Delivery and Activity of Antimicrobial Drugs Released from Human Fibrin Sealant

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Engraftment and healing of native or cultured skin grafts depend on adherence, vascularization, and control of microbial contamination in the wound bed. Fibrin sealant is a biocompatible polymer that may be used to promote skin engraftment by serving as a delivery vehicle for antimicrobial drugs. Human fibrin sealant (25 mg/ml) was polymerized with antibacterial agents (mupirocin [32 μg/ml], nitrofurazone [0.02% wt/vol], polymyxin B [400 U/ml], or norfloxacin [20 μg/ml]) on nitrocellulose (nc) backing and was prepared as 6 mm diameter discs with skin punches. Discs (n = 6) were applied in the Wet Disc Assay to clinical isolates of *Staphylococcus aureus* (mupirocin, nitrofurazone) or *Pseudomonas aeruginosa* (polymyxin B, norfloxacin). Controls included drug applied to 6 mm paper discs (25 μl) and nitrocellulose discs submerged in each drug, blotted, and applied to bacterial cultures on agar in petri dishes. Data were expressed as zone of clearing (mm diameter ± SEM) after overnight incubation at 35° C. Significant differences (ANOVA and Tukey's test, $p < 0.05$) were found for each drug released from the disc of fibrin sealant compared with other vehicles. Release from filter paper discs compared with nitrocellulose was significant for nitrofurazone and norfloxacin. Serial transfer of fibrin discs to fresh bacterial cultures after 24 hours showed no zones of clearing. The data show that fibrin sealant releases topical drugs with no inhibition of antimicrobial activity on burn organisms. Greater zones of clearing from fibrin sealant may result from passive fluid retention or from active binding to fibrin followed by protease digestion by burn organisms. These results suggest that fibrin sealant can act locally for short-term delivery of antimicrobial agents to wounds that may reduce destruction of cultured or native skin grafts by microbial contamination. (J BURN CARE REHABIL 1994;15:251-5)

Management of microbial contamination is an essential component of successful grafting of native or cultured skin for burn wound closure. Fibrin sealant is a biocompatible polymer that may be used as a vehicle for local delivery of antimicrobial drugs.[1,2] Clinical use of autologous or single donor fibrin sealant has been demonstrated for attachment of skin grafts,[3] repair of traumatic injuries to liver or spleen,[4-6] reduction of hemorrhagic sequelae in vascular surgery of great vessels, and reduction of the probability of peritonitis in bowel operations.[7] Allogeneic fibrin has also been demonstrated to be useful for these purposes, but its potential for transmission of disease has curtailed its commercial availability.

Polymerized fibrin is a glycoprotein that forms a semipermeable gel through which soluble molecules may diffuse but through which cells or microorganisms cannot penetrate mechanically. Pore size of the gel is dependent on concentration of fibrinogen, which is approximately 3 mg/ml in plasma and may be regulated in surgical procedures to relatively high concentrations that form rapidly into dense, vulcanized plugs or sheets. Drug delivery from fibrin has been hypothesized to result from ionic bonding of carbohydrate to added compounds or by passive entrapment of soluble compounds in the aqueous phase

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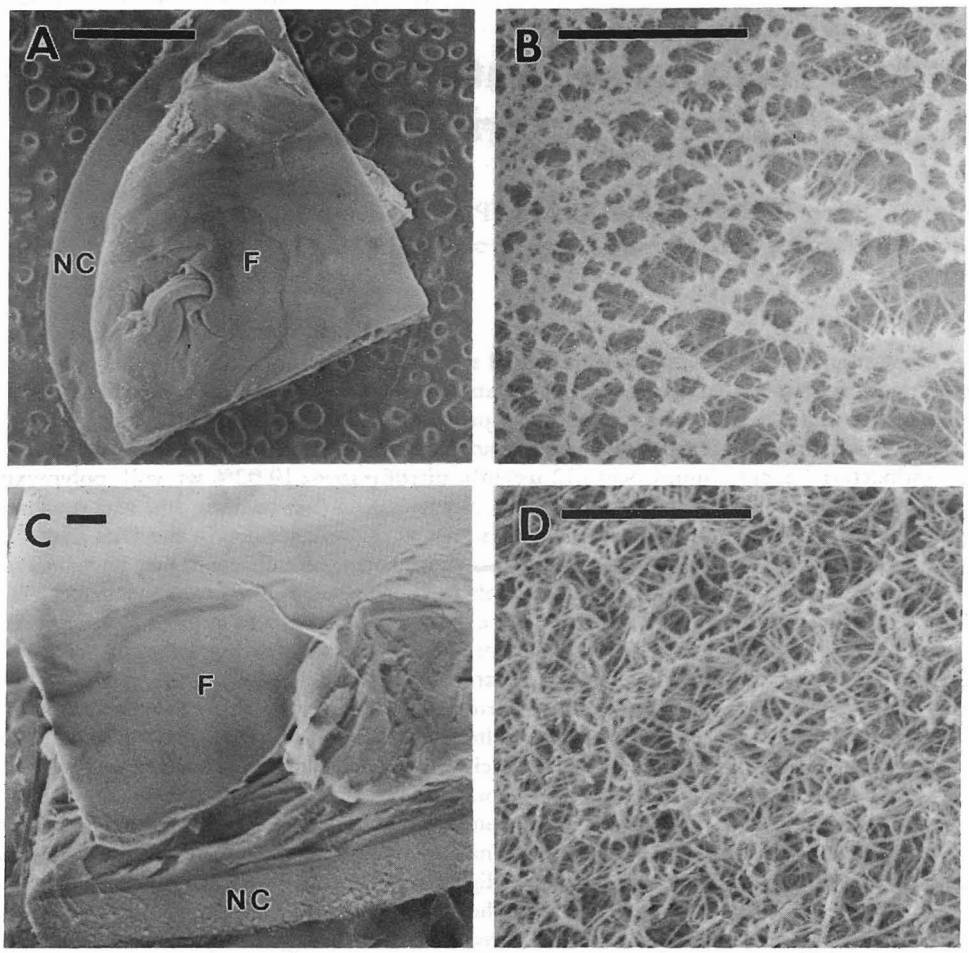

Figure 1. Scanning electron micrographs of discs of fibrin sealant (25 mg/ml) prepared on nitrocellulose paper. **A,** Low magnification image of one quarter of fibrin disc *(F)* on 6 mm punch of nitrocellulose paper *(NC)*. Fibrin has contracted radially. **B,** Surface structure of fibrin disc showing reticulated fiber network joined by aggregations of fibers to form pores 1 to 10 μm in diameter. **C,** Transverse section of fibrin disc *(F)* on nitrocelluose *(NC)* cut at perpendicular axes. Perceived thickness of disc after preparation for microscopy is approximately 750 μm. **D,** Transverse surface of cut fibrin disc showing that disc consists of dense mat of fibers less than 1 μm in diameter, which results in pore size at this surface of approximately 1 to 3 μm. Scale bars: **A,** 1.0 mm; **B** and **D,** 10 μm; **C,** 0.1 mm.

Table. Antimicrobial drugs and burn organisms

Drug	Final concentration	Organism
Mupirocin	32 μg/ml	*Staphylococcus aureus*
Nitrofurazone	0.02% (wt/vol)	*Staphylococcus aureus*
Polymyxin B	400 U/ml	*Pseudomonas aeruginosa*
Norfloxacin	20 μg/ml	*Pseudomonas aeruginosa*

of the fibrin gel.[1,2] The present study tests whether fibrin sealant can act to release antimicrobial drugs that may be useful in management of bacterial contamination of burn wounds. Fibrin polymerized with two antibacterial agents (mupirocin or nitrofurazone) was exposed in the Wet Disc Assay[8] to drug-sensitive cultures of *Staphylococcus aureus,* or fibrin with two other antibacterial agents (polymyxin B or norfloxacin) to drug-sensitive *Pseudomonas aeruginosa.* Compared with drug release from filter paper or nitrocellulose paper, the fibrin vehicle produced the largest zones of clearing in bacterial cultures and was significantly different from other vehicles in all tests performed. However, serial transfer of fibrin-drug discs to fresh microbial cultures produced no zone of clearing. These results demonstrate that fibrin sealant releases antimicrobial agents without loss of

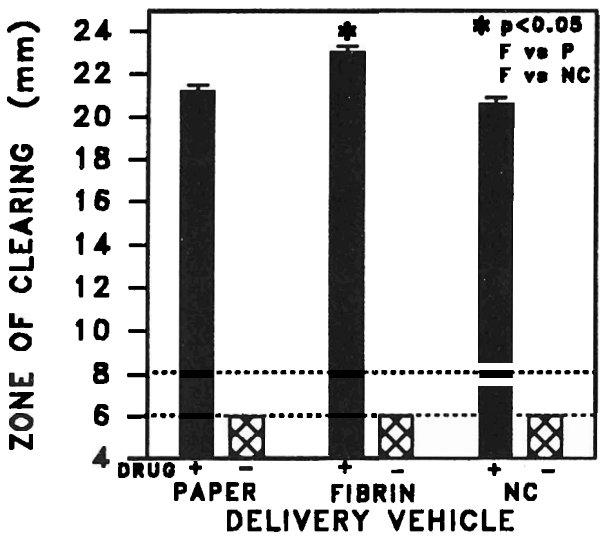

Figure 2. Plot of mupirocin release versus zone of clearing on *Staphylococcus aureus*. Release of 32 μg/ml mupirocin *(solid bars)* from fibrin sealant *(F)* is statistically different compared with vehicle discs of filter paper *(P)* or nitrocellulose *(NC)* paper, although absolute values of differences are small. Discs containing buffer without drug produced no zone from any of the delivery vehicles *(cross-hatched bars)*. *Dashed lines* indicate disc size (6 mm) and zone of clearing considered effective (8 mm).

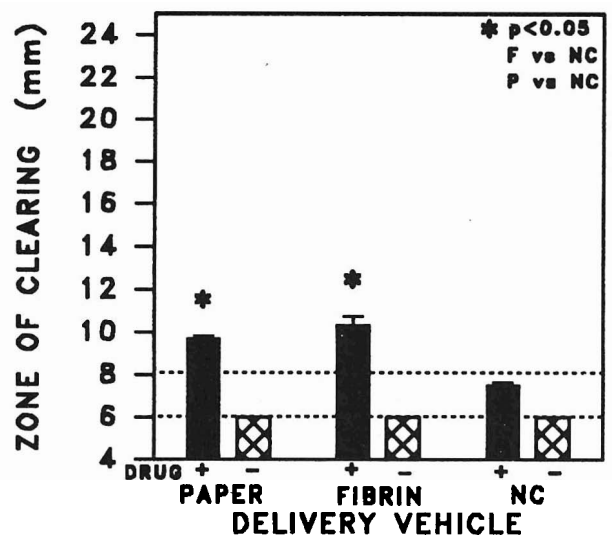

Figure 3. Plot of nitrofurazone release versus zone of clearing on *Staphylococcus aureus*. Release of 0.02% (wt/vol) nitrofurazone from fibrin sealant *(F)*, and vehicle discs of filter paper *(P)* are different statistically from other groups. Release of nitrofurazone from nitrocellulose *(NC)* paper is determined "not effective" by criteria of Wet Disc Assay. Discs containing buffer without drug produced no zone from any of the delivery vehicles *(cross-hatched bars)*. *Dashed lines* indicate disc size (6 mm) and zone of clearing considered effective (8 mm).

activity and that release of antimicrobial agents from fibrin sealant is rapid and short-term. Based on these findings, fibrin sealant may be considered for short-term drug delivery with native or cultured skin grafts.

METHODS AND MATERIAL

Preparation of Fibrin Sealant Discs with Antimicrobial Drugs. Fibrinogen (generously donated by the American Red Cross) was mixed with antimicrobial drugs to result in a solution containing 25 mg/ml fibrinogen and twice the final concentration of antimicrobial drug. A solution of thrombin (250 U/ml) and calcium chloride (45 mmol/L) was prepared, and both solutions were simultaneously dispensed at a volume to area ratio of 0.25 cc/cm^2 onto nitrocellulose discs in 24-well tissue culture plates. After polymerization for 30 minutes, 6 mm discs of fibrin sealant with drug were prepared with skin punches, removed from the wells, blotted to remove excess fluid, and tested in the Wet Disc Assay.[8]

Wet Disc Assays. Fibrin discs with antimicrobial drugs were placed onto petri dishes containing nutrient agar covered with cultures of bacterial strains isolated from patients with burns. After incubation overnight at 35° C, zones of clearing in the bacterial cultures were measured in diameter. Zones of clearing that measured 2 mm or greater than the 6 mm test disc (8 mm total diameter) were scored as effective against test organisms. Fibrin discs that were effective were transferred serially to freshly prepared microbial cultures of the same strain to test for continued drug release. Controls included standard application of 25 μl of drug solutions to 6 mm discs of filter paper or 6 mm discs of nitrocellulose paper only that had been submerged in experimental solutions of antimicrobial agents, blotted, and applied to bacterial cultures. All conditions tested in the Wet Disc Assay were performed in triplicate in duplicate experiments ($n = 6$).

Drugs, Concentrations and Organisms Tested. The Table summarizes the drugs, final concentrations, and organisms included in this study. Mupirocin at 32 μg/ml and nitrofurazone at 0.02% (wt/vol) were tested on a strain of *Staphylococcus aureus* known to be sensitive to both drugs by previous Wet Disc Assays. Polymyxin B at 400 U/ml and norfloxacin at 20 μg/ml were tested on a sensitive strain of *Pseudomonas aeruginosa*. Differences among experimental substrates for each drug were tested for significance ($p < 0.05$) by Analysis of Variance and Tukey's test.

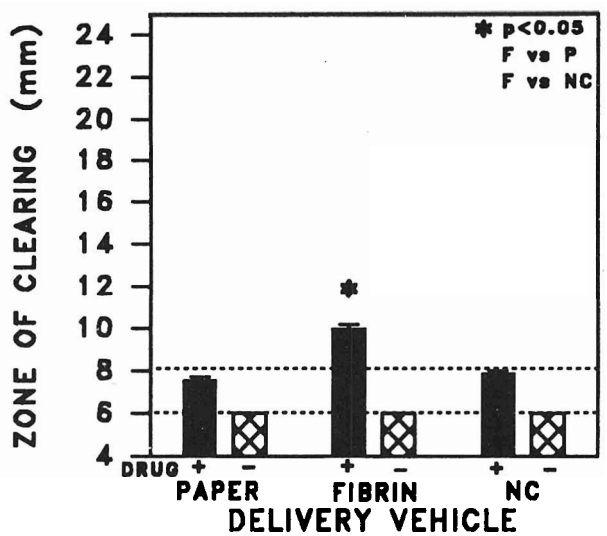

Figure 4. Plot of polymyxin B release versus zone of clearing on *Pseudomonas aeruginosa*. Under these conditions 400 U/ml polymyxin B released from fibrin sealant *(F)* but not from other vehicles *(P,NC)* produces an "effective" zone of clearing. Release from fibrin sealant is statistically different from other vehicles. Discs containing buffer without drug produced no zone from any of the delivery vehicles *(cross-hatched bars)*. *Dashed lines* indicate disc size (6 mm) and zone of clearing considered effective (8 mm).

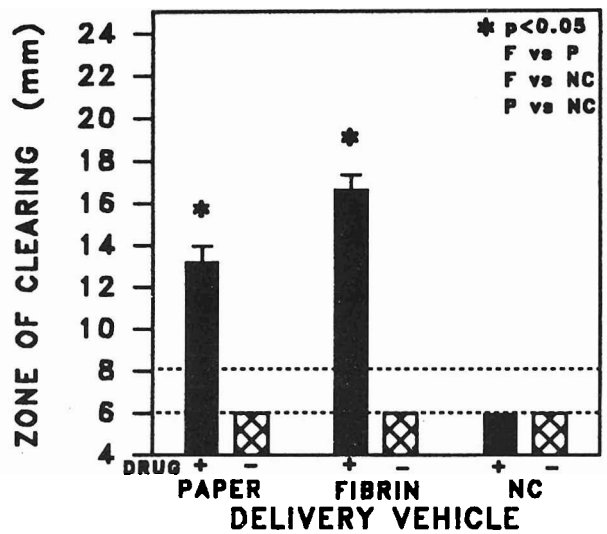

Figure 5. Plot of norfloxacin release versus zone of clearing on *Pseudomonas aeruginosa*. Release of 20 μg/ml norfloxacin from filter paper *(P)* or fibrin sealant *(F)* produce "effective" zones of clearing. No zone is produced from nitrocellulose *(NC)* alone. Zones for each substrate are statistically different from others. Discs containing buffer without drug produced no zone from any of the delivery vehicles *(cross-hatched bars)*. *Dashed lines* indicate disc size (6 mm) and zone of clearing considered effective (8 mm).

RESULTS

Scanning electron micrographs of fibrin discs on nitrocellulose paper are shown in Figure 1. Some shrinkage of the fibrin disc on the nitrocellulose occurs (Figure 1, *A*) as represented by the uniform exposure of nitrocellulose at the edge of the 6 mm punch. Shrinkage may result from contraction of the fibrin after the punch is prepared or from dehydration in preparation for microscopy. Surface structure of the fibrin disc (Figure 1, *B*) shows aggregated fibers with a surface pore size of 1 to 10 μm. Thickness of the fibrin disc measures approximately 0.75 mm (Figure 1, *C*), and the transverse surface of the disc consists of reticulated fibers in the gel with pore size ranging approximately from 1 to 3 μm (Figure 1, *D*).

Mupirocin (32 μg/ml) is highly effective on this strain of *Staphylococcus aureus* after release from each of the three vehicles tested (Figure 2). Although the absolute value of the zone of clearing produced by release from fibrin sealant is not much larger than from filter paper or nitrocellulose, the fibrin zone is significantly different.

Release of 0.02% wt/vol nitrofurazone (Figure 3) shows significant differences for zones resulting from vehicles of filter paper or fibrin compared with nitrocellulose. These zones also show effectiveness against this bacterial strain, but the zone from nitrocellulose alone is scored as not effective.

Activity of 400 U/ml polymyxin B is effective in this test on this strain of *Pseudomonas aeruginosa* only if released from fibrin sealant as shown in Figure 4. The zone of clearing produced from the fibrin disc is significantly different from the two control vehicles.

Figure 5 shows that zones of clearing generated by release of 20 μg/ml norfloxacin from each of the vehicles is significantly different from the others. Release of norfloxacin from fibrin produces the greatest effective zone diameter, filter paper an intermediate and effective zone, and no zone is produced after treatment of nitrocellulose with norfloxacin.

Transfer of fibrin discs with antimicrobial drugs from the initial microbial dishes to freshly prepared dishes containing the same organisms showed no subsequent antimicrobial activity for any of the drugs on either of the organisms (data not shown). Parallel preparations of fibrin/drug discs ($n = 3$) were stored overnight at 4° C and evaluated in the Wet Disc Assay. All fibrin/drug discs demonstrated effective microbial killing, but smaller zones of clearing de-

veloped compared with identical fibrin/drug discs that were not stored (data not shown).

DISCUSSION

Data presented here demonstrate that fibrin sealant releases antimicrobial agents without loss of activity. These findings suggest that fibrin does not chemically inactivate these four antimicrobial drugs. However, the results do not conclusively demonstrate that these drugs are not bound to fibrin with subsequent release by degradation of polymerized fibrin by microbial proteases. In fact, enhanced drug release from fibrin by the actions of microbial proteases is supported by observations that fibrin discs were not visible on the nitrocellulose paper at the time of serial transfer to test for sustained release of drug activity. Although the microbial load in a burn wound at the time of grafting would be expected to be very much lower than in this lytic assay, these results suggest that drug release would be effective clinically only for relatively short periods of time (e.g., 1 to 2 days). Alternatively, these results may be interpreted that drug retention in fibrin is passive entrapment in the aqueous phase of the fibrin gel and that release proceeds by simple diffusion. This possibility may be tested in future studies by serial elution of test drugs from polymerized fibrin in an aseptic solution and evaluation of the eluate for antimicrobial activity. Preliminary studies by ELISA testing with peptide growth factors polymerized in fibrin sealant suggest that release of these relatively large molecules from fibrin proceeds by first-order biochemical kinetics.[9]

Although the clinical utility of fibrin sealant has been demonstrated repeatedly,[10-14] complications, including fibrosis, have also been reported.[15] The biologic response to the implanted polymer would be expected to result, in part, from density of the fibrin and the genetic disposition of an individual to form scar. Although the latter factor is not as easily controlled, the density of the fibrin sealant is dependent completely on the concentration of the fibrinogen solution. The higher the density of the polymerized fibrin, the more mass per unit volume must be degraded by inflammatory and parenchymal cells, and the longer the time for fibrosis to proceed. Therefore, although higher concentrations of fibrin may be more effective for accomplishment of hemostasis, lower concentrations may produce less total inflammation and scar. Similarly, release of drugs such as those tested here would be expected to be dependent on the initial concentration of fibrinogen. Fibrinogen concentration is directly proportional to density of polymerized fibrin and is inversely proportional to the aqueous phase of the fibrin gel. Therefore, if drug retention occurs by passive entrapment of soluble compounds in the aqueous phase of the fibrin gel, then quantitative drug loading and release would be predicted to be inversely proportional to fibrin concentration. These relationships remain to be investigated in future studies.

Delivery of antimicrobial drugs from fibrin sealant may provide rapid and short-term activity against microbial contamination in wounds treated with native or cultured skin grafts. Because of the increased tendency for microbial contamination of cultured skin, application of fibrin sealant containing antimicrobial drugs may reduce microbial colonization during vascularization of the graft, allow greater epithelial survival, and promote more rapid and complete wound healing.

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