsis.

Introduction

The acts of haemostasis and suture have been indissolubly bound to surgery from the beginnings of the art. Technology could only modify these steps in quality, substituting organic materials with new synthetic ones: intertwined polymers, monofibers, special steels. On the contrary fibrin glue, the first sealant and truly biological haemostatic, represents a radical contribution to the development of surgery: its rapid spread is, today, a matter of fact.

For the first time in years a new material not only makes a surgical procedure safer or easier but may considerably change the performance of the operation itself.

In the past 3 years we have used fibrin glue in selected cases, testing its sealing and haemostatic properties. The outcomes of our casuistry led us to extend its application to the many fields of paediatric surgery, so that it is, today, habitually used in our center for almost all major surgical procedures.

Patients and Methods

From November 1982 to June 1985 we used fibrin glue in 242 cases, distributed as follows:

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Maxillofacial surgery	26 cases
Thoracic surgery	31 cases
Abdominal surgery	71 cases
Hepatobiliary surgery	16 cases
Urology	67 cases
Miscellaneous surgery	31 cases

The fibrin glue was applied for haemostatic and/or adhesive purposes to anastomoses, sutures, cavities and parenchymal surfaces.

According to the circumstances, we made use of different dilution rates: lyophylized fibrin glue (Tissucol, Immuno AG, Vienna) was mixed at 37°C in a thermostatic vibrator set (Fibrinotherm S, Immuno AG, Vienna) with the aprotinin solution. Each time the aprotinin rate was chosen in consideration of the characteristics of the tissues and of the operative target.

Since a higher aprotinin concentration determines a slower reabsorption of the fibrin clot and vice versa, aprotinin rates of up to 3000 KIU/ml were used when the sealant was required to last beyond 10 days: e.g. in surgery of the lung there is a rapid reabsorption of fibrin and large surfaces of moving parenchyma have to be stuck together. Aprotinin rates ranging from 100 to 500 KIU/ml were more frequently demonstrated to be useful in the anastomoses of the intestinal tracts or wherever a long persistence of the fibrin clot was undesirable. If a rapid hemostatic action was required (e.g. on large bleeding surfaces) the 500-U thrombin vial was used to activate the aprotinin/Tissucol complex. On the contrary, a deeper adhesive action was achieved (e.g. on high-risk anastomoses) when using the 4-U thrombin vial.

Following these principles the Tissucol kit was activated immediately before surgery, in sterile conditions, and applied by means of the Duploject double-syringe needle.

Results

Owing to the great variety of paediatric surgical operations in which the fibrin glue was tested, it is better to summarize our results by topographical criteria.

In maxillofacial surgery the use of fibrin sealant made possible a perfect adhesion of large mucosal flaps in cleft lips (eight cases) and cleft palates (eight cases) with a minimum number of stitches (or even without stitches as in the case of one coanal atresia operated on through transpalatine access) [1] (Fig. 1).

A quick haemostasis was obtained when bleeding surfaces were treated in disfiguring angiomas (three cases). This resulted in direct healing by uneventful cicatrization: all the palatal flaps were found totally consolidated 5 days after surgery.

In thoracic surgery no bronchial fistula was observed in the cases where fibrin glue was applied to seal bronchial stumps (six cases). Moreover, the use of fibrin glue enabled us to perform, for tumors (four cases), sequestrations (one case) or bronchial cysts (three cases), atypical resections with the smallest loss of parenchyma (Fig. 2).

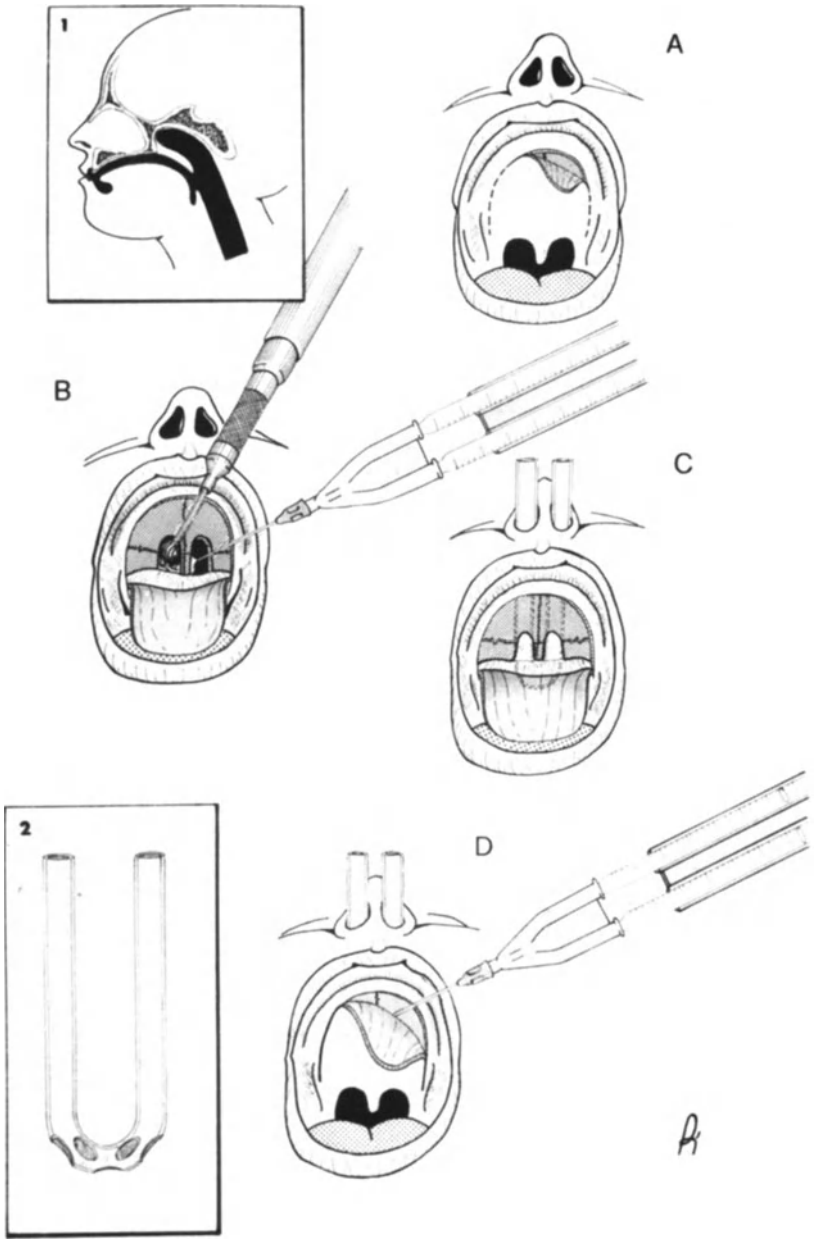

Fig. 1. Coanal atresia: through transpalatine access (**a**) and after opening of the bone septa (**b**), the large palatal flap is stuck to the underlying surfaces (**d**)

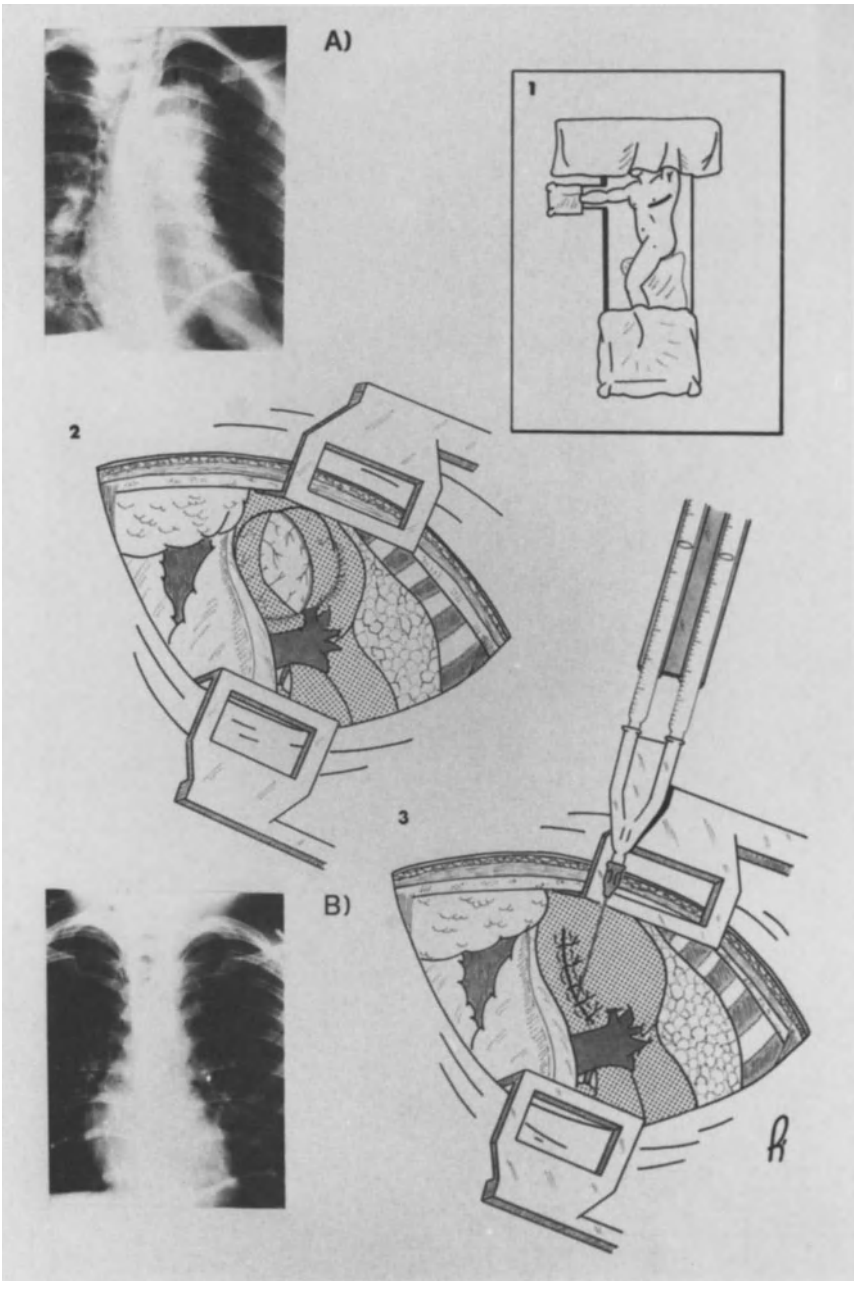

Fig. 2. Atypical resection of the left lung for a bronchial cyst. The fibrin glue is applied to seal the bronchus and to stop the loss of substance

Seven oesophageal anastomoses for atresia were made immediately watertight by sealing the oesophageal edges with fibrin glue: no leakage was recorded not even for high-risk primary or delayed anastomoses where the oesophageal stumps were still under tension after protracted bougienage.

In one case of chylothorax, after electrocoagulation of small ectasiae of the lymphatic vessels on the posterior surface of the right parietal pleura (V-VI-VII space), the application of a thin film of fibrin glue made it possible to stop the lymphatic dripping [1]. The postoperative course was uneventful and the Rx controls remain normal 2 years after surgery.

Abdominal surgery represented, in our experience, the widest field for application of fibrin glue. It is interesting to point out the extremely low rate of complications that occurred in all the anastomoses of the gastrointestinal tract (25 cases) or in the repair of extensive perforations (4 cases). In one case only an end-to-end anastomosis of the sigmoid for segmental aganglionosis disrupted 24 h after surgery, due to concomitant necrotizing enterocolitis. In another case a thin stercoraceous fistula supported by major anorectal surgery (out of 15 cases) was closed, a second time, making use of fibrin sealant injected backwards through its lumen.

No sepsis of the rectal sleeve was observed in five Soave pull-through procedures for Hirschsprung's disease or anorectal malformations when the space between the demucosated rectum and the transposed colon was sealed making use of fibrin glue.

All the loop colostomies performed (three cases) could be opened after a few minutes, thanks to a careful sealing of the cutaneous borders: no wound infection was recorded.

Large cavities left by the removal of tumors (one case), cysts (two cases), haematomas (one case) or abscesses (one case) were immediately obliterated by means of fibrin sealant.

In splenic injuries (two cases) splenectomy could be avoided by sticking the parenchyma along the fracture edges and suturing the capsula to the underlying tissues. In two cases a fragment of spleen was grafted after splenectomy for multiple candidial splenic and renal abscesses, into a pouch of the omentum majus and fixed in place by fibrin glue [5]: a Scintiscan demonstrates a normal captation of the spleen graft 6 months after surgery.

Making use of fibrin sealant any parenchymal loss following hepatic biopsy (mainly in patients with severe defects of coagulation – seven cases), abscesses (one case), injuries (one case) or cysts (one case) was easily mended (Fig. 3). No complication was observed.

In surgery of the biliary tree only one small leakage occurred out of seven anastomoses sealed by fibrin glue and it could be repaired by simple resticking. Similar results were obtained in urological surgery, when parenchymal losses following heminephrectomy for duplication (four cases) or after trauma (two cases) could be repaired, attaining quick haemostasis and reliable adhesion of the tissues.

The use of fibrin glue made possible the reconstruction of one exstrophic bladder in one step: the spreading of sealant underneath a Teflon mesh secured a watertight cystosynthesis against muscular straining.

Multiple abscesses of the kidney in two patients that had previously undergone radiotherapy for neoplasm could be sealed after needle aspiration guided by direct

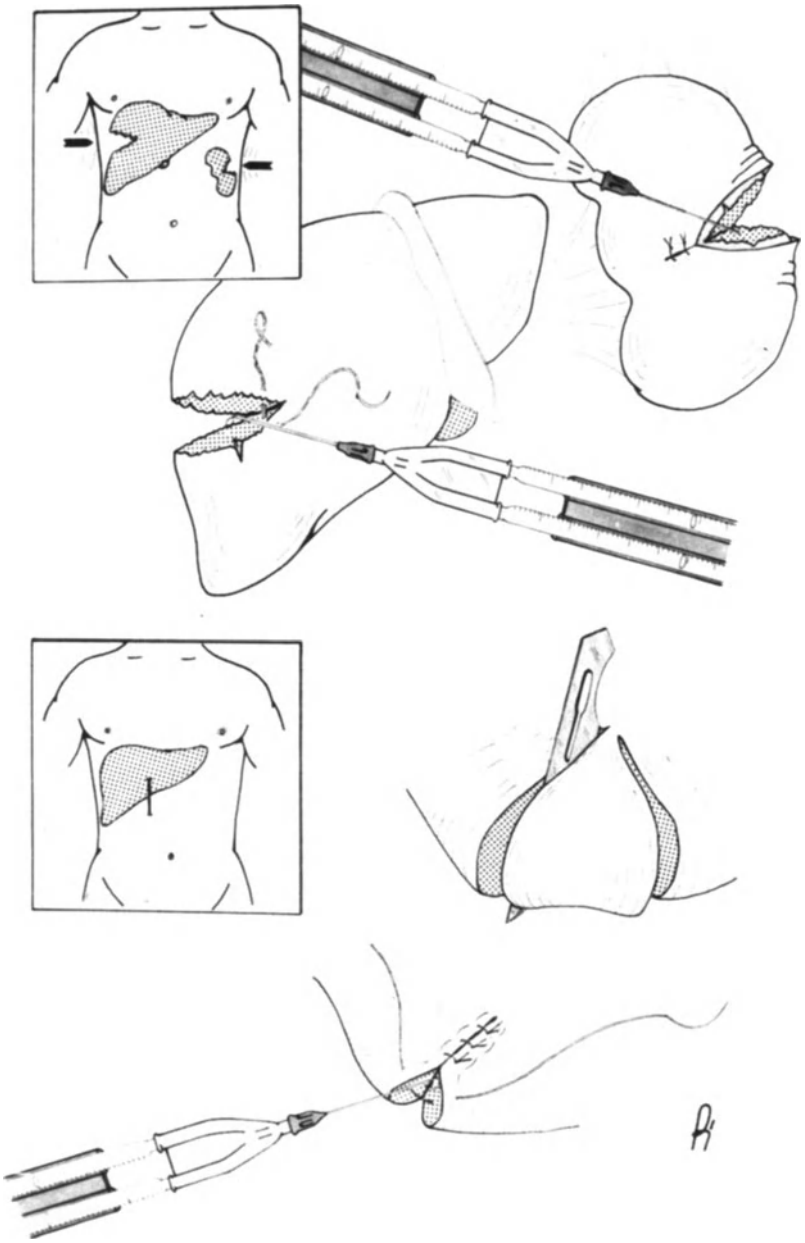

Fig. 3. *Top* Gluing the parenchyma after liver or splenic injuries. *Bottom* Biopsy of the liver in patients with a severe defect of coagulation

intraoperative echography. A CT check 1 year after surgery suggests perfect healing of the kidneys [3].

Fibrin glue is usually employed for sealing anastomoses (10 hydronephroses), sutures (11 plasties for megaureter, 17 vesicoureteral refluxes) and cutaneous flaps (14 hypospadias and 3 epispadias). Small fistulas following surgery for low hypospadias were mended by simple resticking.

In the overall casuistics, including 31 cases of miscellaneous surgery, we observed no complications directly related to the application of fibrin sealant.

Discussion

Surgical results have improved thanks to the introduction of new techniques and materials. In our experience fibrin glue has been shown to have a marked versatility: this fact, together with our first positive results, have enabled us to propose new indications for its use in paediatric surgery.

First of all our clinical trial demonstrates that fibrin glue enables high-risk sutures to be sealed, minimizing tissular trauma by reducing to a minimum the number of stitches. By means of the fibrin glue, large mucosal and cutaneous flaps, anastomoses, and plasties of the urinary tract could be glued, obtaining a useful haemostatic effect at the same time. This fact represents a matter of paramount importance if we consider that whenever a knot is tightened, even using the softest suture materials, microtrauma to the vessels, oedema and formation of granuloma are inevitable.

In all these cases experience led us to apply a very thin film of glue, having observed that a thicker layer does not mean better impermeabilization or better resistance.

Quick and safe haemostasis coupled with adhesive properties represents an advantage if traumatic lesions of noble parenchyma are to be treated: in many cases it is possible to avoid splenectomy, nephrectomy or hepatectomy. By means of a reliable technique large amounts of tissue may be saved [2, 4, 6].

Following the same concepts surgery becomes easier and safer in case of coagulation defects or large bleeding surfaces whose management is usually difficult.

A third possibility is represented by the obliteration of blank spaces: small abscesses (as demonstrated in the cases of candidial infection of the kidney reported in our series), sleeves (as in the case of pull-throughs for anorectal surgery) or large cavities (e.g. after removal of large sacrococcygeal teratomas; Fig. 4) may be sealed by means of fibrin glue. All this means protection against sepsis, direct healing and uneventful postoperative course.

In view of the good long-term results observed in our series, we feel encouraged to carry on the application of fibrin glue in paediatric surgery. The use of an adhesive, haemostatic sealant results in real advantages for both the surgeon and the child. Clinical experience confirms the outcomes of purely experimental trials, opening, maybe, a new era for surgery.

Acknowledgments. The Authors would like to thank Mrs. Christine Lewis for her kind assistance and review of the manuscript.

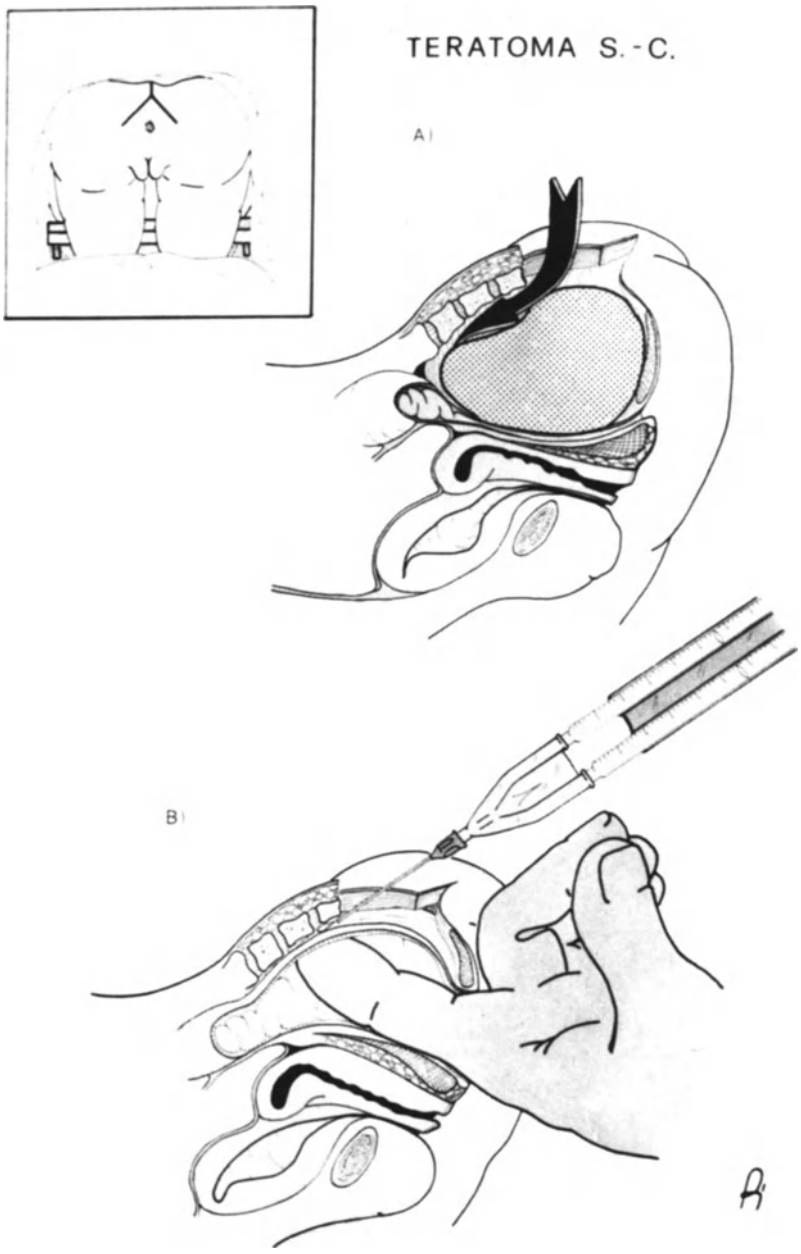

Fig. 4. **a** Removal of a big sacrococcygeal teratoma in a female child. **b** Obliteration of the blank space by injection of fibrin sealant

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The Use of Tissucol (Tisseel) in Pancreatic Surgery

A. MARCZELL

Key words: Rate of complications, fibrin sealant

Abstract

In surgery of the pancreas the rate of complications is high due to the specific properties of pancreatic tissue. A report on a new method, used in more than a 100 cases, proves that fibrin sealant can significantly reduce the complication rate.

Introduction

Apart from the sealing of high-risk anastomoses, the use of fibrin sealant in general surgery is predominantly indicated in the management of bleeding or oozing parenchymatous organs; in abdominal surgery these are the liver, spleen, and pancreas. Successful application of sealant on the liver and the spleen has prompted us to use it increasingly also in interventions on the pancreas.

The tissue-specific characteristics of this organ, e.g. its enormous friability, its tryptic activity together with the possibility of autodigestion, are responsible for the high complication rates (Table 1). Sutures of the parenchyma – especially for haemostatic purposes – lead to ischaemic necroses and these in turn become foci of local inflammation. The resultant typical local complications are postoperative haemorrhages, pancreatitis, and pancreatic fistulae. It is also interesting that even after so-called "minor interventions" the complication rate is high (Table 1).

In view of this grave prognosis in pancreatic surgery – excepting isolated consecutive series [2, 8, 12, 18] – we began to apply fibrin sealant more and more in interventions on the pancreas.

Table 1. Complication and mortality rates [3, 5, 6, 7, 8, 11, 13, 15, 17, 18, 20]

	Complication rate (%)	Mortality (%)
Biopsy or wedge resection	9.5	3.8
Enucleation	0.0–43	4.5–13
Tail resection of extended left pancreatectomy	0.0–41	1.0–11
Whipple's operation	16.4–44	4.5–23
Internal drainage	27.0	6.1
Trauma	25.0–75	?

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Table 2. Possible uses of fibrin sealant in pancreatic surgery

1. Wedge excision and enucleation
2. Tail resection and left pancreatectomy
3. Resection of head of pancreas with pancreatojejunostomy Resection of head of pancreas without pancreatojejunostomy
4. Drainage operations
5. Traumatic lesions

In these procedures, the atraumatic management of the friable parenchyma is the chief problem which has to be reconciled with the ultimate objective of attaining haemostasis and, even more so, fluid-tight sealing of the bleeding and secreting wound area. The fibrin-sealing method lends itself ideally to this purpose.

Applications of Fibrin Sealant (Table 2)

Wedge Excision and Enucleation

The complication rate following wedge excision, but also after enucleating procedures, is as high as 23% [4, 9, 15], pancreatic fistulae with a mortality of 4.5%–6.2% being the most frequent complications [9, 15]. When using the fibrin sealant, seamless sealing of the tissue defect is possible. We have treated more than 30 patients by this method and none of them had any postoperative complications.

Tail Resections and Extended Left Pancreatectomy

Even at hospitals not focussing on pancreatic surgery it is often necessary to manage resection surfaces or extensive superficial defects of the pancreatic parenchyma, e.g. in extended gastrectomies with lymph node dissection, carcinoma of the left flexure, or traumatic lesions.

But also after splenectomies of large spleens for haematological causes it may be required to treat lesions of the tail of the pancreas. After interventions of this type, the rate of pancreatic complications is as high as 41% [6, 15], pancreatic fistulae accounting for two-thirds of these problems. Mortality, too, is comparatively high, ranging between 4.3% and as much as 11% [3, 6, 7, 13]. Superficial lesions are sealed in the same manner as anastomoses, whereas deep defects, such as wedge excisions, are packed with the sealant. Following tail resection or left pancreatectomy without a dissectable pancreatic duct, the resection surface is trimmed angularly, the gaping wound edges are approximated and sealed, and the margins are covered with an additional coat of the fibrin sealant. When the main pancreatic duct can be visualized, it is ligated with non-absorbable suture material prior to sealing. We have used fibrin sealant even in extensive left pancreatectomies performed recently and have had only one postoperative complication.

Table 3. Mortality after Whipple's operation for carcinoma of the Pancreas (collective statistical data)

Warren	1975	15%
Nakase	1977	21%
Kern	1976	23%
Bodner	1979	22%

Resection of the Head of the Pancreas

Following Whipple's operation for chronic pancreatitis both anastomoses are sealed with fibrin sealant. By this method excellent results can be achieved [14]. But this paper is limited to direct sealing of the gland itself. Whipple's operation for carcinoma of the pancreas, the papilla of Vater, and the distal common bile duct is still fraught with a high rate of postoperative complications and, as a result, a high mortality figure (Table 3).

Among the causes of death and non-fatal complications, failure of the pancreato- and biliodigestive anastomosis is the most frequent by far, accounting for 30%, followed by postoperative haemorrhages from the resection surface, which are responsible for 15.5% of such accidents [2, 8, 11, 13, 18]. As for the pancreatoduodenostomy alone, the disparity of the results in chronic pancreatitis and in cancer surgery is certainly attributable to the much more delicate capsule in malignant diseases [19]. For this reason we started, in 1978, to use the fibrin sealant in the management of the latter and departed from the pancreatoduodenostomy in operations for carcinoma of the pancreas.

As early as 1958, Stritzko [16] had described that an atrophy of the excreting portion of the gland was found in all cases, where stasis had prevailed for some time, which was evident macroscopically by a wide main pancreatic duct.

At first we proceeded by the traditional method of ligating the main pancreatic duct and sealing of the cut surface of the remaining pancreas. Nine patients treated in this way had neither immediate nor late postoperative complications, especially no pancreatic fistula [10]. In one patient pancreatitis of the remaining gland necessitated subsequent total pancreatectomy, but, nonetheless, the patient died on the seventh postoperative day. As a consequence we reverted to anastomosing the residual pancreas.

Later the method was modified inasmuch as the duct of Wirsung was filled, today we would say occluded, with the fibrin sealant and, in addition, the residual pancreas was sealed.

This procedure was borne out by the observations reported by Stritzko in 1958 [16] and Gebhardt [4]. By comparative studies performed in 1985, Ascherl showed that Tissucol was superior to prolamin in filling the residual pancreas, which is attributable to the lower viscosity of the fibrin sealant.

The essential point of the sealing method is to apply the sealant continuously, i.e. without interruption. We have continued to use this modified method, but, nevertheless, have had two cases of postoperative pancreatitis of the residual gland; in one of them total pancreatectomy was inevitable. None of our patients had

postoperative haemorrhages. Pancreatic fistulae occurred in one patient and two patients were troubled with abnormal blood sugar reactions postoperatively.

Internal Drainage

In interventions of this type the anastomosis can be sealed by applying the fibrin sealant like a cuff, if necessary also on the inside, to prevent not only anastomotic failure but at the same time arrosion bleeding [17, 20].

Traumatic Lesions

In these cases the rate of complications and mortality depends on the extent of trauma. This explains the grossly divergent figures reported in the literature. Depending on the severity of trauma, 25%–75% of all cases are expected to develop postoperative complications, most commonly pancreatic fistulae. In our experience, the application of the sealant is promising in the following indications:

1. Trauma to the left side including perforations
2. Trauma to head and body with the main pancreatic duct remaining intact
3. Management of all resection surfaces

Results

In 101 applications of the fibrin sealant on the pancreas we have had only seven failures (Table 4).

Table 4. Fibrin sealant application on the pancreas: indications and results (1 April 1977 to 30 December 1984)

	n	Failures
Biopsy	28	1
Enucleation	5	0
Lymph node dissection	14	1
Tail resection / left pancreatectomy	12	1
Head resection	10	1 (1+)
Head resection and duct occlusion	21	2 (1 total)
Drainage operations	7	0
Trauma	4	1
	101	7 (1+)

Discussion

The results achieved in pancreatic surgery by the use of fibrin sealant are highly satisfactory, especially in so-called minor interventions on the pancreas. As the

latter have to be performed not only at specialized clinics, but at every general hospital, fibrin sealant can certainly help to reduce the complication rate.

As for the sealing technique, in pancreatic surgery – unlike most other surgical specialities – the sealant should not be applied as thinly as possible, because in this particular use safe haemostasis and fluid-tight sealing are more important than a delicate scar. A minimum aprotinin concentration of 3000 to 10000 KIU is indispensable.

In pancreas sealings usually no collagen fleece is used. Owing to its haemostatic and sealing effects, fibrin sealant has proved to be a valuable adjunct in the management of bleeding and secreting parenchymatous wound surfaces. Easy handling and the obviation of further tissue trauma sufficiently justify the routine use of this method.

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Securing Pancreatodigestive Anastomoses with Fibrin Sealant

G. ZALAUDEK

Key words: anastomotic leakage, fibrin sealant, pancreatectomy, pancreatic fistula, pancreatic surgery

Abstract

Because of the high rate of postoperative fistulas after partial duodenopancreatectomy, the most important cause of postoperative death, we attempted to diminish the high postoperative mortality by securing the pancreatodigestive anastomosis with fibrin sealant in 23 patients. Because of anastomotic leakage in four patients (17%), an early relaparotomy was necessary, but only in one case was fibrin sealant detected near the anastomosis. The total mortality after Whipple's procedure was 17%. Although statistically not completely meaningful, these results were compared retrospectively with earlier results at our Clinic on the rates for anastomotic insufficiency, relaparotomy, and mortality. We achieved a significantly lower rate of dehiscence ($p < 0.1$) and an insignificantly diminished rate of relaparotomy and mortality.

Introduction

More than 75% of all complications after partial duodenopan-createctomy derive from an insufficiency of pancreatodigestive anastomosis. Consequently more than 40 different modes to reconstruct the intestinal tract have been reported. Insufficiency of pancreatic anastomosis might be due to the lacerability of the parenchyma of the pancreas, that can lead to cutting of anastomotic sutures, or to the high tryptic activity of the pancreas. Local necroses caused by surgery might even induce pancreatic autodigestion [1, 3, 4].

In the literature the rate quoted for postoperative pancreatic fistulas is 20%–50%, representing the main cause of postoperative death. According to the statistics, the lethality of Whipple's procedure is 14%–21% [1].

Having had excellent experience with fibrin sealant repair of spleen and liver injuries at the Surgical Clinic of Graz, we decided to employ fibrin sealant to secure pancreatodigestive anastomoses as well.

Treated Patients

From 1982 to March 1985 23 patients (17 men and 6 women) underwent partial duodenopancreatectomy in our clinic. Twenty surgical interventions were initiated

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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because of malignant diseases. The patients' average age was 61 years, the ages ranging from 40 to 74. All patients had the same preoperative therapy, undergoing enteral and parenteral hyperalimentation via a central venous catheter to normalize the protein and curdle status.

We preferred to perform only one operation if the serum bilirubin was lower than 0.15 g/l, but because of a higher bilirubin value in four patients a preoperative decompression of the bile system was necessary. In one of these cases it was possible neither to normalize the protein status nor to reduce the bilirubin to a value lower than 0.15 g/l.

Surgical Technique

Pancreatodigestive anastomosis was performed with the total pancreatic cross section end-to-end with the approached jejunal loop using a telescopelike suturing technique. For suturing the pancreatic parenchyma to jejunal mucosa and the pancreatic capsule to jejunal seromuscular tissues thread made of polyglycolic acid was used. In a similiar technique using the same suture material the common bile duct was connected end-to-side with the jejunal loop about 10 cm aboral the pancreatojejunal anastomosis. The reconstruction of the gastrointestinal path was completed by gastrojejunal anastomosis with the following jejunal loop and Braun's entero-enteroanastomosis side-by-side (Fig. 1). For accessory tightening of the pancreatojejunostomy first the posterior wall and then the anterior wall of the anastomosis were secured by fibrin sealant like a cuff. The fibrin film was produced by simultaneous application of the fibrin sealant components in aprotinin, using 2–5 ml in a concentration of 3000 KIU [3–5].

The postoperative course and complications were documented until the release of the patients.

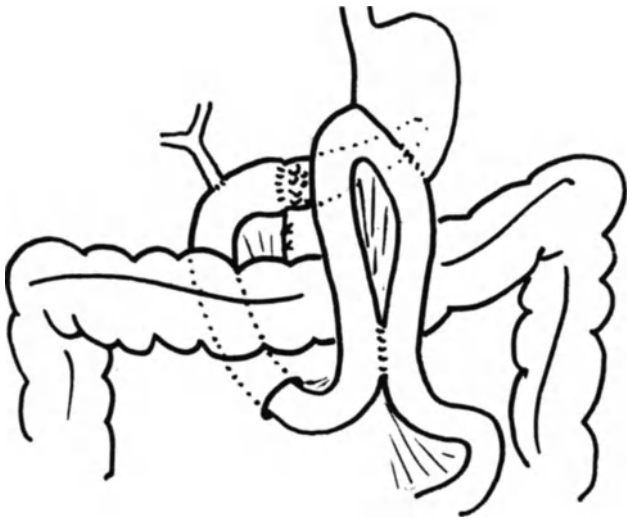

Fig. 1. Our own surgical technique of intestinal tract reconstruction after partial duodenopancreatectomy

Results

Because of anastomotic dehiscence relaparotomy was necessary in 4 out of 23 patients (17%). In one case the insufficiency was localized at the pancreatojejunostomy, in two cases on the biliodigestive anastomosis, and in the fourth case on the side-to-side entero-enterostomy after an intraoperative vascular lesion. In addition, relaparotomy on the 2nd, 3rd, 8th, and 5th postoperative days provided a clear view of fibrin sealant and its change. The surgical repair in our relaparotomies may be seen in Table 1.

Strikingly, on the 3rd day postoperatively fibrin was detected only in one patient and missed entirely in the other cases. We were also surprised by the fact that during insufficiency of the pancreatojejunal anastomosis 2 days after surgery it was not possible to demonstrate the presence of any fibrin at all, either macroscopically or microscopically, using the histological findings of the excised lip of the adjacent jejunum (Fig. 2).

Table 1. Relaparotomy and operative repair

Patient Age	Sex	Disease	Anastomosis	Post-operative day	Therapy by	Fibrin
63	♀	malignant	pancreatic	2nd	blind closure	absent
67	♂	malignant	choledochus	3rd	drainage	present
53	♀	malignant	choledochus	8th	reanastomosis	absent
45	♂	benignant	entero-enterostomy	5th	suturing	absent

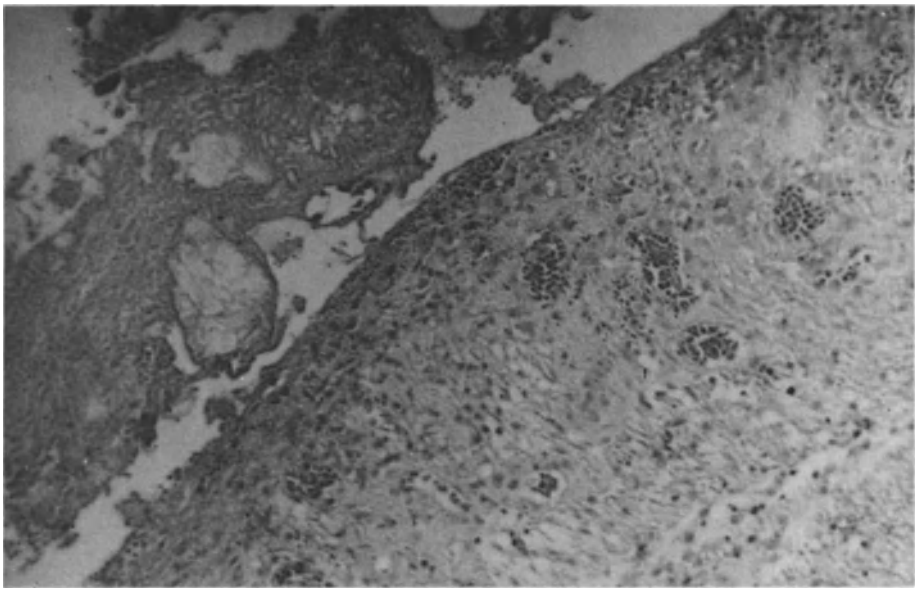

Fig. 2. Histology of the adjacent jejunal lip 2 days after pancreatojejunostomy reoperated because of anastomotic leakage (fibrin sealant completely absent).

Table 2. Death after partial duodenopancreatectomy

Patient Age	Sex	Disease	Cause of death	Postoperative day	Local state
63	♀	malignant	pneumonia	9th	pancreatitis
58	♂	malignant	cardiopulmonary	14th	normal
65	♂	malignant	cardiopulmonary	11th	normal
45	♂	benignant	sepsis	98th	normal

Table 3. Rate of insufficiency, relaparotomy, and lethality. Own results without and with fibrin sealant (FS) compared with data in the literature

Postoperative complication	1975–1979 without FS		1982–1985 with FS		Literature results
	n	%	n	%	%
Insufficiency	5/25	20	1/23	4.3[a]	20–50
Relaparotomy	8/25	32	4/23	17	10–30
Lethality	7/25	28	4/23	17	10–25

[a] $p < 0.1$ (*t*-test)

The rate of postoperative death in our patients amounted to 4 out of 23 (17%). Autopsy 14, 11, and 98 days after surgery showed normal states of healing of the pancreatojejunostomies. In one case (reoperated and treated by blind closure of the residual pancreatic and jejunal stump) a moderate acute pancreatitis was observed at autopsy on the 9th postoperative day. The causes of death are shown in Table 2.

Although not productive of statistically meaningful results, recent results plus earlier ones from our Clinic from 1975 to 1979 have been compared with the data in publications concerning rates of anastomotic leakage, relaparotomy, and lethality (Table 3). We found a significantly lower rate of insufficiency ($p = 0{,}1$ *t*-test). The rate of relaparotomy and mortality were clearly diminished (due to the low number of cases, statistical significance could not be achieved).

Discussion

Successful fibrin sealing of pancreatojejunal anastomoses and reduction of pancreatic fistulas have been repeatedly reported by several authors. As recommended in these reports we applied aprotinin in a concentration of 2000–3000 KIU [3–5]. Using fibrin sealant to secure the pancreatojejunal anastomosis, we approached the aim of reducing anastomotic leakage. Amazingly, in three out of the four cases of early relaparotomy, the fibrin sealant had completely disintegrated, although according to experimental examinations aprotinin is expected to remain detectable 8–10 days after surgery [2]. To successfully secure pancreatojejunal anastomosis we think aprotinin should be employed in a higher concentration.

We draw the following conclusions:

1. The use of fibrin sealant for pancreatodigestive anastomosis has distinctly reduced the rate of fistulas.
2. Due to the high proteolytic activity of the pancreas, aprotinin should be added in concentrations largely exceeding 3000 KIU (corresponding to [4]).
3. The fibrin sealing of the pancreatic resection area should possibly be considered, to protect the pancreatic duct, in addition to the fibrin sealant tightening of pancreatodigestive anastomosis.

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Experimental Fibrin and Cyanoacrylate Adhesion: A Comparative Investigation

O. BRANKOV

Key words: Fibrin adhesive, Cyanoacryl adhesive, experimental surgery, comparative investigation

Abstract

The first experimental comparative studies of two tissue adhesives – Tissucol of the firm IMMUNO, Vienna, and the Bulgarian-made cyanoacryl adhesive, Kanokonlit-B, are reported. The sealants have been applied in liver and kidney wounds, as well as in skin incisions. The author presents the results of the practical application of the two adhesives and a comparative clinical-histological evaluation of their effect.

Introduction

The advantages of tissue glues for tissue union and hemostasis in practical surgery have not been discussed for a long time. The biologically adhesive properties of the chemical substances which are most commonly used, based on cyanoacryl acid or on biological products from human fibrin, have been discussed at many scientific workshops [2, 3].

Our working group (in collaboration with Prof. Dr. med. K. Popov, Dr. M. Paschewa and Dr. med. D. Chinkov, Clinic for Experimental Stomatology, Sofia), which has been working on the experimental and clinical application of the Bulgarian cyanoacryl tissue adhesive Kanokonlit-B, has carried out the first comparative experimental investigations of the two glues in Bulgaria.

The present short communication summarizes some problems of the practical-technical application of the two adhesives.

Material and Method

A total of 14 experimental animals (rats and rabbits) were operated on. Two 12-mm-long skin incisions were made in eight rats bilaterally from the vertebral column on the depilated dorsum. Fibrin adhesive was placed between the adapted wound edges on the right side and cyanoacrylate over the same on the left side. Sharp incisions were made on the two lobes of the liver and on the two kidneys in six rabbits, Tissucol being applied on the right and Kanokonlit-B on the left. An autopsy of the animals was performed at equal intervals between the 1st and 28th days.

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Discussion

1. For the preparation of a multicomponent adhesive not only expensive apparatuses are needed because of the solution and mixture procedures, but also the preparation period is of a longer duration, which is avoided with cyanoacryl adhesive.
2. In order to achieve sufficient tensile strength of the glued wound edges treated with Tissucol, a 4- to 5-min compression is needed, while 30-s compression on the wounds treated with Kanokonlit-B is quite sufficient.
3. No postoperative hemorrhage has been found in the abdominal cavity as a necropsy finding. More pronounced adhesions have been observed in the area of the wounds having been treated with cyanoacryl. All adhesive sites were intact. On the fifth postoperative day parenchymal wounds treated with fibrin adhesive were hardly noticeable.
4. A narrower necrotic area has been histologically observed after fibrin adhesion in the first postoperative days, and later on a smaller connective tissue strip. Nevertheless, much more marginal hematomas have been noticed after fibrin adhesion.

Conclusion

The results of the first comparative studies on experimental animals lead to the following conclusion: the healing process with Tissucol proceeds more biologically and the results completely satisfy the surgeon.

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Use of Fibrin Sealant (Tissucol/Tisseel) in Manual and Stapled Anastomoses

G. Romeo, F. Basile, G. Giannone, A. Iuppa, L. Sandonato, and O. E. Chiarenza

Key words: Stapled anastomoses, Tissucol

Abstract

We present a study on the use of a human fibrin sealant (Tissucol) to prevent anastomotic dehiscence in digestive surgery. The study evaluates:
1. the appropiate modality of application of Tissucol,
2. the intraoperative leakage rate before and after application of Tissucol, and
3. the postoperative leakage rate in 42 patients with gastrointestinal anastomosis.

The results show 25% minor leaks in 32 colorectal and esophagojejunal anastomoses before application of Tissucol and no leaks after application of Tissucol. We conclude in favor of the use of Tissucol in high-risk manual or stapled anastomoses.

Introduction

Anastomotic leakage is the most feared complication after gastrointestinal (GI) surgery, because it is the leading cause of morbidity and mortality in the postoperative course. Despite the advancement in technique and suture materials, anastomotic dehiscence remains a troublesome aspect of esophageal and colorectal anastomoses as well as pancreatic and biliary tract reconstructions [3, 5]. More than 50% of mortality after esophageal surgery is directly related to anastomotic leakage [1, 6, 8, 11, 14]. In colorectal surgery clinically important dehiscences occur in 4–8% of cases [2, 12] while the total number of anastomotic leakages could reach 50% [3, 5].

The use of staplers has not reduced the incidence of anastomotic dehiscences significantly. On a collected series of 3594 anastomoses performed with the EEA stapler, 352 were also found (9.8%) [13]. In the series reported by Graffner [4] the incidence of clinical dehiscences varied from 0% to 14%, while subclinical or radiological leakages were estimated at from 10% to 42%.

The causes of anastomotic dehiscence have been well described, and among them cachexia, local and generalized sepsis, cancer, malnutrition and technical mistakes have been emphasized. While large anastomotic leakages usually lead to severe clinical pictures of grave prognosis, limited leakages, which are probably very frequent, go unrecognized most of the time. Nevertheless microabscesses and phlogosis can result from small dehiscences, resulting in formation of adhesions and possible obstruction. In this respect the use of a human sealant (Tissucol) seems to offer a real advantage. Here we report on our experience during the past 2 years with the use of Tissucol in gastrointestinal anastomoses in GI surgery.

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Material and Methods

A pilot study was undertaken to evaluate:
1. The appropiate method of application of Tissucol
2. The intraoperative leakage rate after application of Tissucol to the anastomoses
3. The postoperative leakage rate of GI anastomoses before and after application of Tissucol

During a 48-month period, 42 GI anastomoses were routinely performed: 16 manual colorectal anastomoses, 12 stapled colorectal anastomoses, 4 esophagojejunal stapled (EEA) anastomoses following total gastrectomy, 8 "Roux-en-Y" hepaticojejunostomies, and 2 pancreaticojejunostomies following the Whipple procedure. We have used a thrombin concentration of 500 IU/ml to obtain a clot within 10 s and therefore achieve a maximum hemostatic potential [7]. The Tissucol therm device has been used for the preheating and reconstitution of the components. After the application of Tissucol the anastomosis was isolated until the clot was firm and not adherent. The same method was used after stapled anastomoses; we used Tissucol only when its application on the stumps before stapling was unnecessary [10].

Result

The application of Tissucol on the completed anastomoses was simple and required an average of 10 min. The use of the Duploject is simple and effective. The complete coverage of the anastomosis was somewhat difficult after hepaticojejunostomy and esophagojejunostomy.

Colorectal and esophagojejunal anastomoses were all tested with diluted methylene blue solution introduced in the rectum or esophagus after the completion of the anastomosis. Minor leaks were present in 8 out of 32 anastomoses (25%) which were sealed off with silk sutures and application of Tissucol. None of anastomoses leaked after the application of Tissucol.

Postoperatively a meglumine diatrizoate (Gastrografin) contrast study was obtained in all the esophagojejunostomies and colorectal anastomoses between the fifth and tenth postoperative day. No leaks were visualized on both upper and lower anastomoses.

One out of 42 patients showed clinical evidence of anastomotic leakage after the Whipple procedure. Pancreatic juice was present for 6 days at the site of the left flank drain.

We have thus found no side effects directly attributable to the use of Tissucol. All patients were discharged from hospital within the 20th postoperative day.

Conclusion

Our pilot study convinced us that the use of Tissucol has some definite advantages in GI tract surgery and specifically:
1. It is easy to use and the technique can be learned quickly.

2. GI anastomoses, either manual or stapled, do have minor leaks when adequately tested, which should be sealed off.
3. The anastomosis which does not have a leak intraoperatively rarely causes problems in the postoperative period unless a problem with vascular supply intervenes.
4. A randomized, controlled trial considering the total cost effectiviness of the use of Tissucol would certainly tell us whether in an era of raising costs this product should be a routine tool in the surgical armamentarium.
5. Presently we feel that the use of Tissucol in high-risk anastomoses is worthwhile, because it reduces clinical and subclinical leaks from either manual or stapled anastomoses.
6. The use of tissue sealant, after the initial enthusiasm with cyanoacrylates, seems to have found a new and promising revival with Tissucol.

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Colonic Anastomoses Protected with Fibrin Sealant (Tissucol/Tisseel)

R. GIARDINO, M. BRULATTI, and A. FRANCHINI

Key words: fibrin glue, colon anastomosis

Abstract

The results of the use of a biological glue (Tissucol) for protection of colonic anastomoses are presented.

With the aim of reducing the incidence of leakages, Tissucol has been used to promote wound healing and to form a waterproof seal around sutures.

The results of 62 anastomoses protected by glue and 62 nonprotected anastomoses are compared.

Introduction

Anastomoses of the colon have traditionally been performed by use of single or multilayered sutures penetrating one or all of the layers of the intestinal wall [8]. Although improvements in suture material and surgical techniques have been made, colorectal surgery is still subject to complications, with leakages being the most serious [1, 2].

The use of mechanical stapling devices has been greeted with mixed feelings [6, 7]. Some believe them to be effective in reducing the number of complications; others, however, believe there has been no improvement compared to the use of sutures [2]. Anastomotic failure continues to occur and is accompanied by elevated morbidity and mortality.

It is well known that suturing material can cause a lot of tissue reactions, microhemorrhages, and areas of ischemic necrosis which may result in a secondary infection and can jeopardize the integrity of the anastomosis. The ideal situation would be one in which anastomosis healing would occur without the use of sutures, thus eliminating the presence of foreign bodies. At present sutureless anastomosis of the colon has been realized only experimentally by the use of synthetic sealants and, more recently, by the use of Tissucol [3, 5, 7, 9, 10]. With this product an improvement has been noted in experimental results [4]. In fact, unlike synthetic glue, human fibrin glue causes neither tissue reactions, nor adhesions and is completely reabsorbed. It has the following properties: hemostatic, adhesive, support of wound healings, and waterproofing of digestive tract anastomosis [3, 5, 11]. Taking advantage of this last property, we carried out a study to evaluate the glue's waterproofing ability in large bowel anastomoses as well as its ability to reduce the incidence of leakages.

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Table 1. Technique of large bowel anastomosis protected by Tissucol in 62 patients (October 1982 – December 1984)

Anastomosis	Sutured	Stapled
Ileocolic	18	3
Ileorectal	5	1
Colocolic	10	1
Colorectal	20	4
	53	9

Materials and methods

We studied 62 patients who underwent colonic surgical resection in our department between October 1982 and December 1984. Of the 62 patients, 52 had colonic resection for neoplasia; the remainder underwent surgery for inflammatory bowel disease.

The anastomosis was manually performed, using mainly monolayer with interrupted stitches, in 53 of the cases. In the remaining cases a mechanical stapling device was employed (Table 1). No protective colostomies were performed.

All the anastomoses were reinforced by 1 ml Tissucol applied with the Duploject. In the handsewn anastomoses the Tissucol was applied along the suture line; in the stapling anastomoses a small quantity was applied to the serosal surface prior to approaching the stumps and clenching the stapler, the additional glue being distributed around the anastomosis.

The maneuver is very easy. It is important to ensure that the entire surface of the suture line is completely covered with a film of glue; the quantity of Tissucol applied, even if abundant, was not found to cause any adhesion with surrounding tissues.

Results

Leakages with peritonitis were observed in two cases (3.2%), on the 7th and 8th postoperative days (Table 2). At reoperation no traces of the glue were found at the level of the anastomosis.

Another patient died 8 days postoperatively from pulmonary embolism. At autopsy, the integrity of the anastomosis was noted and no traces of the glue were

Table 2. Leakages in the large bowel anastomoses protected by Tissucol

	No. of cases	Leakages
Sutured	53	2
Stapled	9	–
	62	2(3.2%)

Table 3. Comparison of frequency of leakage in 124 large bowel anastomoses with or without protection by Tissucol (October 1982 – December 1984

	No. of cases	Leakages
Nonprotected	62	4(6.4%)
Protected	62	2(3.2%)
	124	6(4.8%)

visible. The histological study of the suture line in the specimen showed abundant granulation tissue distributed in a uniform fashion. The presence of giant cells was noted only around the stitches; there was no reaction to the glue.

The results of this group of patients were compared with another group of 62 patients who also underwent colonic and rectal anastomoses during the same period, in whom Tissucol was not utilized to protect anastomoses. The two groups can be considered homogeneous in terms of the criteria of surgical intervention, their general physical condition, and the surgical techniques utilized. In this second group four leakages occurred (6.4%), all on the 5th and 6th postoperative days (Table 3).

Discussion

Tissucol is easily managed and applied; no allergic reactions or side effects were found in our cases. The different results observed in the two groups of the present study are not statistically significant; nevertheless, it would appear that the use of Tissucol may reduce the risk of anastomotic complications and therefore produce results safer than handsewn or stapled techniques alone.

The results seem to confirm that Tissucol is able to create a waterproof seal against the leakage of gas, liquids, and feces during the critical period of healing and consolidation of colonic anastomosis (first 7–8 days).

We feel that in the near future the use of Tissucol will allow anastomosis using fewer sutures, thus resulting in less trauma to the intestinal wall.

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Fibrin Adhesive in Colorectal Surgery

A. ZEHLE and A. WELZ

Key words: Fibrin adhesive, colonic resection, anterior resection of the rectum, rectopexy

Abstract

Between Jan. 1, 1983, and Dec. 31, 1984, human fibrin adhesive was used in 28 colorectal procedures. The indications were colonic resection in high-risk cases such as those involving the ileus or peritonitis, very deep anterior resection of the rectum, and transabdominal rectopexy.

Anastomotic breakdown was seen in one case out of ten patients who had colonic resections and in one case out of 12 anterior resections (height of the anastomosis, 4–7 cm). Six patients had rectopexy for rectal prolapse. During the follow-up period of 6–24 months, there were no failures. We feel that, in the high-risk patient, the human fibrin adhesive makes colorectal anastomosis safer. Its use simplifies transabdominal rectopexy.

Introduction

Since the beginning of modern surgery the surgical suture was the primary tool for closing wounds and stopping bleeding. But first attempts to achieve hemostasis using blood coagulation factors were made as early as 1915 [1, 2]. The experimental work for the development of the modern fibrin gluing technique was done by Lindner and later Spängler and Holle [4, 7, 8].

Today human fibrin adhesive has become a useful aid in multiple procedures in abdominal surgery [9]. The subject of this paper is the use of human fibrin adhesive in colorectal surgery.

The following questions should be answered.

1. What indications can be seen for fibrin sealing in colorectal surgery?
2. Does fibrin sealing make the colonic anastomosis safer.
3. Is there any advantage of combining stapling technique with fibrin sealing.

Patients

Between 1 January 1983 and 31 December 1984 human fibrin adhesive was used during 82 procedures in abdominal surgery (Table 1). In 28 of a total of 117 colorectal operations human fibrin adhesive was used (Table 2).

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Table 1. Use of human fibrin adhesive in abdominal surgery

Diagnosis	Procedure	Number of Patients	Failure	Compli-cations	Deaths
Hiatus hernia	Fundoplication	3	0	0	0
Rectal prolapse	Rectopexy	6	0	0	0
Incomplete descent of the testicle	Orchidopexy	4	0	0	0
Bleeding of the esophagus	Upper gastric dissection	3	0	0	1
Carcinoma of the stomach Gastric ulcer	Gastrectomy/ Resection	16	2	3	2
Carcinoma of the pancreas	Pancreatectomy (Whipple)	6	0	0	0
Crohn's disease	Resection	3	0	1	1
Carcinoma of the colon	Resection	10	1	1	0
Carcinoma of the rectum	Resection	12	0	0	0
Miscellaneous		1	0	0	0
Hepatic tumors	Hemihepatectomy	7	1	0	0
Splenic rupture	Fibrin gluing	7	0	1	0
Hepatic rupture	Fibrin gluing	4	0	0	0
		82	4(4.8%)	8	4

Table 2. Use of human fibrin adhesive in colorectal surgery

Operation	Number of patients	Failure	Complications	Deaths
Colonic resection	10	1	1	0
Anterior resection of the rectum	12	1	2	0
Rectopexy	6	0	0	0
	28	2	3	0

Colonic anastomosis following right or left hemicolectomy or transverse resection was routinely performed using common surgical suture lines. In ten cases the anastomosis was secured by human fibrin adhesive.

Special risk factors were considered such as age over 80 years and emergency operations in patients suffering from peritonitis or ileus.

We feel that it is very important to lay the fibrin adhesive between the colonic stumps before tying the sutures. In this way the anastomosis is additionally glued and not only sealed. Failure of gluing followed by anastomotic breakdown was seen in one patient.

Fig. 1. Deep rectal anastomosis using human fibrin adhesive and stapling technique. Before the application of the fibrin adhesive, the EEA pistol is introduced through the anus and the prepared purse-string sutures are tied

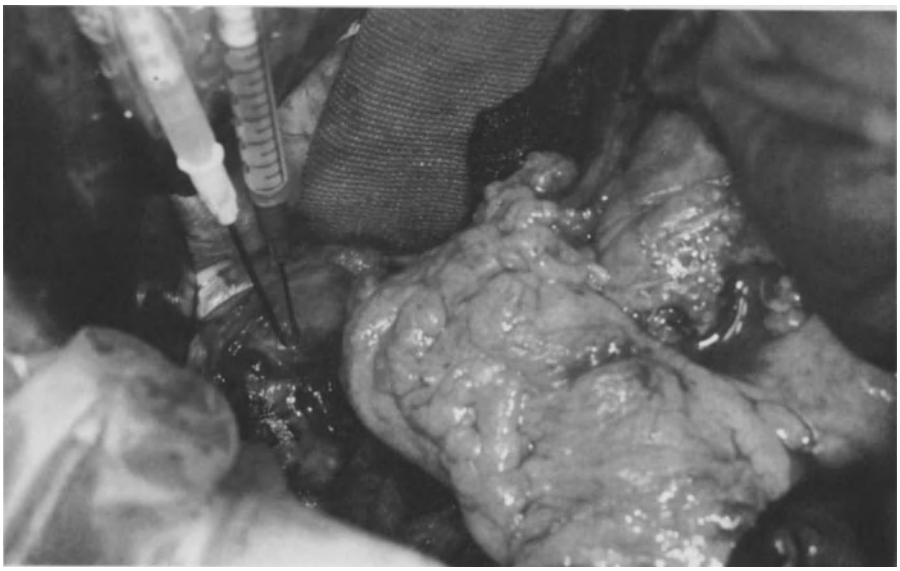

Fig. 2. Intraoperative site of anterior resection of the rectum. Application of the two components of the sealant to the prepared stumps. The EEA pistol is introduced and the purse-string sutures are tied

Table 3. Surgical details of 21 anterior resections of the rectum using stapling technique

Surgical techniques	$n = 21$
Small stapling anastomosis (25 mm)	–
Medium stapling anastomosis (28 mm)	10
Large stapling anastomosis (31 mm)	12
Stapling technique combined with common suture line	4
Fibrin adhesive	12

During the same period 21 anterior resections of the rectum were done. In all cases the anastomosis was performed using stapling techniques (Table 3). In 12 patients fibrin adhesive was used. The indications were resections for diverticulitis and perdiverticulitis and very deep anterior resections for carcinoma (height of the anastomosis 4–7 cm). In these cases, too, the rectal stumps were glued and stapled (Figs. 1, 2). There was one anastomotic breakdown.

Six patients were operated on for rectal prolapse (Fig. 3). In all cases transabdominal rectopexy was performed by gluing the mobilized rectum directly to the fascia of Waldeyer (Figs. 4, 5). No additional sutures were needed. During the follow-up period of 6–24 months no failure was observed.

Discussion

In animal experiments the additional use of fibrin adhesive improved the bursting pressure of colonic anastomosis (by up to 30% in rats) [3]. Clinical experience supported the beneficial effects of fibrin gluing in colorectal surgery. Scheele [6]

Fig. 3. Rectal prolapse

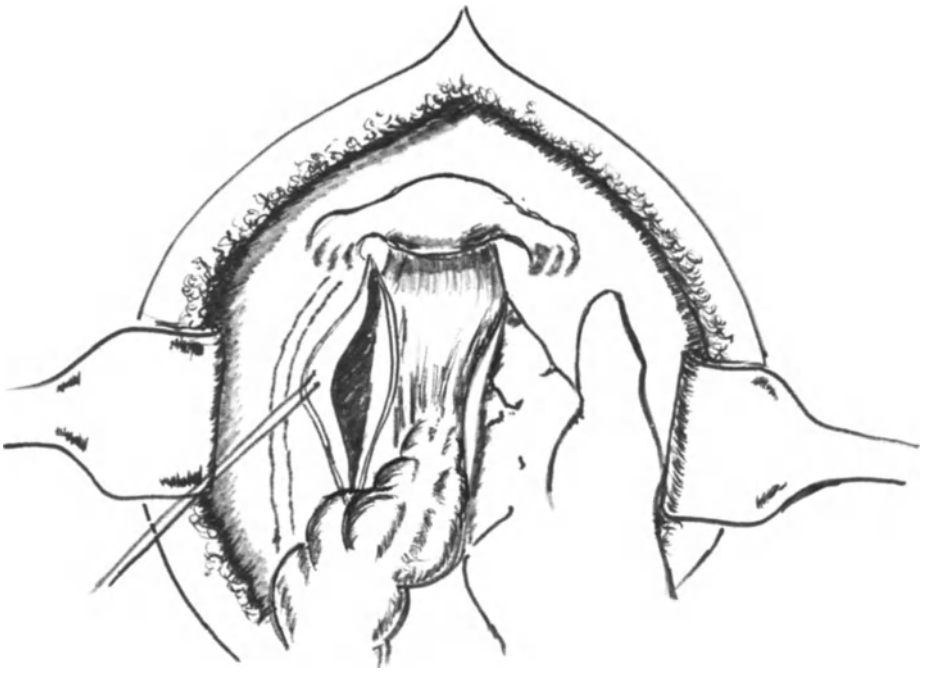

Fig. 4. Transabdominal proctopexy using human fibrin adhesive. The rectum is exposed and the lateral rectal ligaments are dissected

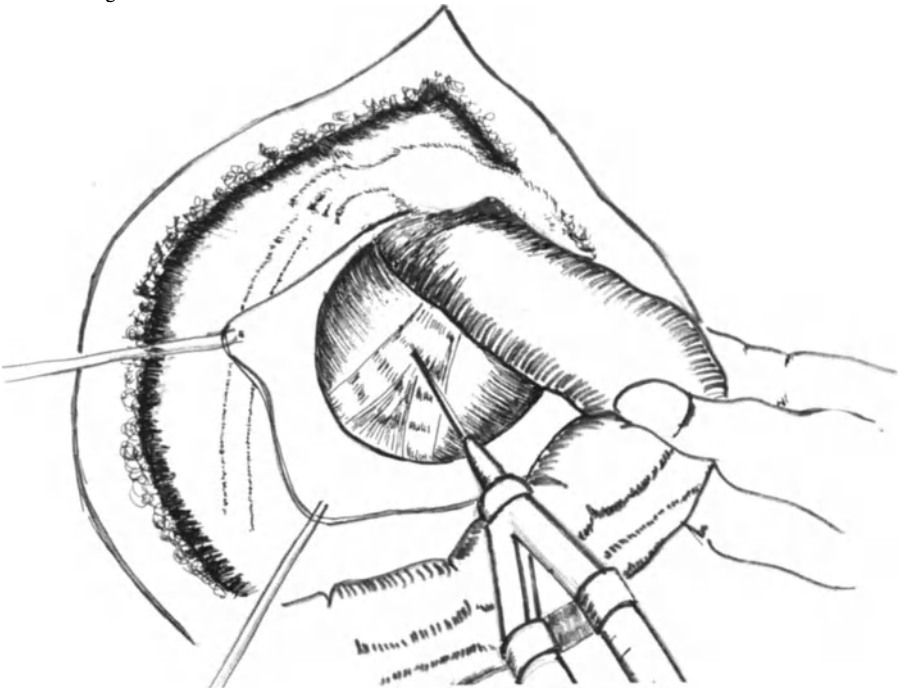

Fig. 5. Transabdominal proctopexy using human fibrin adhesive, the mobilized rectum is elevated and glued directly to the fascia of Waldeyer. With proper technique there is no need for additional sutures

found a rate of anastomotic leakage of 2.8% when fibrin glue was used, versus 15.3% in cases with only sutured anastomosis. The same good results were reported by Marczell [5], who observed an insufficiency rate of 2.5% in resections of the colon and of 3.9% in resections of the rectum.

Because our own patient group is too small for statistical evaluation, the beneficial effect of fibrin sealant will be illustrated by way of two cases.

Case 1: A 45-year-old patient with known Crohn's disease showed acute abdominal illness. Ultrasonic examination revealed an inflammatory tumor with multiple abscess formations in the right lower abdomen. During the emergency operation, resection of the inflammatory conglomerate and an ileoascendostomy were performed. The anastomosis was protected by fibrin adhesive as described.

Case 2: A 60-year-old patient with obstructive carcinoma of the rectum showed complete bowel obstruction. Despite the fully developed ileus an anterior resection was performed during the emergency intervention. Because of an extensive dilatation of the colon, contamination of the rectal and colonic stumps with infectious material could not be completely prevented. Nevertheless, the anastomosis was done using fibrin adhesive and the stapling technique.

These two patients recovered without complications though in both cases an indication for two- or three-stage procedures could have been seen.

Until now only retrospective results have been reported. To clarify the role of fibrin gluing in colorectal surgery, randomized prospective studies are needed.

Today there is no doubt that the transabdominal rectopexy is the safest therapy for rectal prolapse. This procedure is considerably simplified by using fibrin adhesive. In addition the risk of rectal wall necrosis – as is occasionally caused by the common suture lines – is avoided.

Conclusions

We feel that the fibrin adhesive is clearly indicated when resections of the colon are done in high risk patients. It should be combined with the stapling technique for deep anterior resection of the rectum. Additionally, human fibrin adhesive simplifies abdominal proctopexy for rectal prolapse.

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From Conventional Suturing to Sutureless Anastomoses in General Surgery

G. GALLETTI

Key words: Anastomosis, suture, trachea, common bile duct, colon, ovarian tubes, human fibrin sealant

Abstract

In an experimental study the author has analyzed the comparative histopathological differences of suture and sutureless anastomoses of the trachea, common bile duct, colon and ovarian tubes, in 80 pigs and 30 female rabbits. The results of this study show that the anastomoses carried out with human fibrin sealant do not need sutures, favoring tissue healing within 30 days from surgery, while sutures, such as monofilament nylon and polypropylene, induce a state of long-lasting inflammation of tissues with a highly significant delay of tissue healing, also causing lesions at 365 days while being expelled from the tissues. Even reabsorbable sutures seem to limit the healing capacities of injured tissues.

Based on these results the author concludes that Tissucol enhances these biological events that induce prompt and correct tissue healing, becoming the proper safeguard of tissue repair, whereas suture materials seem to represent a conspicuous means of limiting tissue healing, at the same time favoring long-lasting inflammatory cell infiltrates.

The author recommends a more extensive study of sutureless anastomoses for human application, considering it an appropriate technique of modern surgery.

Introduction

Since ancient surgery it has been good custom and safe practice to anastomose hollow viscera with strong and resistant sutures. However, in recent studies [1, 2, 3, 8] it has been proven that sutures, although made of very sophisticated synthetic materials, are often responsible for many of the postoperative complications developing in organs such as the common bile duct, colon, and trachea.

In the past, attempts have been made to explore the feasibility of sutureless anastomoses of hollow organs, but with very poor results because of unsuitable materials [5].

Since biological reabsorbable glues, such as human fibrin sealant, have been available and proved to be effective in living tissues [6, 7], we thought it interesting to conduct extensive experimental research in order to verify whether human fibrin glue can be a good and efficient means of anastomosing hollow viscera without sutures and at the same time to conduct an anatomopathological analysis of tissue

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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reactivity and modality of healing between anastomoses made with sutures and those done without sutures using human fibrin sealant as the only means of keeping tissues together.

Materials and Methods

This study was conducted on a total of 80 young pigs of random sex, weighing an average of 25 kg and 30 adult female rabbits of mean weight of 3.2 kg (Table 1). The organs selected to carry out the experiments were the trachea, the common bile duct and the colon in pigs and the ovarian tubes of rabbits (Table 2). These latter experiments were done employing microsurgical techniques.

Both absorbable and nonabsorbable sutures were used: 6–0 polyglycolic acid and monofilament nylon or polypropylene in pigs and 9–0 in rabbits. The sutureless anastomoses instead were done using fibrin sealant (Tissucol). All sutured anastomoses were made with interrupted single-layer stitches, while the sutureless anastomoses were done in the following manner: in the trachea two nonabsorbable sutures were placed anteriorly and posteriorly at the level of the anastomosis and the fibrin glue was laid in a rather thick layer with a base of 1.0–1.5 cm. The two sutures were left in place in order to prevent diastasis of the stumps due to the stretching movements of the animal neck. The two stumps of the common bile duct were maintained diametrically opposite to each other by using twin autostatic clamps which were removed with particular caution after a thick layer of Tissucol was laid all around the duct. The colon was anastomosed using four cardinal absorbable sutures, which were removed after consolidation of Tissucol and after the strength of the anastomosis was tested by inflating the loop of colon which included the anastomosis, with air at an intraluminal pressure of 500 mm of H_2O. The ovarian tubes were anastomosed employing autostatic microclamps, which maintained correctly the two approximated stumps. They were removed after hardening of the fibrin glue. The Tissucol was used adding 500 IU of thrombin for rapid hardening.

The animals in which the sutures were used were killed at 20, 30, 60, 90, 120, 180 and 365 days after surgery, while those in which the fibrin sealant was employed were killed at 8, 15, 20 and 30 days. The specimens of all groups of animals were

Table 1. Methods of study

Animals: 1. A total of 80 young pigs, random sex and age weighing 25 ± kg[a]
2. A total of 30 rabbits of random sex and age weighing 3.2 ± 0.6 kg[a]
Suture Material: the animals were killed at 20, 30, 60, 90, 120, 180 and 365 days from surgery
Fibrin Sealant: the animals were killed at 8, 15, 20 and 30 days from surgery
All specimens were examined macroscopically at autopsy
All specimens were studied microscopically:
1. Fixation in 10% formalin
2. Staining with:
a) Hematoxylin-eosin
b) Mallory
c) Van Gieson EF

[a] All figures ± are standard errors of the means

studied both macroscopically and microscopically, fixing them in 10% formalin and staining them with hematoxylin-eosin, Mallory and van Gieson EF. The fresh specimens containing sutures were also studied with the reflex light microscope while the histology was also studied with polarizing light filters.

Table 2. Experimental protocol

	Surgical Technique: 1. Isolation and transverse severing of trachea six rings from laryngeal cartilage at the neck 2. End-to-end anastomosis	Interrupted full-thickness single-layer 6-0 PGA and monofilament nylon sutures	Animals: young pigs
		1. Stumps maintained diametrically opposite with two anterior and posterior sutures 2. Layout of Tissucol	Animals: rabbits
	Surgical Technique: 1. Isolation and transverse severing of common bile duct at mid-point between cystic duct and wall of duodenum 2. End-to-end anastomosis	Interrupted full-thickness single-layer 6-0 PGA and monofilament nylon sutures	Animals: young pigs
		1. Stumps maintained diametrically opposite with twin autostatic clamps 2. Layout of Tissucol	Animals: young pigs
	Surgical Technique: 1. Isolation and transverse severing of sigmoid-colon 20 cm from anus 2. End-to-end anastomosis	Interrupted full-thickness single-layer 6-0 PGA and PDS sutures	Animals: young pigs
		1. Stumps kept opposite with four cardinal 6-0 PGA sutures 2. Layout of Tissucol 3. Test of anastomosis strength with 50 cm H_2O intraluminal pressure 4. Removal of cardinal sutures	Animals: young pigs
	Surgical technique: 1. Isolation and transverse severing of both ovarian tubes at the isthmus 2. End-to-end anastomosis	Interrupted full-thickness single-layer 9-0 PGA and monofilament nylon sutures	Animals: rabbits
		1. Stumps maintained diametrically opposite with twin autostatic clamps 2. Layout of Tissucol	Animals: rabbits

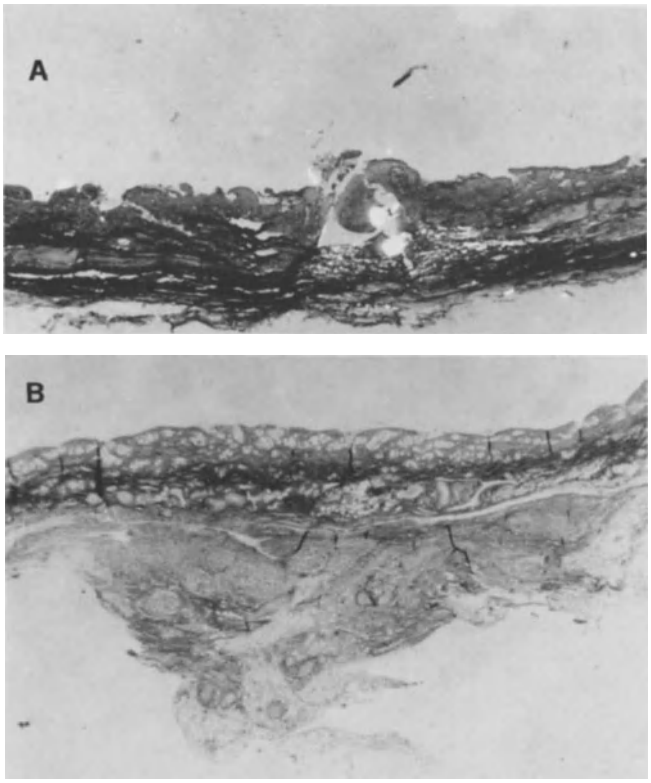

Fig. 1a, b. Histological sections of common bile duct anastomosed with and without sutures. **a** At 365 days from surgery there appears a considerable and evident loss of substance with the suture material made of monofilament nylon being expelled into the lumen. **b** The common bile duct anastomosed with human fibrin sealant appears well healed with the tissue layers completely aligned. There is a little thickening of the wall with adhesion to outer organs. Mallory, x25

Results

All animals of all groups of experiments survived until the date of death, except those operated onto the trachea in which nonabsorbable sutures were used.

The group of experiments in which the anastomoses were accomplished with sutures, particularly with nylon and polypropylene, in all organs tested, showed, independently from their function and anatomical structure, the sutures migrating within the wall, the knot often turning towards the lumen. In those animals killed at 365 days, the nonabsorbable sutures were still present, but were in the process of being expelled into the lumen with evident signs of mucosal loss of substance (Figs. 1a and 2a). In the trachea a rather precocious stenosis developed, with no signs of regression when the nonabsorbable sutures were employed (Fig. 3a). All these animals died within 120 days after surgery due to the stenosis. In the colon even nonabsorbable sutures delayed tissue repair, with persistence of considerable

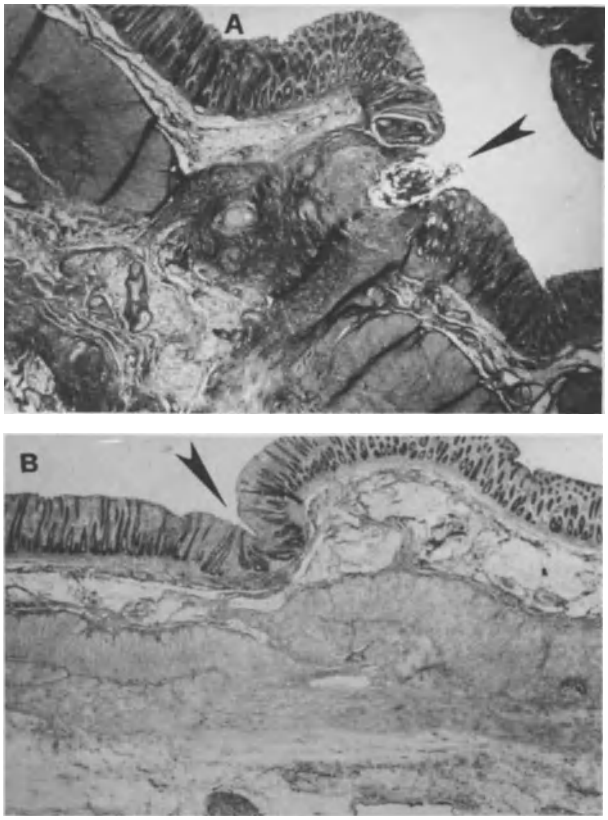

Fig. 2a, b. Histological sections of colon anastomosed with PGA sutures **a** and with human fibrin sealant **b**. In **a** the wall of the colon is still infiltrated by inflammatory cells and evident edema, with the suture being expelled at 90 days from surgery, with evident signs of loss of substance at the mucosa level. In **b** the colon at 30 days appears well healed with complete *restitutio ad integrum* of tissue layers. Also the muscle layer is healed and aligned. Mallory, x100

inflammatory cell infiltrates throughout the wall, with the stitches being expelled into the lumen (Fig. 2a). In the rabbit ovarian tubes sutured there was gross stenosis of the lumen and a remarkable inflammatory cell presence even at 120 and 180 days after surgery.

The group of experiments in which fibrin sealant was used showed a prompt and satisfactory progressive strengthening of all tissue layers. In fact, at 30 days the anastomosis was well consolidated and with excellent alignment of all layers. There were no signs of stenosis in the trachea (Fig. 3b), good healing and normal mucosa folds in the common bile duct (Fig. 1b), good healing and excellent *restitutio ad integrum* of the tissue layers of the colon without residues of cell infiltrates and contemporaneous repair of muscle layer (Fig. 2b). This was also observed in the ovarian tubes, which showed a normal and regular lumen caliber and early and normal appearance of mucosal folds. In all specimens in which human fibrin sealant was used, the cell participation to healing was mainly represented by rather

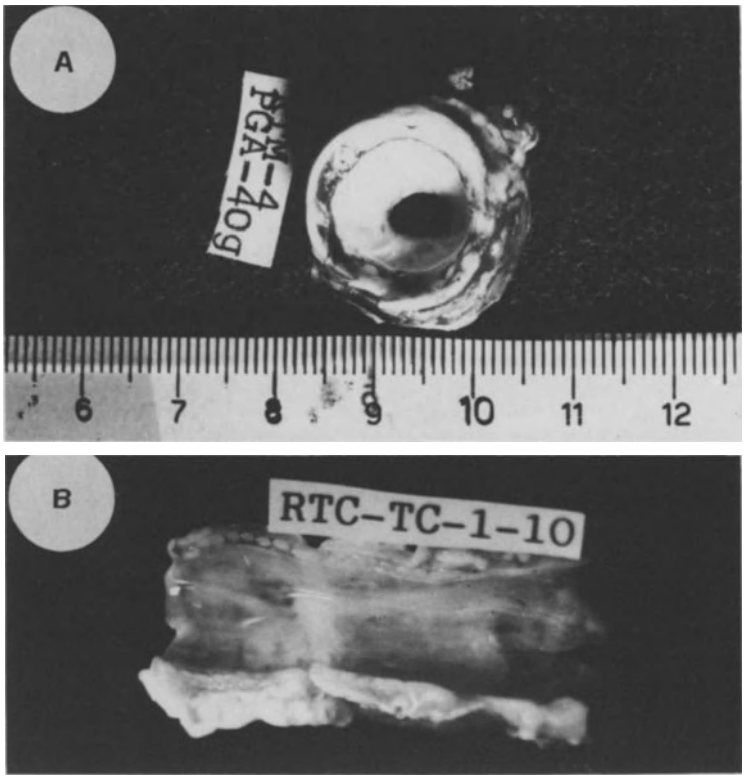

Fig. 3a, b. Anatomical specimens of trachea anastomosed with PGA sutures **a** and with human fibrin sealant **b**. The specimen in **a** shows an evident and conspicuous stenosis at 40 days which, however, will be reabsorbed subsequently; but with nonabsorbable monofilament nylon or polypropylene it will not be absorbed and the animals will have all died within 120 days from surgery. With human fibrin sealant, instead, the healing of the tracheal wall takes place without any stenosis. At 10 days **b** the wall appears already well consolidated.

numerous actively replicating fibroblasts and fibrocytes, the elastic fibers well aligned in an orderly manner, with scanty inflammatory cells which, however, at 30 days after surgery were totally absent.

Discussion

The results obtained in this experimental work indicate that sutureless anatomosis of hollow viscera can be accomplished in animals with a rather simple technique and with quite satisfactory and reliable outcomes. In fact, in no case were animals lost because of dehiscence of the anastomosis or other complications. The human fibrin sealant, besides being a good absorbable glue, seems also to have a protective effect on the tissues and lasts sufficiently to allow tissues to initiate the healing process.

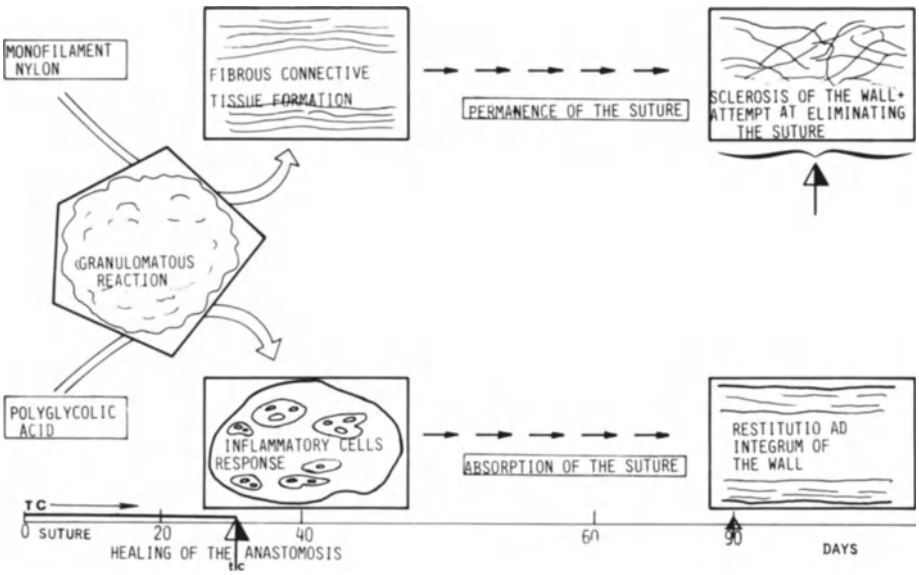

Fig. 4 Liberal schematic representation of tissue and cell response to sutures. The inflammatory response and healing of tissues with absorbable sutures takes place within 40 days while the *restitutio ad integrum* of the tissue layers at the anastomotic line is complete within 90 days with all types of sutures. With Tissucol (TC) the repair of the anastomosis and the *restitutio ad integrum* of tissue layers is complete within 30 days from surgery

This is proven by the limited presence of inflammatory cells, no edema and early active fibroblasts, fibrocytes and orderly elastic fibres. In the experiments in which sutures were used there are rather pronounced inflammatory cell infiltrates and edema which persist for a long period together with a conspicuous delay of tissue healing, with the sutures being expelled out of the tissues, causing remarkable lesions of the mucosa even at 365 days from surgery.

The differences between the two groups are so noteworthy that the time of healing in the presence of sutures was never less than 90 days, whereas with Tissucol the healing time was never beyond 30 days (Fig. 4).

From these results and the histopathological analysis between the two groups of experiments, we can easily deduct that Tissucol is an excellent biological sealant, quite appropriate for sutureless anastomoses of hollow viscera in animals, favoring tissue healing. Sutures instead, should be used in living tissues with great caution, with careful selection of the proper material to be employed.

We are well aware that sutureless anastomosis in humans is not something that can be proposed as a final resolution of the complications due to suture materials. However, our experiments indicate that sutureless anastomosis seems to be an appropriate technique in modern surgery and should be explored more extensively for human application.

As we have postulated on another occasion [4], in order to be optimal the reparative process of damaged tissues needs the intervention of a series of favorable

biological events and situations. Tissucol seems to possess the quality of enhancing these biological events and situations, representing a proper and adequate safeguard for the mechanisms of tissue repair to act without impediment. These mechanisms seem to find rather evident limitations when sutures are present.

Finally, it is also worthwhile to mention the fact that human fibrin sealant has also proven itself to be an excellent experimental model to study the healing potentials and capacities of damaged tissues, which in the presence of sutures have been rather difficult to study and understand properly.

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Endoscopic Therapy of Fistulae with Fibrin Tissue Sealant

M. JUNG, M. RAUTE, and B. C. MANEGOLD

Key words: Esophagotracheal, colocutaneous fistulae, fibrin tissue adhesive sealant, endoscopic closure; indications, limits of the method.

Abstract

Endoscopic fibrin glueing is valid today in congenital esophagotracheal recurrent fistulae, bronchial stump insufficiencies after pneumonectomy, and postoperative colocutaneous fistulae. The two-component tissue sealant Tissucol can be employed successfully in short tubular, uninfected fistulae. Tumor fistulae are difficult to treat since the tissue adhesive sealant promotes the course of physiological sealing and is dependent on normal granulation tissue. Indications and limits of endoscopic fistula therapy with fibrin tissue sealants are illustrated by several examples.

Introduction

Fibrin tissue sealant is widely used today in surgery to safeguard against anastomoses, conserve parenchymatous organs, support wound healing, and to seal organ defects [9]. There has also been repeated success in closure of chronic and postoperative enterocutaneous fistulae [4].

Sealing of a fistula can also be attempted by endoscopy if surgical measures have been exhausted, the risk of anesthesia for the patient is too great, or the fistula itself cannot be approached from the outside. Such situations are not frequently encountered, but endoscopic therapy is then often the meaningful alternative in the individual case. Fistulae in the bronchopulmonary and gastrointestinal region can be closed endoscopically. The fibrin tissue sealant is applied via a four-lumen spray catheter which is 150 cm long (Immuno) and which can be led through all conventional rigid and fiber optic instruments, including small-caliber bronchoscopes.

According to Stemberger and Blümel [12], fibrin glueing imitates the final phase of plasma clotting. Two components (Tissucol and a Thrombin solution) are used. The fibrin tissue sealant consists of highly concentrated fibrinogen, factor 13, fibronectin and albumin. Tissucol is available in a deep-frozen and in a lyophilized form (Tissucol-Kit). Warming of the solution is facilitated by a warming and stirring device (Fibrinotherm). Thrombin is alternatively available in a concentration of 500 IU/ml (rapid glueing) and 4 IU/ml dissolved in 40 mmol/l calcium chloride solution.

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General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Depending on the situation, rapid and slow solidifications of the substance can thus be obtained. Aprotinin as fibrinolysis inhibitor is present in one of the two components at a concentration of 3000 KIU/ml. Both components must be warmed up to 37°C before application in order to be able to manifest their full effect. Due to higher viscosity, low temperatures result in poorer mixing of the two substances and prevent exact glueing [8].

The two disposable syringes with the components are inserted into the Duploject syringe mounting and the connector of the spray catheter is fitted on the syringe conus. The components are not mixed until directly after passing the mouth of the catheter. The process has been described in detail by Redl and Schlag [8]. The fibrin plug which is initially formed solidifies rapidly and attains about 70% of its final strength after 10 min and its maximum strength after 24 h. Stress on the glued areas is to be avoided, especially in the first 5 min. In animal experiments, the resorption by an infiltrative granulation tissue can be demonstrated after three to four days; 15–31 days after application of the substance, there is a scar tissue without remnants of fibrin tissue adhesive sealant [6].

Patients

Fibrin tissue adhesive sealants were employed for the first time by Gdanietz to close esophagotracheal fistulae in children [3]. Our own good experience in closing isolated hair fistulae and esophagotracheal recurrent fistulae after surgical correction of an esophageal atresia in babies led to further specific endoscopic applications of fibrin tissue adhesive sealants in the bronchopulmonary and gastrointestinal region [5, 10] (Table 1).

The convincing results are illustrated in detail by descriptions of two severely ill patiens.

Table 1. Endoscopic therapy of congenital esophagotracheal fistulae

Patient	Sex	Age	Type	Sessions	Course
1	f	28 days	H-fistula	4	healing
2	m	29 days	VOGT III-b-rec.	1	died
3	m	39 days	VOGT III-b-rec.	2	healing
4	f	119 days	VOGT III-b-rec.	3	healing
5	f	809 days	VOGT III-b-rec.	1	healing
6	f	203 days	VOGT III-b-rec.	2	OP

Case 1

This 72-year-old male patient underwent pneumonectomy on August 8, 1985, for a carcinoma of the upper and lower lobe of the right lung. After an initially unproblematic postoperative course in the intensive care and surgical recovery ward, tachycardiac episodes with respiratory distress and tormenting compulsion to cough occurred 8 days after the operation.

There were febrile temperatures up to 39.5°C. The indwelling thorax drainage conveyed abundant air. Bronchoscopically, a stump insufficiency at the amputation edge of the right main bronchus with persistent fistula was diagnosed. After aspiration of secretion which had run over from the left bronchial system, a few milliliters of fibrin tissue adhesive (slow-glueing) were instilled into the fistula site under intravenous sedation in the context of a flexible bronchoscopy. Afterwards, this region was covered by means of "rapid tissue adhesive sealant".

Since leakiness was demonstrated, the procedure was repeated 3 days later (now successfully) and a definitive closure was produced. The urge to cough stopped spontaneously. With a simultaneous high-dose antibiotic treatment, the temperature subsided. The thorax drainage no longer conveyed any air after the second therapy. The patient recovered rapidly and was moved to the general ward on August 26, 1985 (Figs. 1 and 2).

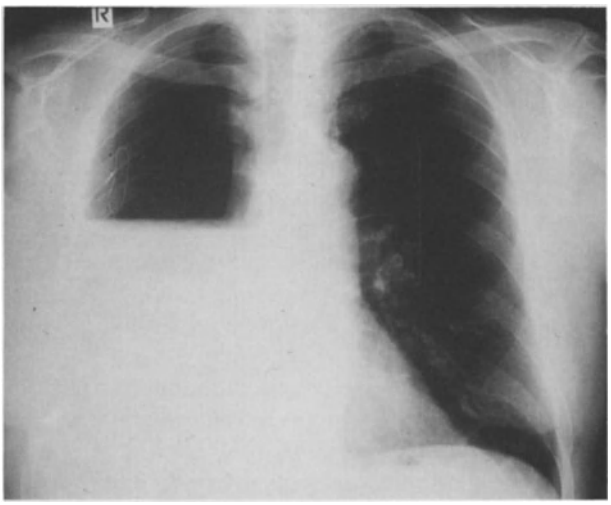

Fig. 1. Bronchial stump fistula 8 days after pneumectomy on the right side because of carcinoma. Chest X-ray at the time of diagnosis. Increasing accumulation of air in the right pleural cavity despite continuous drainage.

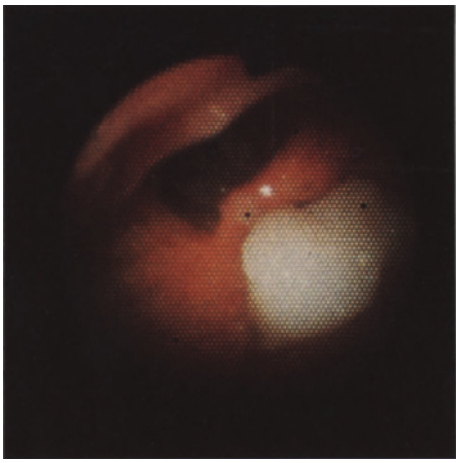

Fig. 2. Endoscopic fibrin sealing with Tissucol

The endoscopic checkup over 3 weeks after fistula glueing showed a normal amputation edge after pneumonectomy and the closed fistula. The patient was discharged from the hospital 5 weeks after the operation. No further problems occurred subsequently.

Case 2

This 65-year-old female patient was operated on for subileus in sigmoid stenosis and covered perforated sigmoid diverticulitis on August 26, 1984. There was a pronounced adhesion in the abdomen with an inflammatory conglomerate tumor in the sigmoid region. The inflammatory tumor was removed and the adhesions were detached by sigmoid continuity resection. Conventional application of an artificial anus was dispensed with in favor of attempted conservative therapy. After slow postoperative recovery, a rare complication arose 5 days later. The silicone drain had perforated through the intestinal wall into the rectum and conveyed feces to the outside.

The result was verified radiologically and endoscopically. The 7-mm thick drainage tube had broadly perforated the rectal wall at a level 11 cm from the anus and had already lead to arrosion of the mucosa opposite. The anastomosis about 5 cm farther up was unirritated. In the context of a partial coloscopy, the drainage was removed under endoscopic control and abundant fibrin tissue adhesive (Tissucol) was immediately instilled into the perforation site, initially with low-concentration thrombin for slow glueing and finally with high-concentration thrombin for rapid glueing. Up to this intervention, there were subfebrile temperature of up to 38.5°C with simultaneous antibiotic treatment. The patient recovered rapidly after the Tissucol instillation, and her temperature subsided; she was discharged home on September 20, 1984, with unirritated wound conditions (Figs. 3 and 4).

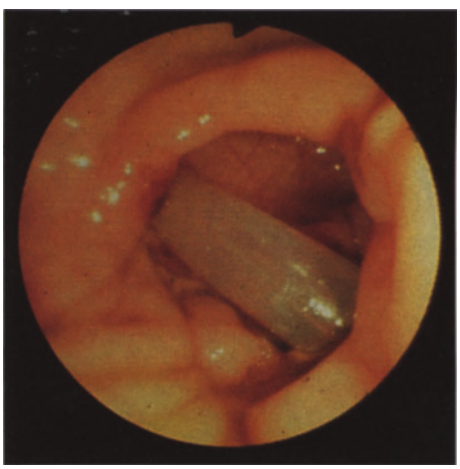

Fig. 3. Sigmoid resection because of a covered perforated sigmoid diverticulitis. Perforation of the silicone target drainage through the rectal wall with arrosion of the mucosa opposite

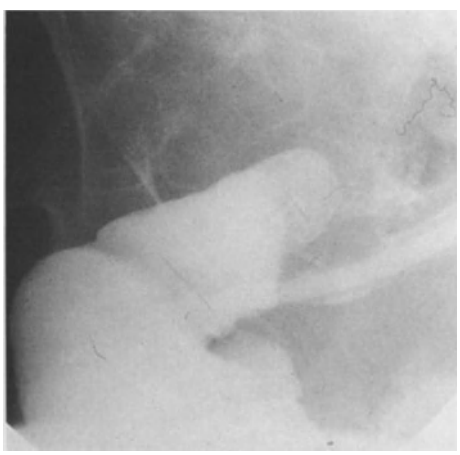

Fig. 4. Radiological representation of the rectal wall perforation (after gastrografin enema). Extraction of the drainage under endoscopic control and application of abundant fibrin tissue adhesive into the fistula site

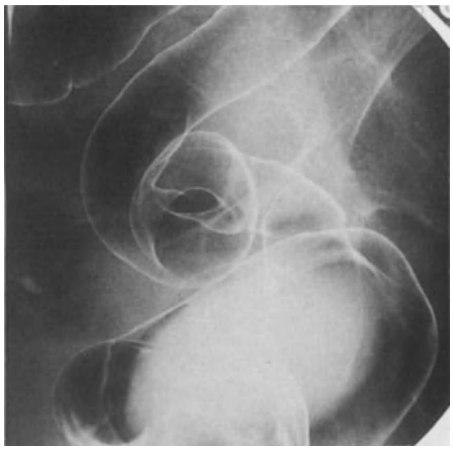

Fig. 5. Normal rectosigmoid in the double contrast technique without indication of fistulation 1 year after the operation and fibrin sealing

The radiological follow-up 1 year later showed a normal condition in the rectosigmoid region without fistulation into the surrounding tissue (Fig. 5).

Discussion

For more than 10 years, tissue sealants have been successfully employed endoscopically, namely in pediatric endoscopy.

Esophagotracheal fistulae in the context of an esophageal atresia which recurred despite thoracotomy with reconstruction of the esophagus and ligature of the fistula could thus repeatedly be closed successfully. Initially Histoacryl and later the two-component sealant Tissucol were used as sealant substances [1, 13]. The substance is applied via a rigid endoscope under conditions of general anesthesia. Histoacryl has the disadvantage that it causes a foreignbody reaction in the region to be closed and hence may not pass into the fistula canal. The plastic glue is only conceived as an external closure [1].

The glueing usually proved to be very tedious and required several sessions spaced out in time. Fibrin tissue sealant has the advantage that it accelerates physiological healing. It appreciably facilitates the procedure and has thus practically displaced therapy with Histoacryl. The glueing can be additionally supported by precedent electrocoagulation of the fistula opening [10]. The de-epithelialization caused in this way provide an additional stimulation for attachment of the fibrin glue.

In our own patients, endoscopic closure of congenital recurrent esophagotracheal fistulae were successful in five out of six babies. One of the babies died of a congenital vitium cordis in the further course, and a further patient was rethoracotomized, since the endoscopic treatment was ineffective after two sessions.

The indication for glueing of fistulae in the bronchopulmonary and gastrointestinal region has been extended in the meantime. Bronchial stump fistulae have been repeatedly closed successfully with fibrin tissue adhesive sealant after pneumonectomy in animal experiments [7, 14]. There are likewise occasional communications

on successful treatment of esophagotracheal fistulae in adults [2]. In the meantime, we have employed the substance in two patients with bronchial stump insufficiency after pneumonectomy on the right side. In the situation described above, it was possible to glue a stump fistula 8 days after pneumonectomy in two sessions. In another case with stumps insufficiency after removal of the right lung diagnosed immediately after the operation, the glueing only held for 8 h. However, rethoracotomy was necessary because of massive bleeding from the right pulmonary vein and the amputation region which had evidently lead to a breakdown of the fibrin plug.

Successful glueing of postoperative fistulae in the alimentary tract after extensive intestinal resection has been described repeatedly [4]. Persistent enterocutaneous fistulae in granulomatous enterocolitis (Crohn's disease) or ulcerative colitis are a worthwhile area of therapy [15]. Whether the fistula type is primarily suitable for such treatment is likely to be decisive. In general, short tubular fistulae without large cavities showed the best tendency to healing, possibly after prior irrigation with fluid containing antibiotics [4]. The case of rectal wall perforation described above provides additional evidence that wide fistulae can also be closed successfully in exceptional cases.

The necessity of maintaining the fibrin glue at an exact temperature when being applied has already been pointed out.

The question as to whether tumor fistulae are generally suitable for tissue glueing cannot be definitively answered, since fibrin glues support physiological wound healing and depend on normally infiltrating granulation tissue. Nevertheless, it was possible to close an esophagotracheal tumor fistula in a 67-year-old male patient in whom it was only possible to seal off the wide wall communication incompletely by a plastic esophageal tube. The patient recovered and survived two further cycles of cytostatic therapy without problems. In another case, repeated attempts to glue an esophagotracheal tumor fistula were unsuccessful, since the substance did not adhere in the region of the tumor.

Therapy with fibrin tissue sealant introduced endoscopically enables closure of fistulae in the bronchial and gastrointestinal region even at sites which are only accessible to fiber optic or rigid instruments.

It does not have to be applied at the beginning of therapy, but attains significance when a burdensome surgical reoperation would have to be accepted as the only alternative.

Apart from the practically standarized techniques of therapy in congenital recurrent fistulae between the trachea and the esophagus, in occasional cases fistula glueing was possible by means of endoscopy. With increasing experience and knowledge of the method, an even wider field of application can be opened up which will extend the indication spectrum of fibrin tissue sealants.

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Lymph Fistulae Following Lymph Node Dissections: Avoidance and Treatment by Use of Fibrin Sealing

H.W. WACLAWICZEK and W. PIMPL

Key words: Lymph node dissection, postoperative lymph fistula, avoidance, treatment, fibrin sealing

Abstract

Fibrin sealing was employed in connection with lymph node dissections – on one hand intraoperatively in order to avoid lymph fistulae (n = 26) and on the other hand postoperatively in treatment of manifest lymph fistulae (n = 9). Thereby lymph fistulae could be avoided in 96.2% (n = 25) or a rapid successful treatment of the lymph fistulae could be achieved in seven of nine cases (77%). On average a quantity of 1 ml fibrin sealant was sufficient; a high concentration of aprotinin (3500 U) and thrombin (500 U) was chosen. No side effects on the part of the fibrin sealing were noted.

Protracted lymph drainage or lymph fistulae following lymph node dissections are troublesome but not serious complications which require long-term treatment and show an increased local infection rate [1, 3]. They frequently occur after lymph node dissections in connection with tumor treatments (melanomas, sarcomas, mamma carcinomas, etc.) as well as after vascular operation, especially in the inguinal region. The incidence amounts to 15% up to 43% [4, 5].

Up to now only the tissue-saving preparation, ligature of the greater visible lymph vessels and drainage of the wound, have been available to avoid lymph seromas or fistulae. In the case of manifest lymph fistulae either the drainages were left until the lymph drainage stopped and/or the lymph seroma was repeatedly punctured.

Methods

Prophylaxis (n = 26)

Greater visible lymph vessels were ligated with reabsorbable sutures after nondestructive preparation in connection with lymph node dissections in the axilla (n = 9) and the inguinal region (n = 17). But before skin closure the whole wound area was sealed additionally with a thin fibrin film in order to close even the smallest lymph vessels using an application set in connection with a spraying head. Therefore a widespread distribution and a better utilization of the fibrin glue were achieved (Fig. 1) [2]. Thereby an average amount of 1 ml fibrin sealant (Tissucol, Immuno, Vienna) was sufficient. Because of the high fibrinolytic activity of the lymph fluid

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Fig. 1. Sealing of the wound area with a thin fibrin film using a spraying head

3000 U/ml aprotinin was used; the high thrombin concentration of 500 U/ml led to a very quick clotting of the fibrin. Finally, suction drainage was inserted into the wound cavity in every case.

Therapy ($n = 9$)

Manifest lymph seromas following lymph node dissections in the inguinal region were treated by tapping. Subsequently the fibrin sealant (average quantity: 1 ml) was applied using the same puncture needle (Fig. 2) Afterwards the patient compressed the wound region for approximately 10 min. In the case of larger lymph caverns ($n = 5$) this procedure had to be repeated two to three times on subsequent days, whereby the sealing of the wound cavity was achieved step by step (Table 1).

Table 1. Number of fibrin sealant applications and results in lymph fistulae following lymph node dissections with fibrin sealing ($n = 9$)

Fibrin sealant applications	Successful	Ineffective
$1 \times (n = 4)$	2	2
$2 \times (n = 4)$	4	0
$3 \times (n = 1)$	1	0
Total ($n = 9$)	7	2

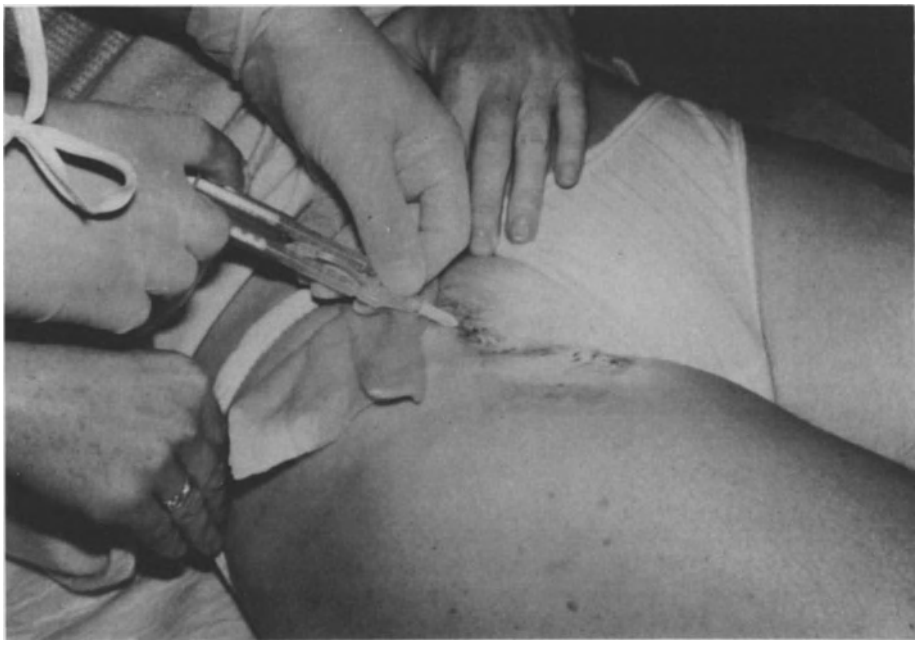

Fig. 2. Application of fibrin sealant into the lymph cavern through the puncture needle

Results

Prophylaxis

Only in one case (3.8%) did we observe a lymph seroma on the seventh postoperative day, whereas this complication rate amounted to 15.4% in the control group. In all other cases (n = 25) the duration and also the quantity of lymph drainage postoperatively was significantly smaller than in the control group (Table 2).

Table 2. Additional fibrin sealing of the wound regions following lymph node dissections (controlled study: n = 52)

Postoperative lymph drainage	Fibrin Sealing group (n = 26)	Control group (n = 26)
Duration	1.8 days	4.7 days
Quantity	23 ± 10 ml	105 ± 35 ml
Lymph fistula	3.8% (n = 1)	15.4% (n = 4)

Therapy

In seven of nine patients with manifest lymph fistulae in the inguinal region the treatment with fibrin sealing was successful. Suction drainage had to be inserted again in two cases after the ineffective fibrin sealing, until the secretion ended. However, these two cases originated from the initial period of our method, when the repeated application of fibrin sealant had not been taken into consideration.

Discussion

Lymph seromas or fistulae following lymph node dissection are rarely serious complications but often need long-term treatment. They are mainly dangerous for artificial grafts after vascular operations because of an increased local infection rate. Nevertheless, we consider that the tissue-saving preparation and ligature of bigger lymph vessels are the most important factors for the avoidance of lymph seromas and fistulae [1, 3, 4]. But additional fibrin sealing achieves a tight closure even of the smallest lymph vessels [5].

In a controlled study we were able to prove a significantly lower lymph drainage and a reduced rate of lymph fistulae using fibrin sealing prophylactically. In manifest lymph seromas the sealing of the lymph caverns was possible by the use of fibrin glue.

The main factors for the effectiveness of this method seem to be a high aprotinin and thrombin concentration as well as the compression on the sealed wound cavity for up to 10 min. As the handling is simple and no side effects could be observed, fibrin sealing can be recommended for the prophylaxis and treatment of lymph fistulae due to lymph node dissection. The significant question of cost is answered by a shortened time of clinical treatment.

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Fibrin Sealant in Skin Necroses Induced by Cytostatic Drugs and in Superinfected Wounds

H. KÖSTERING, J.U. WIEDING, J. GRAUDINS, G. EISINGER, J. KUSSMANN, and J.-H. BEYER

Key words: cytostatics, fibrin sealant, skin necroses, superinfected wounds

Abstract

Normal wound closure and optimal cicatrisation which is equally satisfactory from the medical and from the cosmetic standpoint is contingent on the early formation of fibrin, which has to be sufficiently stabilised by factor XIII and is subjected to gradual, generalised or local fibrinolysis. Thus, the fibrin sealant – at one time just wishful thinking among the medical profession – has become an indispensible treatment modality for patients with congenital or acquired bleeding disorders, and has helped to improve certain operative procedures. The indications for which fibrin sealant is used at the Göttingen University Medical Hospital are listed in Table 1; nearly all of them have been described in the spate of literature on fibrin sealant published over the past few years.

Table 1. Proven indications for the fibrin sealant

Impervious sealing of	**Reinforcement of**
Vascular prostheses	Enteroanastomoses
Vascular anastomoses	Tendinosutures
Suture lines of major vessels	Tympanoplasties
Pericardiolysis	Cordopexies
Microvascular anastomoses	Trauma to the tympanic membrane
Pleural leaks in spontaneous pneumothorax	Tonsillary bed
Carcinomatous pleurisy	**Packing of**
Liquor-tight sealing of	Bone cavities (e.g. osteomyelitis)
Dura grafts	Soft tissue cavities
Open dural lesions	'Sterile' fistulae
CSF fistulae	Endoprostheses
Neuroanastomoses	Dental alvaeoli (extraction wounds)
Sealing of	**Repair of superficial tissue lesions, e.g.**
Liver ruptures	Skin necroses induced by cytostatics
Liver biopsies	Crural ulcers
Gallbladder bed	Decubitus ulcers
Renal ruptures	Skin grafts
Partial renal ruptures	Split thickness skin grafts
Prostatic bed	Secondary sutures
Bone and cartilage	

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
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Introduction

In this paper we shall report on the management of skin necroses caused by cytotoxic drugs and comment briefly on our experiences with superinfected wounds, such as decubitus ulcers, crural ulcers and wound healing on second intention. In all these cases it is essential to be aware of the fact that superinfected wounds like that can never be cleared of bacteria. Moreover, fibrinogen and fibrin are known to be an excellent culture medium for certain strains of bacteria. On the other hand, thrombin and factor XIII, i.e. the fibrin stabilising factor, have a bacteriostatic effect, so that in 1977 we decided to use fibrin sealant in the management of skin necroses induced by cytostatic medication. The following considerations have prompted the use of the fibrin sealant in these cases:

1. Every other form of treatment involves considerably higher costs and/or risks.
2. The patients, whose pains cease instantly, are far better able to move and their skin necroses soon start to heal.
3. The treating physicians emphasise that sealing leads to satisfactory scar formation and is an optimal and time-saving method to manage such skin ulcers.

Methods and Results

In Table 2 preparations known to have a high incidence of skin necroses are listed. It should be pointed out that skin necroses of the greatest severity were seen upon treatment with adriamycin and mitomycin C, whereas vinca alkaloids mostly led to the destruction of the superficial skin layers and a burning pain, but caused no deep necroses reaching to the periosteum. In this respect adriamycin is the most widely and carefully studied preparation (Table 3).

Table 3 shows that it is often difficult to recognise the extent of an extravasation of, for example, adriamycin in time to take effective steps. It is of prime importance that cytostatic drugs are infused with the utmost caution, but nonetheless, patients should be warned that in the case of pains, heparin and methyl prednisolone should be administered by deep local injection as soon as possible. In our experience, injection should be started upon the mere suspicion of an extravasation of cytostatic

Table 2. Cytostatics with an increased incidence of skin necroses. It is noted that solar and X-ray irradiation as well as repeated injection of the cytostatic tend to exacerbate the condition

Skin necroses caused by:	Exacerbation by:
1. Adriamycin	a) Solar irradiation
2. Mitomycin C	b) X-ray irradiation
3. Actinomycin D	c) Repeated injection of the causal substance (up to 5 weeks)
4. Dacarbacin (DTIC)	
5. Vinca alkaloids	
a) Oncovin	
b) Vinblastine	
c) Vindesine	
6. Lyovac-Cosmegen	
7. Piperazindion	

Table 3. Clinical and histomorphological findings after the extravasation of adriamycin

1. Immediate, burning pain in the injection area and restricted ability to move, usually of several articulations
2. Occasional reddish, discoloured skin
3. After 1 h: vasodilation, sludged erytrhrocytes, increasing pains
4. After 24 h: formation of a papule, in rare cases symptoms of inflammation (rubor, calor)
5. 48–72 h: degenerative changes of the vascular endothelium, thrombosing of vessels, extravasation of blood corpuscles
6. 2nd–7th day: piloerection, scaling
7. 3rd–7th day: necrobiosis of collagen and vascularisation
8. Adriamycin was locally detectable for 7 days
9. After about 3 weeks: avascular sclerotic collagen with crusty skin
10. Indolent ulceration of skin after 1–13 weeks
11. No evidence of cells at any time
12. Progressive necrosis of the skin, exposing the tendons, sapping the skin; oedematous swelling of the periosteum
13. Mostly it takes 3–5 months until ulceration stops
14. Frequently skin grafts are rejected
15. Delayed healing of the ulcer often after 6 months or more; discomfort and pain caused by movement
16. Permanent lesions: painful movement, disturbance in articular function, contractures, scars

drugs, as thereby skin necroses can be avoided in most cases. It should be emphasised that by the local injection of heparin and methyl prednisolone within the first 6 days, the appearance of skin ulcers has been invariably prevented in our patients.

In order to discuss the results presented in Table 3, which are based on histomorphological investigations and clinical observations, it is important to consider that massive changes of the vascular endothelium, thrombosing even of minute arterial and venous vessels, and sometimes also extravasation of blood cells occur at an early stage. Mostly, the progressive necroses can be brought to a standstill only after 3–5 months. Skin grafts are often rejected and there are reports of necroses caused by cytostatic preparations which only healed after 18 months in spite of surgical management and concomitant measures. Moreover, kinesalgia and contractures persist for a long time and the remaining scars may be highly disfiguring.

The fibrin sealant is applied in the usual manner (Fig. 1). As it is sought to achieve rapid wound closure we nearly always use 500 U thrombin/ml. In cases of insufficient wound closure or defective adhesion of the fibrin seal, fibrinogen is mixed with a thrombin solution containing 4 U thrombin/ml and is rapidly injected through the fibrin film into the subcutaneous tissue underneath. Thereby, adhesion of the fibrin seal is substantially improved and wound granulation accelerated in most cases.

The disinfection of skin necroses induced by cytostatics is a particular problem, as a large variety of contaminating germs have been noted in all cases we have treated. Doubtlessly, it is of advantage that thrombin and factor XIII have a bacteriostatic effect so that massive growth of bacteria under the fibrin film is rare.

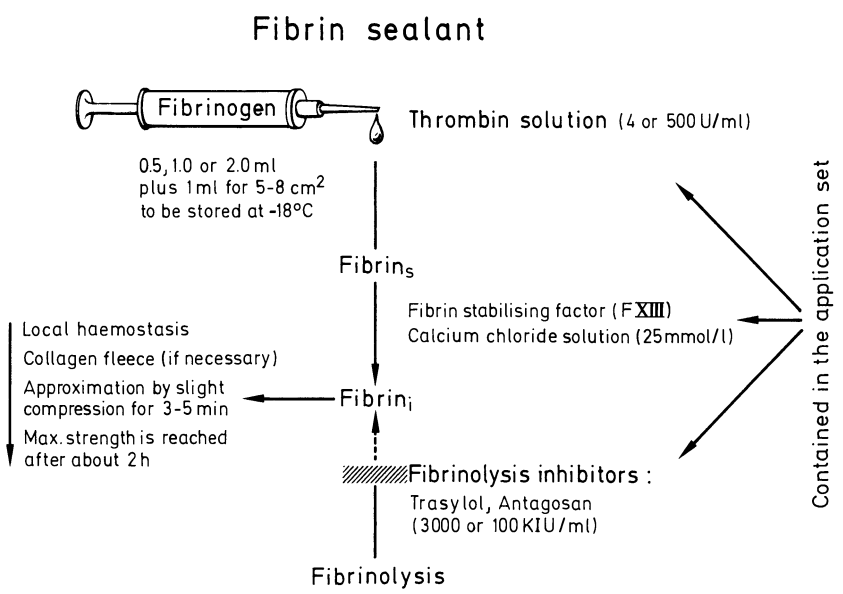


Fig. 1. The action of the fibrin sealant

Another problem is detachment or premature lysis of the fibrin seal which – in spite of the addition of aprotinin – is seen more often in superinfected wounds. In our experience, the local, but also the additional oral administration of synthetic fibrinolysis inhibitors, such as 1 tablet of Cyclocapron taken 4 times a day, or E aminocaproic acid, has proved beneficial (Table 4). This not only prevents the conversion of plasminogen into plasmin but leads to a stabilisation of the fibrin matrix formed.

This leads us to our patients who presented with skin necroses induced by cytostatic treatment. Figure 2a is a typical necrosis caused by vindesine at the beginning of treatment. The photograph shows the patient after the deep injection of heparin and cortisone and application of the fibrin sealant. Figure 2b shows his forearm, which 4 days after the onset of therapy has started to heal.

Table 4. Step-by step management of necroses induced by cytostatics

1. Cleanse necrotic areas
2. Rinse necroses with physiological saline and ε-aminocaproic acid and dab with sponge or swab
3. Apply fibrinogen and mix in situ with solution of concentrated thrombin (500 NIH units/ml), kallikrein inhibitor and $CaCl_2$
4. Mix fibrinogen and solution of thrombin (4 NIH units/ml), kallikrein inhibitor and $CaCl_2$ in the syringe and inject subjacently around the ulcer. Work fast to avoid clogging of the syringe. Then compress injection area
5. Top with ε-aminocaproic acid
6. To inhibit fibrinolysis, 1 g ε-aminocaproic acid, or a corresponding quantity of Cyclocapron (4 times 1 tablet), should be given at hourly intervals for 3 days

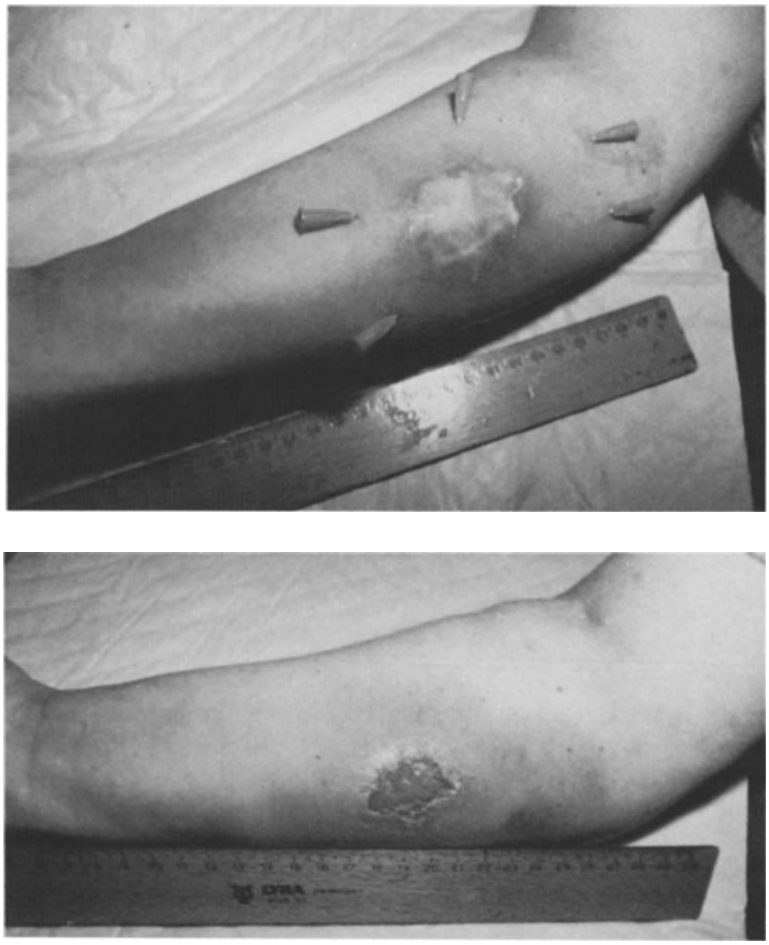


Fig. 2. **a** Skin defect after subjacent injection and coating with fibrin sealant. **b** Condition when followed up 4 days after treatment with fibrin sealant

Clinically, the extravasation of vindesine is not dramatic, contrary to the case of another patient who had received adriamycin (Fig. 3). It took 20 days until she consented to heparin treatment by deep injection. Her case illustrates the sufferings caused by the extravasation of a cytostatic drug which was treated too late. In spite of injection therapy progressive necrosis still persisted after 14 weeks. It took 26 weeks until complete healing of this severe necrosis was attained. It must be taken into account that in this severely ill patient the therapy with adriamycin had to be continued, which certainly led to an exacerbation of the necrosis time and again.

There are a few more cases to illustrate our method of treatment: Figure 4a shows a severe ulceration in another patient suffering from a necrosis caused by adriamycin. In Figure 4b the marked improvement of the skin necrosis is clearly visible.

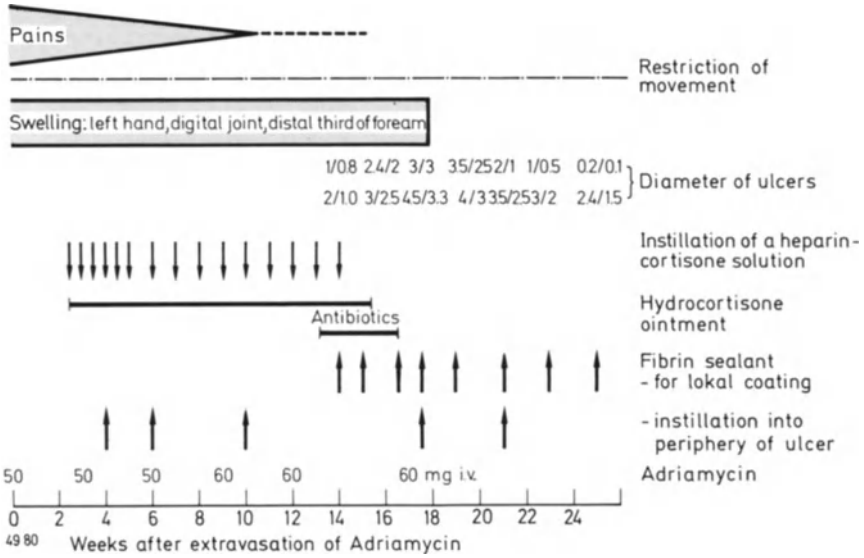
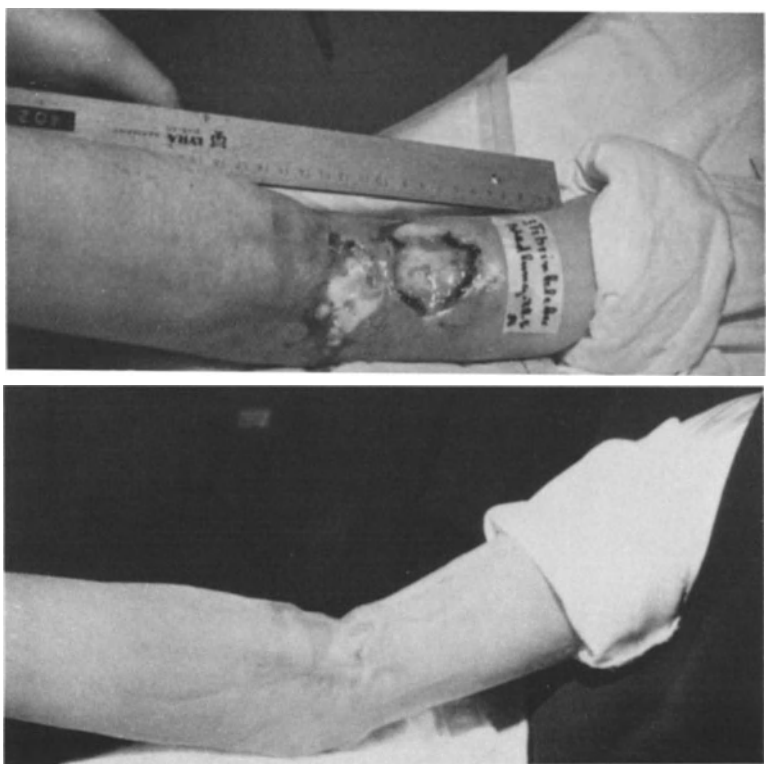


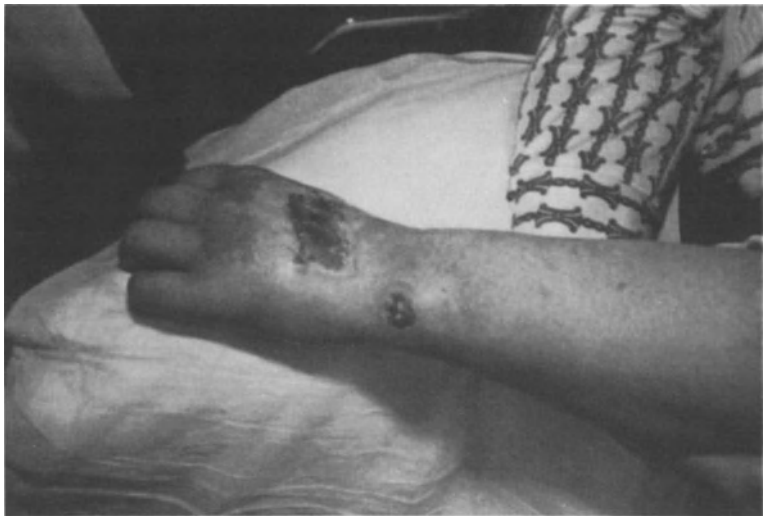
Fig. 3. Course of treatment in a patient who consented to subjacent injections only 20 days after the extravasation of adriamycin. By the repeated administration of adriamycin a skin necrosis had occurred after 98 days. The duration of antibiotic therapy is shown, as well as the applications of fibrin sealant. Healing was obtained after as long as 126 days



a

b

Fig. 4. **a** Patient with necrosis and severe ulceration caused by adriamycin. Here, the ulcers have been covered with fibrin sealant. **b** After 98 days the necrosis had largely healed



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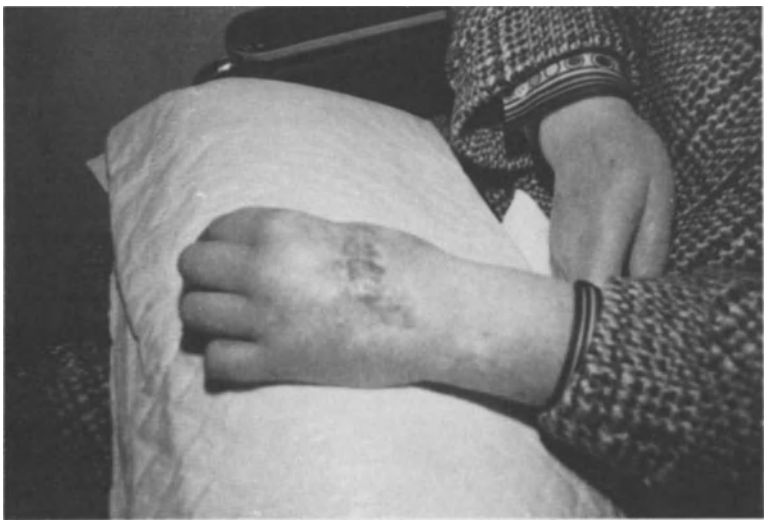


b

Fig. 5. **a** Skin necroses on the back of the hand and the caput ulnae. **b** Here, a slight mitomycin necrosis on the back of the hand is still visible. Complete healing was achieved after 115 days

After 95 days the necrosis had completely healed. Another patient (Fig. 5a) had a necrosis on the hand and in the region of the head of the ulna. In this case healing took 115 days (Fig. 5b). This shows that healing is often attained very slowly, but since the treatment can be carried out on an out-patient basis and the patients are promptly free from pains, we see no problems in long-term therapy.

Table 5 includes the cases we treated for necroses induced by cytostatics before the end of 1982, fortunately only ten in all. The table also shows great differences in the length of the healing periods, which was partly attributable to the type of therapy

Table 5. Survey of the necroses induced by cytostatic therapy which we have successfully treated with fibrin sealant

Patient		Age	Diagnosis	Preparation	Period between extravasation and ulceration (days)	Size of necrotic area (cm)	Operation	Healing period (days) following sealant application
1.	B., A.	77	Breast CA.	Adriamycin	25	5 × 5, 3 × 3	24 March 1980	126
2.	L., E.	51	Breast CA.	Adriamycin	16	3 × 3, 3 × 4	—	37
3.	N., A.	68	Breast CA.	Mitomycin	77	7 × 4, 1.5 × 2	6 June 1980	117
4.	H., A.	71	Breast CA.	Mitomycin	54	5 × 4	21 March 1980	19
5.	H., W.	55	Breast CA.	Adriamycin	98	3.5 × 2.5	—	95
6.	W., A.	72	Colon CA.	Vindesin	4	4 × 2	—	11
7.	E., H.	46	Ethmoid bone CA.	Vindesin	5	6 × 4	—	14
8.	B., A.	57	Larynx CA.	Vindesin	3	5 × 3	—	12
9.	N., F.	56	Breast CA.	Adriamycin	28	3 × 2	—	38
10.	G., J.	40	Breast CA.	Adriamycin	15	4 × 2.5	—	61

Table 6. Superinfected wounds treated with fibrin sealant since 1983

Skin necroses induced by cytostatc medication	$n = 2$
(Extravasations)	$(n = 11)$
Crural ulcers	$n = 6$
Decubitus ulcers	$n = 4$
Fistula	$n = 1$
Healing on secondary intention following:	
Nucleus pulposus operations	$n = 24$
Craniotomy	$n = 10$
(Thereof: use of Palakos)	$n = 8$
Appendectomy, back of the foot	$n = 3$

administered in each case. Even with the utmost caution in cytostatic treatment necroses will not be prevented entirely. Some of the patients listed in Table 5 were referred from other hospitals to our clinic in Göttingen after extravasations of cytostatics had occurred.

Since the end of 1982 just two further necroses caused by cytostatics had to be treated (Table 6), though extravasations occurred in a total of 11 patients. This shows that we succeeded in preventing such necroses in most cases by the immediate deep injection of heparin and methyl prednisolone. Among the patients successfully treated were six with crural ulcers, four with decubital ulcers in whom operation was not indicated, and one paraplegic patient with a large fistula having a volume of 15 ml in the region of the sacral bone. In the meantime the fistula has largely healed due to the repeated application of fibrin sealant.

Most patients were treated for dehiscent sutures following surgical interventions. Especially after nucleus pulposus operations wound dehiscence is frequent and often occurs after 7 to 15 days post-operatively. Such wounds are prone to secondary infection. Reoperation could be prevented in these cases by repeated wound sealing with fibrin sealant. It should be emphasised that these patients are always given saluretics and positioned in such a way that the flow of CSF is transiently reduced or completely interrupted. Ten patients were treated following craniotomy, but it should be mentioned that in eight of them Palakos had been applied for closure of the craniotomy wounds. Similarly, two cases of secondary healing in patients with bleeding disorders, namely an appendectomy wound and a lesion in the region of the back of the foot, could be closed with fibrin sealant.

From our experiences we conclude that in superinfected, badly healing wounds and especially in necroses induced by cytostatic treatment the local application of fibrin sealant leads to satisfactory scar formation and at the same time helps to reduce the number of operations and graft rejections. Nevertheless, all our attention must be focussed on the prophylaxis of decubitus ulcers, of crural ulcers and especially of skin necroses caused by cytostatic treatment. As for the latter (Table 7), it is imperative that cytostatics are administered by trained, experienced hospital personnel and that all safety precautions are taken to avoid extravasations of highly aggressive cytostatics in our patients. It is equally important that the patients are

advised to see a doctor immediately if they have pains in the injections area in order to prevent skin necroses by the prompt injection of heparin and corticosteroids into the underlying tissue (Table 8).

According to our experiences, the use of fibrin sealant in this indication is of obvious benefit to the patients.

Table 7. Prophylactic measures of a general nature to avoid paravenous injections

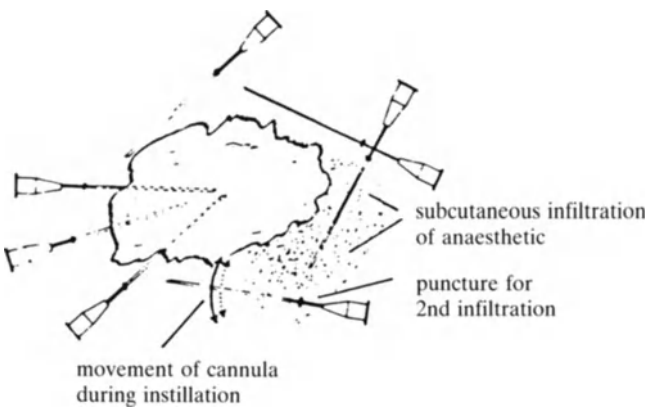
1. Injections should be given by experienced personnel only
2. Patients should be informed in advance of side effects
3. Patients should be advised to present immediately in the case of pains or other vexation
4. Fixation of the limb to be treated
5. Intravenous injections should be avoided
6. No prolonged infusions of cytostatics without adequate supervision
7. Add drugs causing tissue damage to another infusion by injection into the infusion tube:
   a) Use butterfly needle and short soft catheter
   b) Start infusion at a brisk rate and check if there is any extravasation
   c) Inject cytostatic material into the infusion tube without interrupting the intravenous drip
   d) Irrigate the vessel before removing the infusion needle
8. Immediately stop injection if there is any back pressure
9. Never give any injection distal to a ruptured or thrombosed vessel
10. Give injections into the large brachial vein only; avoid the back of the hand or the volar forearm

Table 8. Treatment schedule following the extravasation of cytostatic preparations; the illustration below is to illustrate the procedure

Immediate treatment (via an already inserted cannula and/or by ample subjacent injection) following the extravasation of cytostatics

1. Local anaesthetic (without adding a vasoconstrictive)
2. Heparin solution (7500 IU Liquemin/5 ml physiological saline)
3. Methylprednisolone-21-acetate (crystal suspension, 40 mg)
4. Bicarbonate solution (approx. 5 ml, 8.4%); (optional)
5. Generous application of icebags (for 24 h)
6. Inunctions with hydrocortisone preparations (1%, twice a day)

In the case of pains repeat steps 1 and 2;
or, after 7 days, steps 1, 2 and 3



The Kinetics of Antibiotic Release from a Fibrin-Clotting System: An Animal Experiment

R. POINTNER, J. KOFLER, Ch. OFFER, and G. SCHWAB

Key words: Fibrin glue, antibiotics, diffusion test

Abstract

In colon surgery fibrin glue is commonly used for the additional sealing of high-risk colonic anastomoses. As every anastomosis must be considered contaminated, and fibrin is a culture medium for pathogens, it is natural to suggest antibiotics should be added to the adhesive for its application to an anastomosis.

After preliminary studies concerning the influence of antibiotic combinations on the clotting and adhesive properties of fibrin glue, we investigated the release time of a clindamycin-cefotaxim combination from a fibrin clot under standardized conditions.

One millilitre of fibrin glue was mixed with 25 mg clindamycin and 25 mg cefotaxim, and the fibrin-antibiotic complex was implanted into the free peritoneal cavity of Wistar rats. The clot was removed after 1, 3, 5, 8 and 24 h and the remaining quantity of antibiotic in the fibrin clot was measured. *Escherichia coli* was used for the assessment of cefotaxim release; clindamycin release was tested by *Staphylococcus epidermidis*. After 24 h we still found sufficiently high concentrations of antibiotic in the fibrin clot. This demonstrates that without impairment of clotting behavior and adhesive qualities of the fibrin glue it is possible to mix the adhesive with an antibiotic combination, which provides for extremely high local concentrations of antibiotic agents by slow release from the fibrin clot.

Introduction

After its successful employment in abdominal surgery [5, 7, 9], fibrin glue now offers a new range of application by its utilization as a physiological carrier substance of antibiotics. The response of fibrin to the aerobic and anaerobic bacterial invasion caused by an inflammatory process in the free peritoneal cavity is still poorly defined [1, 2]. Since fibrin must be regarded as a culture medium for pathogens [10], it is natural to demand that it should be mixed with an antibiotic combination for the application in a contaminated environment. The object of our animal experiment was to investigate the release time of an antibiotic combination from a fibrin-antibiotic complex.

Previous studies for the investigation of clot rigidity, clotting time, and release time of various antibiotic combinations from a fibrin clot had shown that among the

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
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six tested combinations the one of clindamycin and cefotaxim had the longest release time. No significant differences were found in clot rigidity and clotting time. The present study was carried out to validate these results in vivo.

Material and Methods

One hundred milligrams cefotaxim and 100 mg clindamycin hydrochloride, both dry substances, were mixed with thrombin 500. Then this antibiotic-thrombin mixture was dissolved in 2 ml 3 000 IU aprotinin-calcium and, after warming to 37°C, filled into a Duploject system together with 2 ml of the warmed fibrin seal, which had previously been deep frozen. Now we pipetted four equal portions of 1 ml into Eppendorf test tubes. After consolidation the clots were weighed, one of them was kept as a blank to recheck, and three were used in the animal experiment.

We experimented with 15 Wistar rats weighing 350 g each. For the implantation of the fibrin-antibiotic clot an operative anaesthesia was obtained with 100 mg/kg BW Ketamin and 16 mg/kg BW Rompun. After shaving and disinfection with Merfen we implanted the fibrin clot through a 1-cm midline incision into the peritoneal cavity. The wound was closed in two layers. We removed the clots in groups of three animals after 1, 3, 5, 8 and 24 h under the anaesthesia described above and under sterile conditions.

The fibrin clot, which had been mixed with 25 mg clindamycin hydrochloride and 25 mg cefotaxim before the implantation into the peritoneal cavity of the rats, was added to 25 ml buffer after the explantation and stored for 24 h at 4°C. Pre-experimental studies had shown that more than 95% of the antibiotic agent was released to the buffer during this period.

The concentration of antibiotic in the buffer was determined by means of the agar diffusion test. The weight of the fibrin clots ranged from 950 to 980 mg, which means that the amount of antibiotic measured in the buffer corresponded with the activity per gram of the fibrin clot.

An appropriate agar-containing culture medium, which had been cooled down to 50°C after autoclaving, was inoculated with testing bacteria. The bacteria concentration amounted to 106 bacteria/ml agar medium. Petri dishes were filled with 25 mg agar medium each. After the agar medium had set, six holes were imprinted (∅ 5 mm). Three holes were filled with 50 µl of a falling concentration of standard solution. The testing solution was diluted until it corresponded to the expected antibiotic concentration. The antibiotic was released to the agar medium, producing circular inhibiting zones around the testing bacteria, provided that it met sensitive testing bacteria. The diameter of this circle was proportional to the antibiotic concentration and exhibited a straight line on semilogarithmic paper. Then we calculated the values for the standard graph: the antibiotic content of the testing solution was determined by interpolation of the inhibiting zones. As our testing solution contained two different kinds of antibiotics, we had to select two special testing bacteria, each of them resistant to one of the antibiotics, in order to allow measurements of the antibiotic concentration without any disturbing influence.

Escherichia coli was used to examine cefotaxim release. Since *E. coli* is not affected by clindamycin, the measured inhibiting zone was entirely due to the effect

of cefotaxim. To determine the diffusion of clindamycin we used *Staphylococcus epidermidis*, which is not influenced by cefotaxim, so that the measured inhibiting zone could be entirely attributed to the effect of clindamycin.

Standard concentrations were 20 µg/litre, 10 µg/litre and 50 µg/litre. The quantity per imprinted hole was 50 µg, so that the amount of antibiotic was 1 µg, 0.5 µg and 0.25 µg. The testing solution was diluted until the expected amount of antibiotic corresponded to the concentration of the standard solution: The 1-h result was diluted 1:20, the 3-h result 1:10, and the 5-h result 1:1. The 8- and 24-h results were instilled undiluted. The inhibiting zones were read with an Antibiotic Zone Reader, an optical instrument with an accuracy of 0.1 mm. The antibiotic release was determined separately for each of the two antibiotics incorporated in the fibrin seal. The values set out in Figures 1 and 2 are the mean values of the quantities released from the three simultaneously explanted fibrin-antibiotic polymeres.

Results

After 1 h we still found 12.15 mg clindamycin in the clot, this is about half of the initial quantity. The detectable amount of clindamycin was 1.45 mg after 3 h, 0.9 after 5 h, and 0.62 mg after 8 h; after 24 h the value dropped to 0.05 mg (Fig. 1). The kinetics of release was similar in the case of cefotaxim: The 1-h value was below that of clindamycin (8.44 mg); after 3, 5, 8 and 24 h, however, we found about the same quantities (Fig. 2). This means that with an initial dose of 25 mg clindamycin and 25 mg cefotaxim, 29.4 mg is released during the 1st h, and that after 24 h there is still 0.1 mg of these antibiotics released and acting upon the environment.

Discussion

The broad range of application of fibrin glue in abdominal surgery [5, 7, 9] suggested that the primary sealing of colonic anastomoses might be helpful in reducing the incidence of anastomotic dehiscence [3, 6, 8]. Anastomotic dehiscence is caused by internal and external contamination, followed by abscess formation and disseminated parietal necroses, and the respective inflammatory metaplastic repair processes.

As fibrin must be considered a potential culture medium for bacterial pathogens [1, 10], it seems to be reasonable to mix it with an antibiotic combination with a broad spectrum of activity when used for the administration to a contaminated anastomosis.

The selection of antibiotics must mainly be determined by the pathogens present in the intestinal flora. We chose a combination of clindamycin and cefotaxim, with the latter being exchangeable for mezlocillin with regard to release time, clotting time and rigidity. Clindamycin was preferred to other preparations, because its effect can more easily be examined than that of other anaerobic antibiotics. Besides, it has hardly any negative influence on the properties of fibrin glue, whereas other antibiotic combinations were found to have a considerably negative effect on either clotting time or rigidity of the fibrin glue. With no other fibrin-antibiotic complex

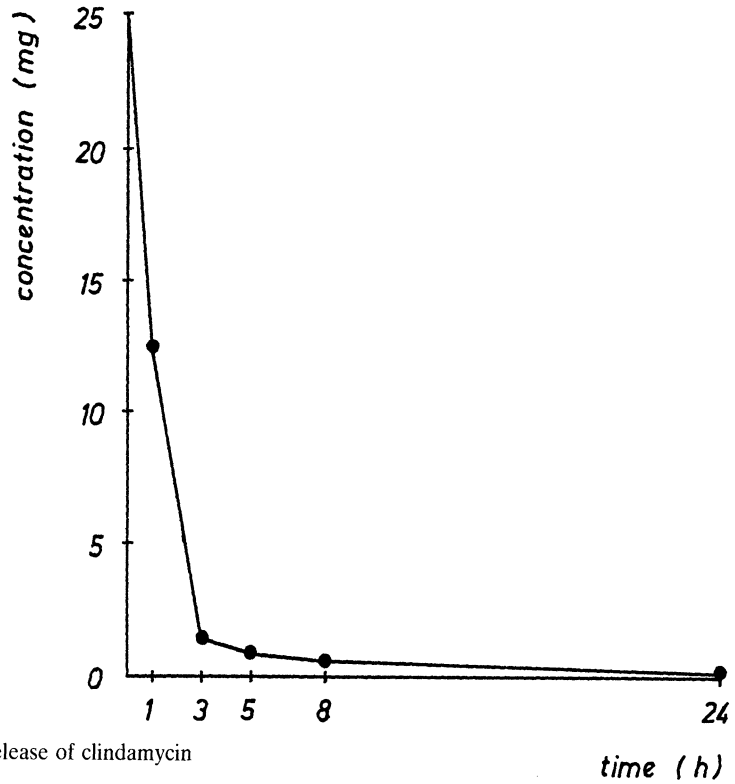

Fig. 1. Kinetics of release of clindamycin

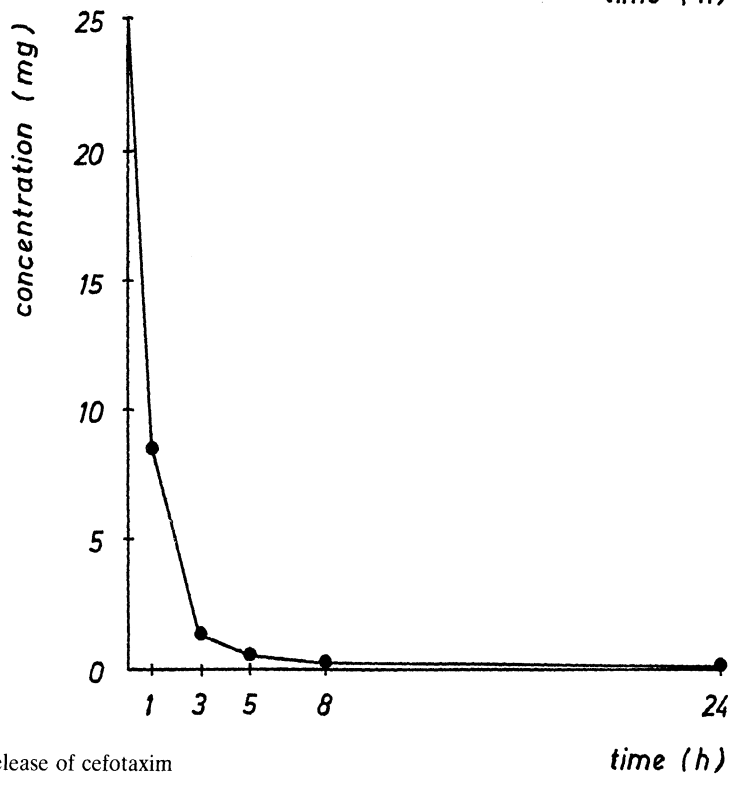

Fig. 2. Kinetics of release of cefotaxim

was it possible to maintain high concentrations of active substance within the implantation area for a prolonged period. Apart from making an anastomosis impervious to gas and liquid, the fibrin glue can also serve to render a high-risk colonic segment aseptic by a high level of active antibiotic agent. When 25 mg clindamycin and 25 mg cefotaxim/ml are added to the fibrin glue, the level of active substance at the site of application is still high enough to have an antibacterial effect after 24 h. With a fibrin-antibiotic complex it is possible to achieve concentrations of antibiotic agents at the site of infection in the free peritoneal cavity that cannot be achieved by any conventional method of administration without exposing the organism to toxic doses.

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Haemostatic Effect of Fibrin Sealant in Patients with Congenital and Acquired Bleeding Disorders

F. BAUDO and F. de CATALDO

Key words: bleeding disorders; tooth extraction; local hemostasis

Abstract

Fibrin sealant was used for local hemostasis in 405 patients with various hemostatic disorders (thrombocytopenia, chronic liver disease, hemophilia A and B, von Willebrand's disease and oral anticoagulants) undergoing tooth extraction. Prophylactic replacement therapy (platelets or plasma concentrates) and antifibrinolytic agents were not administered. Oral anticoagulants were not discontinued. Minor postextraction bleeding occurred only in severe hemophilia A and occasionally in the oral anticoagulant group.

Introduction

Fibrin sealant has been used for its local hemostatic effect in various clinical conditions, in patients with either normal or abnormal hemostasis. Fields of application range widely: cardiac and vascular surgery [1, 2], hepatectomy [3], neurosurgery [4], orthopedic surgery [5], stomatology [6] and otorhinolaryngology [7].

Tooth extraction in patients with hereditary and acquired hemostatic disorders requires specific replacement therapy in order to avoid severe bleeding. It is therefore particularly appropriate to demonstrate the hemostatic effect of this product when locally applied. We shall discuss our experience with 405 patients with various types of bleeding disorders who underwent tooth extraction.

Patients

A total of 405 patients undergoing tooth extraction (for diagnosis and relevant laboratory data, see tables) have been treated. Of these, 150 patients had multiple extractions in the same session. Prophylactic replacement therapy with platelets or plasma concentrates and antifibrinolytic agents was not given. Oral anticoagulants were not discontinued.

Fibrin Sealant in Operative Medicine
General Surgery and Abdominal Surgery – Vol. 6
Edited by G. Schlag, H. Redl
© Springer-Verlag Berlin Heidelberg 1986

Table 1. Tissucol in tooth extractions in patients with chronic liver disease and thrombocytopenia

Patients	33	
Platelets	30–90 × 10^9/l	($\bar{x}$ 65)
Normotest	18%–35%	($\bar{x}$ 24)
Extractions	62 (1–6/patient)	
Prophylaxis	0	
Bleeding complications	0	

Technique

Fibrin sealant was applied into the alveolar cavity that was successively filled with collagen felt (B. Braun AG, Melsungen, FRG) kept in place by suture.

Results and Comments

Data on patients affected by chronic liver disease and acquired hypoprothrombinemia are given in Table 1. There were 62 extractions in 33 patients, 18 of whom had multiple extractions. The platelet count was 30–90 × 10^9/l, and the Normotest values ranged from 18% to 35%. No prophylactic replacement therapy with concentrates (prothrombin complex or/and platelets) was given; no bleeding occurred.

Data on patients affected by thrombocytopenia are given in Table 2. There were 1 case of bone marrow hypoplasia, 6 chronic lymphocytic leukemia, 7 acute nonlymphocytic leukemia, and 4 chronic idiopathic thrombocytopenia. The platelet count was 10–70 × 10^9/l. No prophylaxis was given and no bleeding occurred.

Table 2. Tissucol in tooth extractions in thrombocytopenia

Cases (*n*)	Diagnosis	Platelets (× 10^9/l)	Extractions	Bleeding complications
1	Bone marrow hypoplasia	20–32	9 (in 4 sessions)	0
6	Chronic lymphocytic leukemia	10–40	16	0
7	Acute non lymphocytic leukemia	15–45	17	0
4	Chronic idiopathic thrombocytopenia	40–70	6	0

Data on patients on oral anticoagulant therapy are in Table 3. There were 319 patients with prosthetic heart valves who had 478 extractions; 108 of them had multiple extractions. In these patients the oral anticoagulant therapy was not discontinued because of the high risk of thromboembolic complications. The

Table 3. Tissucol in tooth extractions in patients on oral anticoagulants

Patients	319
Thrombotest	5%–13%
Extractions	478 (1–5/patient)
Bleeding complications	25
Replacement therapy[a]	2

[a] Single infusion of "prothrombin" concentrate 10 u/kg

Table 4. Tissucol in tooth extractions in hemophilia and von Willebrand's disease

Patients (*n*)	Factor deficiency	u/ml	Extractions	Bleeding complications	Replacement therapy
12	VIII	<0.01	29	7	7[a]
16[b]	VIII	<0.10	24	1	0
1	IX	<0.01	1	0	–
6	vW	<0.05	7	0	–

vW, von Willebrand

[a] Single infusion of Factor VIII concentrate (15 u/kg)

[b] A patient with chronic liver disease (Normotest 30%, platelets 90×10^9/l)

therapy was monitored by the Thrombotest mantained in the therapeutic range (5%–13%). While 25 patients had bleeding, only two required replacement therapy: a single infusion of prothrombincomplex concentrate (10 u/kg). In the remaining 23 patients bleeding ceased after the stitches were removed.

Data on hereditary bleeding disorders (hemophilia and von Willebrand's disease) are in Table 4. These were 12 cases of severe hemophilia A (factor VIII: $C < 1\%$), 16 mild hemophilia A (Factor VIII: $C < 10\%$), 1 severe hemophilia B (Factor IX: $C < 1\%$), 6 von Willebrand (bleeding time $> 20'$). Bleeding occurred in 7 severe hemophiliacs 24–28 h after extraction and was controlled by removal of the stitches and low dose replacement therapy (single infusion of 15 u/kg of Factor VIII concentrate). Tooth extraction was uneventful in mild hemophilia and von Willebrand patients.

Tooth extraction in these clinical settings is a remarkable challange to the hemostatic mechanism. These data substantiate the efficacy of Tissucol in controlling traumatic bleeding when hemostasis is impaired.

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