Use of microfibrillar collagen as a topical hemostatic agent in brain tissue

JOHN D. RYBOCK, M.D., AND DON M. LONG, M.D.
Department of Neurological Surgery, Johns Hopkins Hospital, Baltimore, Maryland

Microfibrillar collagen, a recently introduced topical hemostatic agent, was used to obtain hemostasis in suction-evacuation lesions of canine cortex. Gelatin foam was used as a control in identical lesions on the opposite side. Microfibrillar collagen was found to be faster acting and more effective than gelatin foam. Histological evaluation of the lesions at 2, 4, and 6 months postoperatively showed no significant difference in the amount or type of tissue reaction to the two agents.

KEY WORDS • hemostatic agent • microfibrillar collagen • gelatin foam

The hemostatic techniques available to the neurosurgeon are markedly limited by the nature of the central nervous system and the location of the hemorrhage. Proximal ligation, chemical cautery, packing, and suction drainage are of little use in hemorrhage related to the brain and spinal cord. Magnification and bipolar cautery have proved highly effective in certain situations, but the techniques available for control of diffuse, small-vessel bleeding are neither completely reliable nor always effective.

Multiple surface hemostatic agents have been tested, but gelatin foam, introduced over a quarter of a century ago, remains the standard agent. Recently, a new agent, microfibrillar collagen (MFC), has been developed; initial work suggests that it can provide more effective hemostasis than current agents.  

This project was primarily undertaken to assess the long-term effects of applying MFC to neural tissue; in the process, we have been able to observe its effectiveness and unusual handling characteristics.

Materials and Methods

Microfibrillar collagen is prepared from purified bovine corium. It is a water-insoluble partial acid salt of collagen, processed into microcrystals of submicron size. The resultant product is a dry, fluffy white substance which is self-adherent, such that a large bolus can be easily transferred with smooth forceps. This is the “flour form.” It is also available in a “sheet form,” which is a non-woven web formed by compression of the “flour form.”

Experimental Injury

Twelve mongrel dogs weighing 10 to 15 kg were used in the experiments. Anesthesia was induced with pentobarbital sodium, and a 10-cm midline excision was made. The galea was incised and the temporalis muscles and pericranium were stripped laterally. Bilateral symmetrical craniotomies were made by

*Avitene microfibrillar collagen hemostat supplied by Avicon, Inc., Fort Worth, Texas.
crown trephination, burr-hole perforation with rongeur enlargement of the defect, or flap formation with an electric craniotome. In some cases, the bone flap or fragments were replaced before closure.

The dura was opened in a cruciate manner and the dural flaps electrocoagulated. Pial incisions were made with the cold scalpel and suction-evacuation of the underlying cortex was carried out.

Application of Hemostatic Agents

We attempted to obtain hemostasis with MFC alone on one side and gelatin foam alone on the other. Only if several attempts failed did we use more standard techniques such as bone wax or bipolar coagulation.

A plug of the appropriate hemostatic agent was placed in the bleeding cortical defect and suction applied through a wet pledget. When hemostasis was obtained, the pledget was removed, and a sheet of the appropriate hemostatic agent used to cover the dural defect. Bone was replaced if indicated by the protocol and the scalp was closed in layers.

Histological Methods

The dogs were sacrificed in groups at 2, 4, and 6 months postoperatively. The surgical sites were visually inspected and blocks of tissue incorporating the cortical lesion and overlying tissue taken. After fixation in 10% formalin, serial sections were cut and stained with hemotoxylin and eosin and with Masson trichrome stains. Slides were studied without knowledge of treatment type or sacrifice interval. Qualitative assessment of the degree of gliosis, amount of fibrous reaction, and extent of meningocortical cicatrix was made. The presence of foreign material and amount of inflammatory response were also noted.

Results

Handling Characteristics

Microfibrillar collagen was noted to have an initially frustrating affinity for moist surfaces. If gloves and instruments were not dry, large clumps of MFC adhered to them and could not be shaken off. There is no need, however, for anything except forceps tips to contact the material because of its self-adherence. Either drying the tips with a sponge or simply wiping the excess material off the forceps tips periodically alleviated this problem. This affinity for moist surfaces was also apparent when MFC was applied to a bleeding surface. It immediately conformed to the surface and was not dislodged by irrigation or gentle suctioning. The surface layers of material could be easily teased away with suction, but a thin, hemostatically effective layer remained adherent to the wound and vigorous suctioning was required to remove it. Removal of an overlying pledget never dislodged the MFC.

Hemostatic Effectiveness

When using gelatin foam, we routinely found it necessary to use bone wax to control diploic hemorrhage and bipolar coagulation to control bleeding from pial vessels. Except occasionally in case of a large pial vessel, MFC alone was effective in providing hemostasis. When gelatin foam was applied to an actively bleeding surface, it became saturated with blood and increased in volume. Blood continued to filter through the gelatin foam until coagulation occurred within the material. Even then, the gelatin foam continued to leak plasma. The gelatin foam did not form a tight bond with the wound, as demonstrated by leakage of fresh blood between the clot-filled foam and wound and

FIG. 1. Photomicrograph of a 2-month-old MFC-treated lesion demonstrating small islands of dark fibrillary material within an otherwise typical eosinophilic matrix. This material was identified as residual MFC. In specimens taken later postoperatively it was not present; such islands provided the only histological distinction between MFC- and gelatin foam-treated lesions. H & E, × 125.
Microfibrillar collagen for hemostasis

by the ease of dislodgement with pledget removal.

Microfibrillar collagen appeared to achieve hemostasis in an entirely different manner. As soon as it was applied to a wound, it adhered tightly and hemostasis was usually immediate and complete; blood rarely leaked from beneath it. Only over larger vessels did blood penetrate the material. In most cases, the surface remained white and dry. No plasma leaked through. There was no observable swelling of the MFC.

Gross Effects

Eleven dogs were alive at the assigned time of sacrifice. One dog died shortly postoperatively. A reexamination of the wounds demonstrated no hematoma, evidence of increased intracranial pressure, or mass shifts. The dogs were sacrificed and the wounds inspected without knowledge of which side had been treated with MFC. The two sides were compared by appearance and palpation.

In the six animals without bone replacement, two demonstrated qualitatively identical healing bilaterally; in the remaining four, there was a definite increase in the amount of fibrous tissue filling the defect on the MFC-treated side. Whereas a thin diaphragm of scar tissue bridged the defect on the side treated with gelatin foam, a plug of fibrous tissue of about the thickness of the skull was present on the MFC-treated side. There was, however, no increase in extracalvarial or intracranial scar.

In two of the five dogs with bone replacement, healing was symmetrical. In two MFC-treated specimens and one gelatin foam-treated specimen, a more solid fusion relative to its counterpart was present. These flaps showed no movement with digital pressure, while the opposite flaps could be depressed 1 or 2 mm.

There were no cysts, hematomas, or abscesses. The reconstituted dura was delicately bound to the pia at the lesion sites; no difference in the degree or extent of adhesion was noted between the two sides. Skull x-ray films of the 6-month survivors showed no difference from side to side.

Histological Findings

Because of the variability of lesion size and depth and the obliquity of some tissue sections, absolute measurements of factors such as glial concentration and collagen thickness were not taken; relative estimates were made of the degree of gliosis and inflammatory cellular response and the presence of foreign material, fibrous reaction, and meningo-cortical adhesions. The gelatin foam- and MFC-treated lesions were indistinguishable by any criterion except the presence of residual foreign material at 2 months.

Marked glial proliferation was present at the lesion margins in the 2-month specimens; astrocytic processes invaded the lesion, which was filled with a delicate reticulin matrix. Large numbers of macrophages and lymphocytes were present within this matrix. The matrix became progressively coarser in moving away from the lesion; in the most superficial portion, true collagen fibers and abundant fibroblasts were present.

No residual gelatin foam was detected. On the MFC-treated side, small islands of densely eosinophilic fibrous material was present. A strong collagen-type staining reaction without birefringence identified this material as residual MFC. There was no cellular response to this material; the immediately surrounding ground substance was remarkably free of cells (Fig. 1).

At 4 months, a much better defined superficial collagen layer was present. This was clearly separated from the cortical lesion by a mixture of reticulin fibers and arachnoid-type cells. A moderate chronic cell infiltration persisted. Marked gliosis defined the lesion margins. No foreign material was observed.

At 6 months, a well defined collagen layer with few fibroblasts replicated the dura mater. Multiple sheets of delicate cells and scattered fine collagen fibers separated this layer from the cortex; this layer bound the “dura” to the edges of the lesion, simulating an adhesive arachnoiditis. Few chronic inflammatory cells remained and the gliotic margin of the lesion was unremarkable.

Discussion

The hemostatic requirements in neurological surgery are highly exacting. Bone bleeding can be well controlled with wax, and identifiable bleeding vessels can be clipped or coagulated. However, multiple, small-vessel surface bleeding remains difficult to control. Many agents have been used from fibrin
and charged gold leaf to oxidized cellulose and gelatin foam, but none are highly effective or reliable. Our study suggests that MFC is better able to provide hemostasis than the agents in common usage. It is thought that MFC exerts its hemostatic effect largely through promotion of platelet aggregation. This is theoretically appealing, since it is known that platelet adhesion to exposed perivascular collagen, followed by the release reaction and platelet agglutination, is the primary step in physiological hemostasis. Electron microscopic studies have demonstrated that, in vitro, platelets adhere to MFC and undergo the morphological changes of the release reaction. Similar studies of splenic injury in vivo have shown tight approximation of MFC to the site of injury with marked entrapment of platelets.

Microfibrillar collagen has been shown to be effective in the presence of heparinization, as well as intrinsic clotting defects, but much less effective in thrombocytopenia. These observations lend further support to a major role for platelet agglutination, as opposed to activation of the intrinsic clotting system, in MFC-induced hemostasis. The strong affinity of MFC for tissue surfaces may contribute to its effectiveness by physically blocking injured vessels.

The difference between the mechanisms of action of gelatin foam and MFC is obvious in the clinical setting. Gelatin foam swells with blood and is minimally effective until coagulation occurs; even then, its bond to the injured surface is tenuous. On the other hand, MFC usually stops bleeding immediately, does not swell or leak blood, and remains tightly bound to the wound. The bulk of the MFC can be removed by suction-delamination, leaving only a thin, hemostatically effective layer.

Although MFC is a “foreign protein,” the potential for induction of antibodies appears small. Hait found no evidence of skin sensitization or accelerated graft rejection in dogs treated with MFC. Wilkinson, et al., studying patients in whom MFC had been repeatedly applied to skin graft donor sites, found no sensitization by intradermal testing and weak-to-absent sensitization by hemagglutination testing.

In our study, no detectable histological difference was found between MFC- and gelatin foam-treated injuries. While some residual MFC was present at 2 months, it appeared to elicit no cellular response and was gone at 4 months. The degree of gliosis and fibrous reaction was unaffected by the hemostatic agent used. The subjective observation of more complete fibrous and bony healing of the craniotomy defects with MFC was not evaluated histologically for technical reasons. Excessive healing, that is, exostoses or extracalvarial fibrous masses, was not seen.

Abbott and Austen studied the effects of wrapping common iliac anastomoses with no hemostat, with MFC, and with oxidized cellulose. By the criteria of flow rate, histological appearance, and distensibility of the repaired area at intervals up to 4 months, no difference was noted between control and MFC-treated anastomoses, suggesting that MFC does not significantly alter physiological healing. In contrast, oxidized cellulose led to progressive narrowing and stiffening due to excessive scar formation. It thus appears that residual MFC provides an essentially benign, non-fibrogenic scaffold for physiological healing mechanisms.

With gelatin foam serving as the current standard, MFC appears as good or better by most criteria. It is absorbable and does not provoke a major tissue reaction. It is not significantly antigenic or neurotoxic. Smaller quantities remain in the wound if excess material is delaminated and removed. Microfibrillar collagen does not swell significantly within the wound. It is rapidly effective even in the face of intrinsic clotting disorders. Its unusual handling characteristics are counterbalanced by its firm adherence to the wound.

Controlled studies in human patients will be needed before a final conclusion can be reached; however, prior studies and this study indicate that MFC provides a definite improvement in the control of surface bleeding.

References
Microfibrillar collagen for hemostasis

17. Woodhall B: Fibrin foam as a hemostatic agent in rehabilitation neurosurgery. JAMA 126:469-471, 1944

This paper was presented at the Annual Meeting of the American Association of Neurological Surgeons, San Francisco, California, April 6, 1976.

Address reprint requests to: John Rybock, M.D., Department of Neurological Surgery, The Johns Hopkins Hospital, 601 North Broadway, Baltimore, Maryland 21205.