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The objective of the present study was to determine the effects of a fibrin sealant (Tisseel) on wound healing. The sealant tested is produced from human plasma. This study involved 12 adult beagle dogs, all of which underwent creation of full-thickness palatal wounds. Six animals received immediate application of a fibrin sealant as a wound dressing.

Polaroid photographs were taken immediately after creation of the palatal wounds and at regular intervals during the healing period. Six measurements were obtained from these photographs.

Longitudinal comparisons of the profile of the mean curves for the relative changes during the 38-day study period indicated that the six parameters investigated have similar curve profiles in both the fibrin-sealant treated wounds and the untreated control wounds. The overall direction of healing was not significantly different in the two groups. However, comparisons of the curve magnitudes indicated that there was a significantly greater reduction in wound size in the fibrin sealant treated group.

Cross-sectional comparisons (analysis of variance General Linear Models procedure) indicated that in the first 2 weeks there was a relatively greater reduction in wound width in the fibrin sealant treated group of animals. No significant differences were present at the end of the experimental period.

The findings of this study indicated that the fibrin sealant when used as a dressing on palatal wounds of adult beagle dogs resulted in greater wound contraction in the early stages of wound healing.

Those involved in the different aspects of maxillofacial surgery and cleft palate rehabilitation realize that surgical management of the oral structures could result in the denudation of palatal bone. The presence of denuded bone has been related to increase incidence of infection, wound contraction, and scar formation, each of which can cause inhibition of the normal growth of the adjacent structures.

There are two important aspects of palatal wound healing: first, the need to enhance, facilitate, and stimulate the process by which the denuded bone is reinvested with mucoperiosteum, and second, the need to inhibit skeletal deformation to minimize the undesirable effects on maxillary size and shape. As a result, there
is a need both to evaluate different biological materials which have the potential for covering the denuded palatal bone at the time of surgery and to enhance wound healing during the immediate postsurgical period without adversely influencing adjacent structures. The use of such material should be particularly advantageous during the surgical repair of clefts of the lip and palate.

Several different procedures have been suggested to minimize any untoward effects of wound healing on adjacent structures during palatal surgery. These procedures have included pharmacologic treatment of wounds to reduce contraction, changes in surgical design to minimize the amount or alter the location of the denuded bone, and finally, changes in the timing of the surgical intervention to minimize its effects on growth. A detailed literature review which sheds a historical perspective on wound healing was published by Kremenak (1984).

A fibrin sealant produced from human plasma has been found to be effective in face-to-face sealing of tissue, wound sealing, and in establishing hemostasis. The fibrin sealant Tisseel is a two-component system. The sealant proper contains highly concentrated fibrinogen and Factor XIII, in addition to other plasma proteins, such as albumin and cold-insoluble globulin. The second component is a solution of thrombin and calcium chloride. A fibrinolysis inhibitor is contained in one of the two solutions. The two components of the sealant can be applied to the wound surface by several methods either simultaneously or consecutively (Redl et al, 1980).

Matras (1982) and Wepner et al (1982) outlined some of the uses of the fibrin sealant including nerve repair, bone grafting, hemostasis during liver surgery, and skin grafting. The actions of the fibrin sealant are to provide hemostasis, provide adhesion, improve vascularization, and accelerate healing.

It should be noted that the reconstituted fibrin sealant resembles a blood clot minus its cellular components. The application of such a sealant on fresh wounds should provide an immediate and homogeneous fibrin covering over the denuded palatal bone. Some of the possible benefits of sealing the bone and the wound surfaces are to minimize infection, minimize discomfort that accompanies the presence of denuded bone, and provide proliferating cells at the wound margins with an immediate and homogeneous fibrin matrix that may enhance surface wound healing.

In general, the effects of different types of pharmacologic or surgical treatments on wound healing should be examined from two perspectives: (1) the immediate effects (5 to 7 weeks) of the procedure on wound contraction and healing, and (2) the long-term effects of the procedure on the adjacent structures in growing and nongrowing individuals.

The objective of the present study is to determine the effect of a fibrin sealant on the healing of palatal wounds in adult beagle dogs over a 38 day period. The relative changes in surface wound contraction were evaluated in two groups of dogs that underwent surgical excision of a strip of palatal mucoperiosteum. The wounds in one of the two experimental groups were treated with the fibrin sealant.

**MATERIALS AND METHODS**

This study involved 12 adult beagle dogs, each of which underwent creation of a full thickness palatal excision wound. Six animals received application of the fibrin sealant Tisseel as a wound dressing within 10 minutes of the time of excision. The other six animals were used as experimental controls. They received no wound dressing after their operations. All other conditions were kept constant. Xylazine was used as a sedating premedication and Biotol was used for induction and maintenance of general anesthesia.

**Placement of Tattoos and Wound Size**

The margins of the planned wound were tattooed using a hypodermic needle (25 gauge) and India ink. Three pairs of tattoo points delineated the lateral wound margins. One pair of tattoo points marked the anterior and posterior wound margins (Fig. 1). The anterior and posterior tattoos were placed just anterior to the crest of the 5th ruga and just posterior to the crest of the 7th ruga. The distance between the anterior and posterior tattoo points averaged 15 mm with a range of 13.5 and 17.0 mm. The lateral tattoos were 6.5 mm apart. The points for the tattoos were marked by a bow divider. The divider was centered on the median raphe. Tattoo ink was

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1Tisseel® Fibrin Seal, Immuno AG, Vienna, Austria
WOUND MEASUREMENTS:

Widths = A-B, C-D, and E-F in millimeters.

Length = G-H in millimeters.

Calculated Surface Area = \( \frac{AB + CD + EF}{3} \times GH \) mm²

Traced Surface Area = the margin of each wound was traced, at each period, and the surface area was calculated in mm².

FIGURE 1 Wound measurements

injected into the bleeding puncture-point wounds.

The incisions were placed on the crest of the ruga for the anterior and posterior margins and approximately 0.75 mm medial to the lateral tattoos. This provided for a wound approximately 5.0 mm wide. Using the tattoo points as a guide, a full-thickness palatal wound was created which measured approximately 20 percent of the palatal width at the premolar region. Hemostasis was achieved using direct pressure to the wound.

In the experimental group of 6 animals, a two-component fibrin sealant was applied to the wound within minutes after excision of the palatal mucoperiosteum. The sealant was applied with a Duploject applicator (Redl et al, 1980) which provides a mechanism for simultaneously applying the two components of the sealant. The sealant forms a "clot" which covers the wound surface and adheres to the hard and soft tissues.

Records Collected

Polaroid photographs were taken immediately after creation of the palatal wound and at regular intervals during the healing period. The photographs were taken using an occlusal mirror and a fixed object-to-lens distance, which allowed accurate measurements to be made from the photographs. The photographs were standardized to produce a 1:2, object-to-image ratio. The polaroid photographs were obtained at 13 different time periods namely: pre-surgery, within 10 minutes after surgery, first day after surgery, then at days 2, 3, 7, 9, 11, 17, 22, 29, 32, and 38.

Parameters Measured

The following parameters were measured from the photographs (Fig. 1):


4. Wound length: G–H in mm.

5. Surface area between tattoos calculated as:

\[ \text{Calculated Surface Area} = \frac{AB + CD + EF}{3} \times GH \text{ mm}^2. \]

6. Traced surface area: The wound margin was traced, and the inside area of the wound margin was measured in mm² using a quantimet image analyzer.

Each linear parameter was measured independently by two investigators. The allowable limit for intra- and interexaminer disagreement was arbitrarily established at 0.25 mm. When a discrepancy between the two investigators exceeded this limit, new measurements were made by each. The tracings, as well as the quantimet measurements were independently made by two investigators. When the discrepancy between the two investigators exceeded 5 percent, new tracings were made, and the two closest measurements were averaged.

Evaluation of Absolute vs Relative Changes

Instead of comparing the actual measurements of width, length and surface area of the two groups of animals, the relative change in each dimension was calculated and compared. The relative change, at any period, was calculated as where \( Mo \) is the measurement before surgery and \( Mx \) is the measurement at any subsequent postsurgical time period evaluated. For this study, relative changes in the parameters are considered to be particularly relevant as compared to the absolute changes. This is because differences, however small, in either the wound size or the distances between tattoos might influence the comparisons.
The relative change, on the other hand, converts the absolute value into a percentage change for each wound in each animal regardless of its original size.

Statistical Analyses

Longitudinal Comparisons. The mean growth profile curve for the changes of each parameter was compared between the two experimental groups. In the statistical analysis of the growth curves there are two aspects that need to be evaluated: (1) the shape of the curves, i.e., the slope which describes growth direction. In this respect, the curves might show a parallel relationship indicating that the growth trends are the same. On the other hand, lack of parallelism indicates different growth trends. (2) The magnitude of the curves, i.e., the height of the curve which represents the amount and duration of change with time as measured by the intercepts of each curve. This method of analysis was described in detail by Kleinbaum and Kupper (1978).

Cross Sectional Comparisons. Descriptive statistics for the relative changes in the various parameters were calculated for both groups. The calculations were performed for each of the 12 different time periods evaluated. The analysis of variance General Linear Models procedure was used to compare the different measurements cross sectionally at each time interval. Statistical significance was predetermined at the 0.05 level of confidence.

It should be pointed out that when interpreting the longitudinal and cross sectional results, the longitudinal comparisons take into consideration the cumulative changes that occur in the parameter in the overall time period investigated. The cross sectional data compare points along the continuum of the curve.

RESULTS

Longitudinal Comparisons of the Relative Changes in the Treated and Untreated Wound Groups

The relative changes in the six parameters evaluated were plotted and the six curves are presented in Figures 2 to 7.

Curve Profile Comparisons. The comparisons between the relative changes over the 38 day period indicate that the six parameters investigated have similar curve profiles in the fibrin sealant treated and untreated wounds. This indicates that the overall direction of healing is not significantly different in the two groups.

Curve Magnitude Comparisons. There were statistically significant differences between the fibrin sealant and control groups in the comparisons of the linear measurement of wound width A–B and C–D. These results indicate that

FIGURE 2 Relative changes in wound width A–B
FIGURE 3 Relative changes in wound width C–D

FIGURE 4 Relative changes in wound width E–F

FIGURE 5 Relative changes in wound length G–H
there is a significantly greater overall reduction in the width dimensions of the fibrin sealant treated wounds as compared to the control wounds.

Cross Sectional Comparisons of the Relative Changes

The various parameters were compared at twelve different periods using the analysis of variance. Comparisons of the linear parameters between the treated and control wounds (Table 1) indicated that the greatest differences between the two groups occurred during the first 9 days, particularly in those measurements which describe wound width (A–B, C–D, and E–F). The differences are more pronounced in the middle of the wound (C–D), i.e., this is the area of greatest contraction. Wound length (G–H) showed no statistically significant differences between the treated and control wounds. The differences between the calculated surface area (Table 2) are essentially a reflection of the differences in wound widths.
<table>
<thead>
<tr>
<th>Time Period</th>
<th>A - B</th>
<th>C - D</th>
<th>E - F</th>
<th>G - H</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Tisseel</td>
<td>Control</td>
<td>Tisseel</td>
</tr>
<tr>
<td>Pre S-10 min Postop</td>
<td>-5.0</td>
<td>4.9</td>
<td>-2.2</td>
<td>3.2</td>
</tr>
<tr>
<td>10 min-1 day</td>
<td>-3.5</td>
<td>6.1</td>
<td>2.7</td>
<td>2.9</td>
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<td>4.4</td>
</tr>
<tr>
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<td>-1.9</td>
<td>5.0</td>
<td>4.3</td>
<td>3.9</td>
</tr>
<tr>
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<td>4.7</td>
<td>5.3</td>
<td>3.7</td>
</tr>
<tr>
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<td>3.2</td>
<td>8.3</td>
<td>4.9</td>
<td>3.3</td>
</tr>
<tr>
<td>11-17 days</td>
<td>0.4</td>
<td>4.9</td>
<td>5.6</td>
<td>3.9</td>
</tr>
<tr>
<td>17-22 days</td>
<td>0.4</td>
<td>6.4</td>
<td>6.9</td>
<td>4.2</td>
</tr>
<tr>
<td>22-29 days</td>
<td>-0.4</td>
<td>6.3</td>
<td>5.1</td>
<td>3.6</td>
</tr>
<tr>
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<td>1.5</td>
<td>5.5</td>
<td>4.4</td>
<td>3.4</td>
</tr>
<tr>
<td>32-38 days</td>
<td>1.4</td>
<td>7.4</td>
<td>3.4</td>
<td>4.5</td>
</tr>
</tbody>
</table>

* A negative (−) relative change indicates that the subsequent measurement is on average larger than the initial measurement.
** Significant at the 0.05 level.
† Significant at the 0.01 level or greater.
$\bar{x}$ = mean, SD = standard deviation, P = probability of a statistically significant difference, NS = not significant.
TABLE 2 Relative Changes in the Calculated and Traced Surface Area Wound Measurements

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Control</th>
<th>Tisseel</th>
<th>Control</th>
<th>Tisseel</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>SD</td>
<td>$\bar{x}$</td>
<td>SD</td>
<td></td>
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<tr>
<td>Pre 5-10 min Postop</td>
<td>-5.3</td>
<td>4.4</td>
<td>-6.3</td>
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<td>S*</td>
</tr>
<tr>
<td>10 min-1 day</td>
<td>0.1</td>
<td>5.0</td>
<td>7.0</td>
<td>3.9</td>
<td>S**</td>
</tr>
<tr>
<td>1-2 days</td>
<td>0.8</td>
<td>5.1</td>
<td>9.8</td>
<td>4.1</td>
<td>S</td>
</tr>
<tr>
<td>2-3 days</td>
<td>-0.1</td>
<td>7.1</td>
<td>9.2</td>
<td>4.8</td>
<td>S*</td>
</tr>
<tr>
<td>3-7 days</td>
<td>1.4</td>
<td>8.8</td>
<td>12.8</td>
<td>6.8</td>
<td>S</td>
</tr>
<tr>
<td>7-9 days</td>
<td>4.9</td>
<td>8.4</td>
<td>16.1</td>
<td>6.4</td>
<td>NS</td>
</tr>
<tr>
<td>9-11 days</td>
<td>9.9</td>
<td>10.9</td>
<td>17.0</td>
<td>6.6</td>
<td>NS</td>
</tr>
<tr>
<td>11-17 days</td>
<td>13.5</td>
<td>9.2</td>
<td>20.6</td>
<td>4.8</td>
<td>NS</td>
</tr>
<tr>
<td>17-22 days</td>
<td>16.3</td>
<td>7.6</td>
<td>21.9</td>
<td>5.6</td>
<td>NS</td>
</tr>
<tr>
<td>22-29 days</td>
<td>15.5</td>
<td>8.9</td>
<td>20.4</td>
<td>5.0</td>
<td>NS</td>
</tr>
<tr>
<td>29-32 days</td>
<td>18.0</td>
<td>8.9</td>
<td>19.9</td>
<td>4.6</td>
<td>NS</td>
</tr>
<tr>
<td>32-38 days</td>
<td>14.0</td>
<td>10.7</td>
<td>19.9</td>
<td>4.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level.
** Significant at the 0.01 level or greater.

The lack of significant differences between the two groups of animals in the traced surface area (Table 2) indicates that the changes in the appearance of the wound surfaces are similar in the two groups. This lack of statistical difference might also be the result of the large standard deviation, i.e., variation in the control wounds.

It is of interest to note that by the end of the experimental period (38 days) no significant differences were present between the two groups. This indicates that the effects of the fibrin sealant are more apparent in the early stages of wound healing.

**DISCUSSION**

As indicated in Tables 1 and 2, there is an initial increase in the distance between tattoos after the wound is made, regardless of whether or not a wound dressing was placed. The original increase in wound dimensions might result from either pressure on the wound margins from the gauze sponges used to control bleeding or from an initial retraction or pull on the wound margins in the attempt to control bleeding following the excision of the mucoperiosteum.

After this initial relative increase, wounds treated with fibrin sealant demonstrated a consistently greater contraction in the width measurements A-B, C-D, and E-F until approximately the tenth postoperative day. The center of the wound (C-D) showed the most relative change in both the treated and control wounds. The differences in wound contractions were not as distinct in the measurements of wound length (G-H).

Currently, it is of interest to note that in the control wounds the greatest reduction in wound size occurred after the tenth postoperative day. However, in the fibrin sealant treated group there was a more gradual and consistent change in wound size.

One possible explanation for the more gradual and consistent change in wound size in the treated group is the adhesive properties inherent in the sealant, which are similar to those of a regular blood clot, providing the wound with an immediate and homogeneous fibrin clot. While adhesion is a property of a normal clot, the added adhesive effect of the sealant may explain the differences found in the treated group.

This preliminary study provides some insight into the difficulty of applying the fibrin sealant to superficial, fresh, and bleeding palatal wounds. Although the fibrin sealant causes hemostasis and coagulates in a fairly short period of time, the presence of profuse bleeding does not allow the fibrin sealant to form a clot that completely covers the wound surface. Therefore, the successful application of the fibrin sealant is partly dependent on effective control of bleeding.

The present findings indicate that the applica-
tion of the fibrin sealant results in greater wound surface contraction during the first 11 days after surgery. Further studies are needed to determine the long-term effects of the fibrin sealant on the growth of adjacent structures, i.e., is the effect of the sealant limited to the wound area or does it result in greater constriction of the dental arches? In addition, comparisons of the effects of the fibrin sealant and other materials presently used as wound dressing at the time of cleft palate surgery, such as microfibrillar collagen, should be investigated.

The ultimate objective is to find a wound treatment that can provide optimal palatal wound contraction, rapid and uncomplicated healing, and minimal long-term effects on the growth of adjacent structures.

**CONCLUSION**

The findings from this pilot study indicate that the fibrin sealant Tisseel, when used as a wound dressing on full thickness palatal wounds of adult beagles, results in a greater wound contraction particularly in the first 2 weeks of wound healing. At the end of the experimental period (38 days), no differences were noted in the relative changes of the wound between the two groups. Therefore, it can be concluded that the use of the fibrin sealant enhanced wound contraction during the early stage of the healing process.

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**References**


