



FERRIS MFG. CORP.

16W300 83rd Street • Burr Ridge, IL 60527-5848 • U.S.A.

Direct 630.887.9797 **1.800.POLYMEM** Fax 630.887.1008

info@ferrispolymem.com

JOURNAL OF PAIN, February 2004

Alvin J. Beitz, PhD and Alan R. Kahn, M.D.

- **“The PolyMem Plus[®] dressing showed an analgesic effect in addition to preventing hyperalgesia.”**
- **“Histologic observations indicated that the PolyMem plus[®] dressing reduced the spread of inflammatory cells from the stab wound site into the surrounding muscle.”**
- **“Our results show that there is a robust, reproducible, and statistically significant decrease in both secondary mechanical and thermal hyperalgesia when the stab wounds were wrapped with PolyMem Plus[®] dressing.”**
- **“Useful for both increasing wound healing and reducing pain associated with stab wounds, deep lacerations, surgical incisions, or other types of penetrating injuries.”**

JN0404
ISO 9000 | CE MARK CERTIFIED

www.FERRISPOLYMEM.com

A Polymeric Membrane Dressing With Antinociceptive Properties: Analysis With a Rodent Model of Stab Wound Secondary Hyperalgesia

Alvin J. Beitz,* Allison Newman,* Alan R. Kahn,[†] Timothy Ruggles,* and Laura Eikmeier*

Abstract: The putative antinociceptive properties of a commercially available polymeric membrane dressing were tested by using a hind limb penetrating stab wound model in which secondary hyperalgesia could be evaluated from the hind paw. We examined the responses to mechanical and thermal stimuli applied to the hind paw remote to 2 small penetrating stab wounds of the calf. Application of the polymeric membrane dressing, but not gauze dressing, significantly reduced the development of both mechanical and thermal hyperalgesia induced by the penetrating stab wounds. In addition, animals with stab wounds showed a significant decrease in cage activity, and this decrease was prevented by application of the polymeric dressing. Analysis of spinal cord Fos expression demonstrated that the polymeric membrane, but not gauze, dressing significantly decreased stab wound-induced Fos expression in laminae I to VI of the ipsilateral L3-L5 cord segments. In addition, application of the polymeric membrane, but not gauze, dressing to the hind limb of naïve animals elicited Fos expression in laminae III and IV of the lumbar spinal cord. The data indicate that this model might be useful for evaluation of the mechanisms underlying deep tissue injury-induced secondary hyperalgesia, but they also demonstrate that the polymeric membrane dressing tested is capable of significantly reducing secondary hyperalgesia.

Perspective: Surgery and other types of penetrating wounds cause pain that is not always relieved by opioids and/or less potent analgesics. The present results suggest that the polymeric membrane dressing tested here may be used alone or in conjunction with analgesics to relieve pain caused by penetrating tissue injury.

© 2004 by the American Pain Society

Key words: Mechanical hyperalgesia, thermal hyperalgesia, spinal cord, tissue injury, antinociception, incision.

Pain accompanies most types of penetrating wounds including those caused by bullets, sharp objects, lacerations, and incisions.^{14,20,23,31,34} The extent and quantity of pain in most cases are directly related to the severity of the injury, particularly to deep tissue structures. Most penetrating wound injuries including those caused by surgical incisions are covered with a protective dressing to promote wound healing and to reduce the risk of infection. Although there are a wide variety of commercially available dressings, it is known that the type of wound dressing can play a role in the generation

of localized pain,^{6,36} particularly during dressing changes.^{8,12,32,36} The majority of studies to date have evaluated different dressing types with respect to the pain associated with dressing changes, but not with ongoing pain.^{2,8,12,16,17} Despite the advances that have been made in the treatment of pain caused by surgery, injury, or penetrating wounds, the incidence of pain remains high.^{7,22} The possibility of treating such pain with an antinociceptive dressing alone or in combination with analgesics is an interesting possibility that has received little attention. To our knowledge there have been only a limited number of studies that have examined the possible antinociceptive properties of commercially available dressings in humans,^{15,21} and there are no studies that have tested such properties in animal nociceptive models. The purpose of this study was to evaluate the antinociceptive effects of a polymeric membrane wound dressing by using behavioral tests to determine changes in secondary hyperalgesia and immunohistochemical/histologic analysis to evaluate dressing effects on incisional stab wound-induced spinal cord Fos expression and on tissue morphology at the site of injury. Secondary

Received June 3, 2003; Revised September 4, 2003; Accepted September 15, 2003.

From the *Department of Veterinary Pathobiology and Neuroscience Program, University of Minnesota, St Paul, Minnesota, and [†]Department of Lab Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota.

Address reprint requests to Alvin J. Beitz, PhD, Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, 295 Animal Science/Vet Med Bldg, 1988 Fitch Avenue, St Paul, MN 55108. E-mail: beitz001@tc.umn.edu

1526-5900/\$30.00

© 2004 by the American Pain Society

doi:10.1016/j.jpain.2003.09.003

Table 1. List of All Animal Groups Used for Behavioral Studies Indicating What Procedures Were Done to the Left and Right Hind Limbs

GROUP NUMBER	NUMBER OF ANIMALS	LEFT SURGERY	LEFT TREATMENT	RIGHT SURGERY	RIGHT TREATMENT
1	6	Naive	None	Naive	None
2	6	Sham	Polymeric dressing	Sham	None
3	6	Sham	Gauze dressing	Sham	None
4	6	Sham	Gauze dressing	Sham	Polymeric dressing
5	6	Incisional stab wounds	None	Incisional stab wounds	None
6	6	Incisional stab wounds	None	Incisional stab wounds	Polymeric dressing
7	6	Incisional stab wounds	None	Incisional stab wounds	Gauze dressing
8	6	Incisional stab wounds	Gauze dressing	Incisional stab wounds	Polymeric dressing
9	6	Sham	None	None	None
10	6	Incisional stab wounds	Polymeric dressing	None	Polymeric dressing
11	6	Incisional stab wounds	Gauze dressing	None	Gauze dressing
12	6	Incisional stab wounds	None	None	None

rather than primary hyperalgesia was measured because measurement of primary hyperalgesia required removal of the dressing at each time point tested, whereas measurement of secondary hyperalgesia from the foot did not.

Materials and Methods

The experiments were approved by the University of Minnesota Animal Care and Use Committee and were consistent with the ethical guidelines of the National Institutes of Health and of the International Association for the Study of Pain.

Experimental Animals

Adult, male Sprague-Dawley rats weighing between 250 and 400 g were housed 2 per cage and maintained on a normal 12:12-hour light-dark cycle in a temperature-controlled room (23°C ± 0.5°C) in the Veterinary College research animal facility. Rats had access to food and water ad libitum. The experimental and control groups used in this study are summarized in Tables 1 and 2.

Experimental and Control Dressings

The PolyMem Plus® dressing used in these experiments is a product of the Ferris Manufacturing Corporation

(Burr Ridge, IL). PolyMem Plus® dressing consists of a polyurethane membrane matrix on a semipermeable thin-film backing. In addition, the hydrophilic membrane contains F-68 Surfactant, which acts as a mild wound cleansing agent; glycerin, which serves as a moisturizer and keeps the dressing from adhering to the wound bed; and a superabsorbent starch co-polymer. Collectively these agents eliminate the need for manual debridement and cleaning during dressing changes. Because there is clinical evidence suggesting that this dressing reduces pain after routine knee arthroscopy,²¹ the present study was designed to test directly whether this polymeric membrane dressing is antinociceptive in a stab wound injury model in the rat. As a control, several groups of animals with or without penetrating stab wounds were wrapped with standard gauze dressing (Curity 8 ply, type VII gauze; The Kendall Company, Boston, MA).

Experimental Groups and Dressing Application

Groups for Behavioral Analysis

Rats were randomly assigned into 12 groups (Table 1). Animals were tested before and after bilateral, unilateral, or sham surgery. For the sham groups, both hind limbs were shaved (sham groups 2 to 4), whereas the bilateral incisional stab wound groups were shaved and then received surgically placed stab wounds (incisional stab wound groups 5 to 8). PolyMem Plus® dressing or gauze dressing was applied to one (groups 2, 3, 6, and 7) or both (groups 4 and 8) hind limbs, and the dressing was secured firmly to the limb with elastic tape (Elastikon; Johnson and Johnson, Arlington, TX) without interfering with limb movement or circulation. To assess specifically whether limb movement was affected by the dressing application, both calves were shaved, either wrapped with dressing or not wrapped, but only the left limb received surgical incisional stab wounds (groups 9 to 12, Table 1).

Table 2. List of All Animal Groups Used for Fos Studies Indicating What Procedures Were Performed on the Left Hind Limb

GROUP NUMBER	NUMBER OF ANIMALS	LEFT SURGERY	LEFT TREATMENT
1	6	Naive	Naive
2	6	None	Polymeric dressing
3	6	None	Gauze dressing
4	6	Incisions	None
5	6	Incisions	Polymeric dressing
6	6	Incisions	Gauze dressing

Groups for Spinal Cord Fos Analysis

As an extension of spinal cord Fos analysis induced by incisional stab wounds (described in the accompanying article³), we quantified the number of Fos-positive neurons in the lumbar spinal cord after unilateral incisional stab wounds and dressing application in 6 groups of animals as summarized in Table 2. Two surgical stab wounds were made, and/or dressing was applied unilaterally to the left leg in all 6 animals in groups 2 to 6.

Surgical Procedure

Rats were anesthetized with isoflurane before surgery and were maintained with isoflurane throughout the surgery or application of the wound dressing. The hind legs of the rat were shaved, and incisional stab wounds were made either unilaterally (groups 4 to 6 for the Fos studies and groups 10 to 12 for the behavioral studies) or bilaterally (groups 5 to 8 for the behavioral studies) by using a carbon steel surgical blade (B-P no. 11; Bard-Parker, Rutherford, NJ). Two 0.5-cm long (1 mm deep) incisions were made 1 cm distal to the stifle (knee) joint, one on the cranial (anterior) and one on the caudal (posterior) aspect of the leg. The surgical blade was then used to make a 1-cm deep penetrating stab wound through the incision into the tibialis cranialis muscle on the cranial side of the leg and into the gastrocnemius and flexor digitorum superficialis muscles on the caudal side of the leg. The wound dressing (gauze or PolyMem Plus[®]) was secured over the incisional stab wound with elastic tape. After surgery the animals were moved to their home cages and allowed to recover until behavioral testing was performed.

Behavioral Measures

Mechanical thresholds were measured by using von Frey filaments, and thermal latencies were measured by using a Hargreaves box as described below. For both the mechanical and thermal behavioral assays baseline scores were recorded the day before surgery or application of wound dressing, and the behavioral test sessions were repeated 1, 3, 5, and 24 hours after surgery.

von Frey Test for Mechanical Hyperalgesia

Unrestrained rats were individually habituated to a clear plastic cage before testing. Two different von Frey filaments (a 5.46 filament, 254.97 mN, and a 5.88 filament, 588.40 mN) were applied 10 times each, perpendicularly to the plantar surface of the rat's hind paw at a rate of 1/s. Paw withdrawal responses were counted each time the rat lifted its paw in response to the mechanical stimulus, and the data were expressed as percent of stimuli giving rise to a withdrawal response as described previously.³⁵ Measurement of percent response by using 2 von Frey monofilaments was used in the present study because responses to these monofilaments were reproducible and sufficiently sensitive to allow detection of changes in stab wound-induced hyperalgesia.

Hargreaves/Dubner Test for Thermal Hyperalgesia

The time taken by the rat to withdraw its paw (paw withdrawal latency, PWL) in response to a radiant heat source¹⁸ was used to evaluate the presence of secondary thermal hyperalgesia induced by the incisional stab wound and to determine whether application of wound dressing altered the secondary hyperalgesia. Baseline paw withdrawal values were calculated from an average of 3 consecutive withdrawal latencies of both the left and right hind paws measured at 3-minute intervals as previously described.^{24,25} The order of paw testing varied randomly. Voltage to the light source was adjusted to yield baseline latencies ranging from 9.5 to 11.5 seconds in normal rats. A cutoff time of 30 seconds was imposed to avoid tissue damage.

Movement Analysis

To measure movement objectively, animals were placed in 43 × 43 × 21 cm Plexiglas activity boxes (Columbus Instruments, Columbus, OH) surrounded by movement recording sensors as previously described.¹ Each box was equipped with 6 sensor housings that included 32 XY emitter/detector sensor pairs and 16 Z emitter/detector sensor pairs. Animals were placed in the activity box and allowed to adapt to the novel environment for 3 to 4 hours before obtaining data. The total number of X, Y (horizontal), and Z (vertical, ie, rearing) movements were recorded continuously during an 18-hour period in all rats to obtain baseline values. Animals then received unilateral stab wounds or sham surgery and/or were wrapped with PolyMem Plus[®] or gauze dressing. Movements were again recorded for an 18-hour period. Changes in the average number of movements per 15-minute increments were determined for groups of animals with unilateral incisional stab wounds with or without dressing.

Fos Protein Immunohistochemistry

Four hours after incision, the animals were deeply anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally) and perfused transcardially with 800 mL 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (pH, 7.2). L3-L5 segments of the spinal cord were removed, post-fixed in the same fixative at 4°C for 4 hours, and then cryoprotected in 30% sucrose in 4% paraformaldehyde overnight at 4°C. Frozen serial transverse sections (40 μm) were cut and collected in 0.1 mol/L phosphate-buffered saline for immunohistochemistry. Free-floating sections were incubated with rabbit anti-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA; 1:2500) at 4°C overnight. The sections were subsequently processed by using the avidin-biotin-peroxidase/DAB procedure as previously described.²⁶ For quantitative analysis of Fos-like immunoreactive neurons, lumbar spinal cord sections were examined at 400×, and the 10 sections with the greatest number of labeled cells at the L3-L5 level were selected from each animal. For quantitative analysis of Fos-like immunoreactive neurons, indi-

vidual sections were digitized with 4096 gray levels by using a cooled CCD video camera (Hamamatsu XC77; Hamamatsu, Hamamatsu City, Japan) that was interfaced with a computer-assisted image analysis system (Metamorph; Universal Imaging Co, West Chester, PA). All Fos quantitation procedures were performed as previously described.²⁵

Histology

Animals in groups 10 to 12 were anesthetized and perfused with fixative immediately after the 24-hour behavioral testing time point. Hind limbs were removed, post-fixed, decalcified, and paraffin-embedded, and 5- μ m transverse sections through the incision were cut by using a rotary microtome and were stained with hematoxylin-eosin. The average number of inflammatory cells (macrophages and neutrophils) in the incision area was quantified by using a computer-assisted image analysis system (Metamorph). To accomplish this the number of inflammatory cells in 4 randomly selected 625- μ m² areas located at the incision site (or at a comparable site from the contralateral limb) were quantified from each of 4 sections/animal for all animals in the 3 groups. Metamorph parameters were set to exclude muscle cell and fibroblast nuclei; thus, all cells (excluding myocytes and fibroblasts) within each 625- μ m² area were counted as a measure of inflammation. The mean circumferential spread of inflammation along the incision site was also quantified from each of 4 sections per animal per group. The extent of the inflamed area at 8 randomly chosen locations along the incision was measured by using the "trace region tool" in the Metamorph program. This allowed calculation of the actual area of inflammation at each of the 8 locations, and these data were then averaged across sections and animals to create a mean cross-sectional area of inflammation.

Statistical Analyses

Overall effects for changes in paw withdrawal responses and latencies from baseline values were compared for statistical significance by using parametric analysis. The time course data were converted into a single point area under the curve. The areas under the curve were then used to test for significance between treatment groups by using two-way analysis of variance (ANOVA). If significant ($P < .05$), the analyses were followed by Scheffe or Bonferroni-Dunn post hoc tests. Comparisons between legs were determined by using the paired t test. The Fos, activity box, and histologic data were analyzed by one-way ANOVA. In all cases the data were expressed as means \pm standard error of mean, and P value less than .05 was considered significant.

Results

Thermal Hyperalgesia

Baseline PWL to radiant heat was not significantly different among groups before initiation of leg dressing or incisional stab wound (mean, 10.41 ± 1.05 seconds). As indicated in the accompanying article,³ thermal hyperal-

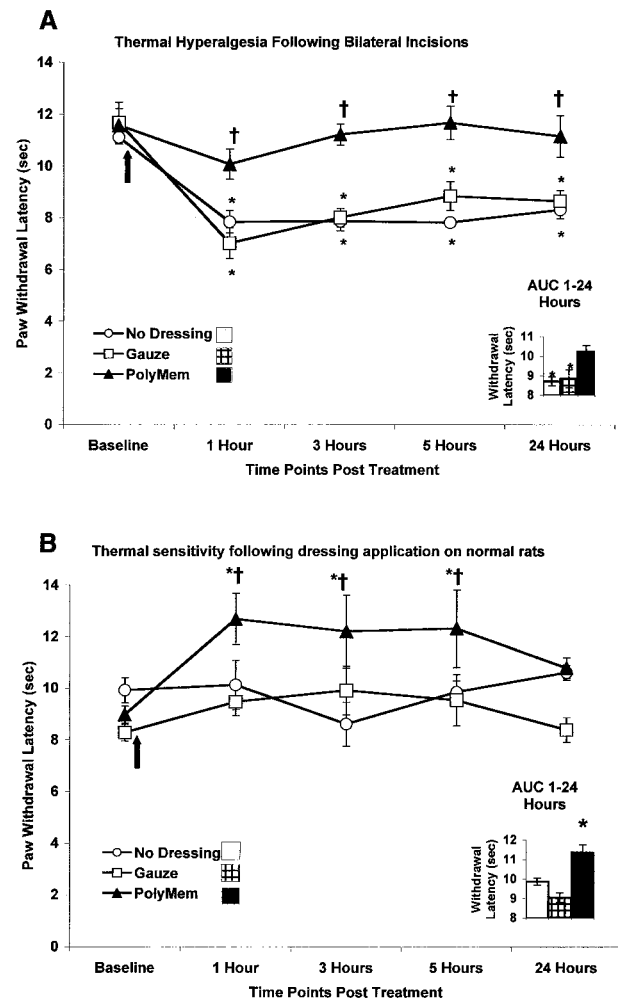


Figure 1. (A) PolyMem Plus[®] dressing application, but not gauze dressing application, reverses the decrease in PWL induced by bilateral incisional stab wounds. Stab wounds were made bilaterally, but the PolyMem Plus[®] dressing (group 6) or gauze dressing (group 7) was only applied to the right leg. The arrow indicates the time point of incisional stab wound placement and dressing application. *Significant difference from baseline values; †significant difference from the contralateral limb (data not shown). Note that the PolyMem Plus[®] dressing prevented the decrease in PWL from baseline (mean, 10.278 ± 0.38) on the wrapped leg, whereas the unwrapped leg still showed a significant decrease in PWL across all time points (mean, $7.81 \pm .46$; $P < .05$). Finally, a Bonferroni/Dunn post hoc analysis of the PWL showed a significant difference between the PolyMem Plus[®] dressing and gauze ($P < .0001$) and between the PolyMem Plus[®] dressing and no dressing ($P < .0001$). (B) Effect of the PolyMem Plus[®] dressing on paw thermal withdrawal latency in normal animals. Application of PolyMem Plus[®] dressing (group 2), but not gauze dressing (group 3), significantly elevated the PWL to a thermal stimulus at 1, 3, and 5 hours after application compared to baseline levels or to sham-treated animals without dressing. Sham surgery did not cause any changes in PWL when compared to baseline levels or to PWLs from naive animals (data not shown). Arrow indicates the time point of sham surgery and dressing application. *Significant difference from baseline values; †significant difference from the contralateral limb.

gesia could not be detected in animals with unilateral incisional stab wounds; therefore, the data presented here are from animals with bilateral stab wounds exhib-

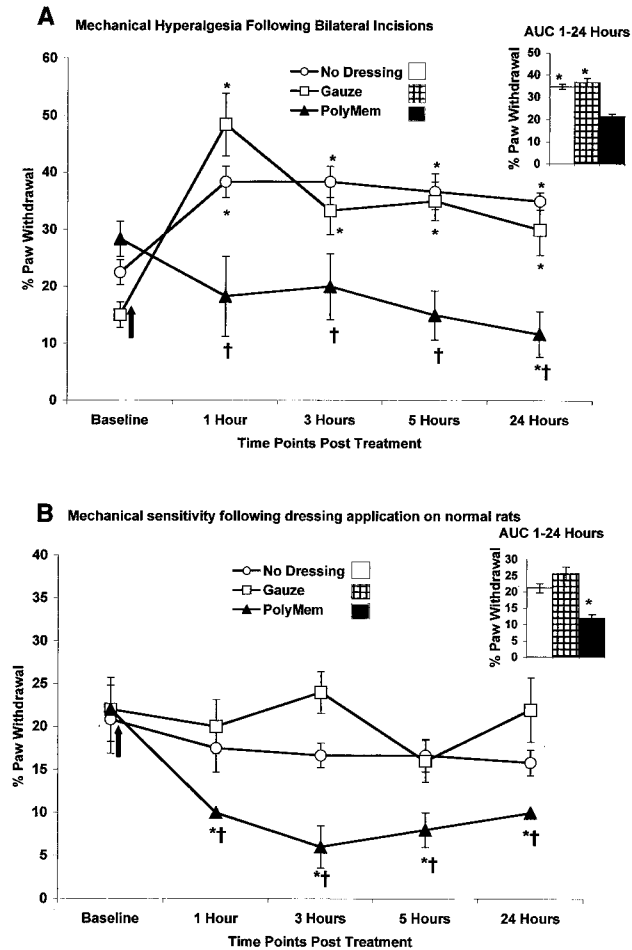


Figure 2. (A) Effect of different dressings on mechanical hyperalgesia associated with bilateral incisional stab wounds. PolyMem Plus® dressing (group 6) or gauze dressing (group 7) was applied to the right leg, whereas the left leg was left unwrapped. The incisional stab wound (no dressing) caused a significant increase in the percent paw withdrawal of the right limb (tested with the 588.40-mN filament) that was prevented by application of the PolyMem Plus® dressing (filled triangles) but not the gauze dressing (open squares). The arrow indicates the time point of incisional surgery and dressing application. *Significant difference from baseline values; †significant difference from the contralateral, unwrapped limb. A Bonferroni/Dunn post hoc analysis of the mechanical sensitivity over all time points showed a significant difference ($P < .0001$) between the PolyMem Plus® dressing and gauze and the PolyMem Plus® dressing and no dressing (see inset). (B) The polymeric dressing produces an analgesic effect in naïve animals. The left hind limb of naïve animals was wrapped with either PolyMem Plus® membrane dressing or gauze dressing or was left unwrapped; the percent withdrawal of the limb to a 588.40-mN von Frey filament was compared to baseline levels. Polymeric dressing, but not the gauze dressing, significantly reduced the percent paw withdrawal compared to baseline. In addition, withdrawal frequencies for the ipsilateral and contralateral hind limbs differed significantly from one another for the animals in groups 2 and 4 ($P > .001$). The arrow indicates the time point of dressing application. *Significant difference from baseline values; †significant difference from animals with gauze dressing.

iting bilateral thermal hyperalgesia. Bilateral incisional stab wounds with no dressing (group 5) or with gauze dressing on 1 leg (group 7) significantly decreased PWL in both hind limbs at 1, 3, 5, and 24 hours, and this decrease

was prevented by application of the polymeric dressing (group 6; Fig 1A). It is important to note that the PolyMem Plus® dressing prevented the decrease in PWL from baseline (mean, 10.278 ± 0.38) on that limb, whereas the unwrapped leg still showed a significant decrease in PWL across all time points (mean, $7.81 \pm .46$; $P < .05$). Moreover, the PWLs for the 2 hind limbs were significantly different from one another ($P < .0001$). Animals with bilateral stab wounds and a gauze dressing on one limb and experimental dressing on the other leg (group 8) produced results similar to group 6 (data not shown).

Surprisingly, the PolyMem Plus® dressing showed an analgesic effect in addition to preventing hyperalgesia. The PWLs of naïve rats with no incisions and polymeric dressing (groups 2 and 4) but not gauze dressing (group 3) were significantly greater than baseline values and contralateral (unwrapped) limb values at 1, 3, and 5 hours after dressing application (Fig 2; $P = .0005$, Fig 1B).

Mechanical Hyperalgesia

No differences in baseline withdrawal frequencies to mechanical stimuli were observed among any of the groups before incisions (mean value across groups for the 2 von Frey filaments tested = vF 5.46, 254.97 mN: $8.37\% \pm 2.02\%$; vF 5.88, 588.40 mN: $18.33\% \pm 1.05\%$). After bilateral incisional stab wounds, animals with no dressing (group 5) or gauze dressing (group 7) application exhibited a significant increase in paw withdrawal frequencies at all time points. In contrast, PolyMem Plus® dressing applied to 1 leg after bilateral incisions (group 6) prevented this increase in percent response for the wrapped limb (Fig 2A), whereas the contralateral, unwrapped limb showed a significant increase in withdrawal frequency. In addition, paired *t* tests indicated a significant difference between the 2 legs when tested with 2 different von Frey filaments (vF 254.97 mN: $P = .0004$; vF 588.40 mN: $P < .0001$). Note that at the 24-hour time point the percent paw withdrawal of animals in group 6 with stab wounds and polymeric dressing was significantly less than baseline values. It should also be noted that animals with stab wounds on both legs and gauze dressing on the left leg and PolyMem Plus® dressing on the right leg (group 8) showed similar results. Specifically, there was no change in withdrawal frequency across time points for the leg covered with the PolyMem Plus® dressing (vF 254.97 mN: $11.42\% \pm 1.51\%$; vF 588.40 mN: $21.50\% \pm 2.14\%$), but there was a significant increase in withdrawal responses for the leg covered with gauze dressing (vF 254.97 mN: $33.50\% \pm 1.91\%$; vF 588.40 mN: $47.01\% \pm 2.61\%$). The difference between the ipsilateral and contralateral legs was highly significant ($P < .0001$). In addition, a Bonferroni/Dunn post hoc analysis of the mechanical testing data compressed over time showed a significant difference ($P < .0001$) between the PolyMem Plus® dressing and gauze and the PolyMem Plus® dressing and no dressing.

As shown in the previous article,³ a unilateral stab wound was sufficient to evoke mechanical hyperalgesia. The effect of different dressing application after unilateral stab wounds was similar to that seen after bilateral

wounds. In groups 11 (unilateral incisional stab wound/bilateral gauze dressing) and 12 (unilateral incisional stab wound/no dressing), the ipsilateral hind limb showed significantly higher percent response compared with the contralateral hind limb at all times after baseline (data not shown). In contrast, application of the PolyMem Plus® dressing prevented this mechanical hyperalgesia; there was no difference in response between ipsilateral and contralateral limbs for the animals in group 10 (incisional stab wound left leg/bilateral PolyMem Plus® dressing).

Again, PolyMem Plus® dressing was shown to have an effect on baseline mechanical sensitivity. In group 3 (no incisional stab wound/gauze dressing on 1 limb), there was no change in paw withdrawal response for either limb (Fig 2B). Group 2 and group 4 (bilateral sham surgery, one leg unwrapped or wrapped in gauze dressing, the other in PolyMem Plus® dressing) showed a decrease in percent withdrawal for the limb with the PolyMem Plus® dressing.

Movement Alterations

Analysis of spontaneous vertical and horizontal movements (activity box data) indicated that rats with leg incisional stab wounds had significantly reduced vertical (Fig 3A) and horizontal (Fig 3B) movements during an 18-hour period when compared to baseline values. Application of polymeric dressing, but not gauze dressing (Fig 3), reversed this stab wound–induced decrease in spontaneous activity.

Fos Expression

Results of the Fos experiments are summarized in Fig 4. Analysis of spinal cord sections from naïve animals showed an average of 3.6 ± 1.6 (in laminae I and II), 2.0 ± 0.9 (in laminae III and IV), and 0.8 ± 0.5 (in laminae V and VI) Fos-positive cells per section, respectively; data for naïve animals are the same in Fig 4A and 4B. Analysis of Fos groups 4 (incisional stab wound/no dressing) and 6 (incisional stab wound/gauze dressing, Fig 4A) indicates a significant increase in Fos-immunoreactive neurons in laminae I to VI of the ipsilateral L3-L5 spinal cord segments after leg stab wounds (naïve, 9.8 ± 1.0 ; group 4, 41.3 ± 3.1 ; and group 6, 46.6 ± 2.8 ipsilateral Fos cells/section in laminae I to VI). In contrast, those animals with incisional stab wounds that were wrapped with the PolyMem Plus® dressing (Fos group 5) exhibited a significant reduction in Fos expression (18.5 ± 2.1 ipsilateral Fos cells/section in laminae I to VI) compared to groups 4 and 6. Surprisingly, naïve rats wrapped with PolyMem Plus® dressing (Fos group 2) showed a significant increase in spinal Fos neurons (36.1 ± 2.9 Fos cells/section in laminae I to VI), suggesting that the experimental dressing itself causes spinal cord Fos activation (Fig 4B).

Incision Histology

Examination of histologic sections through the incisional stab wound sites showed a significant infiltration

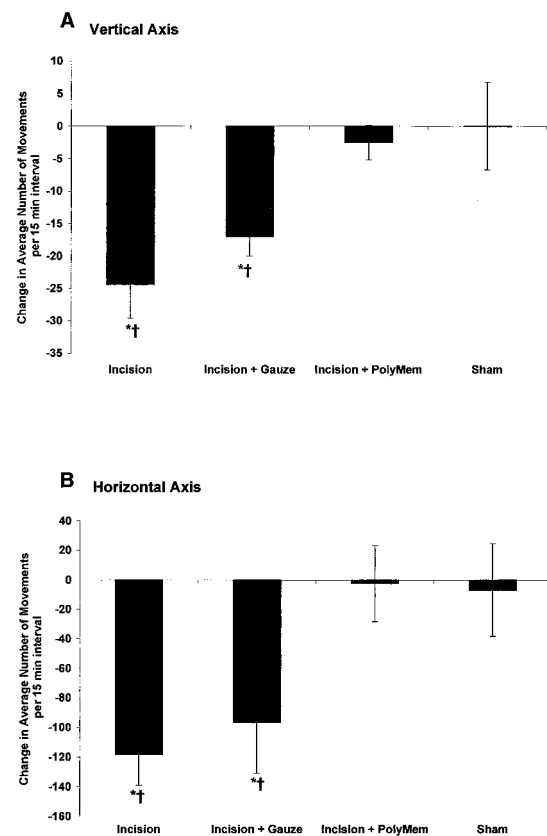


Figure 3. Activity box recordings of movements in both the vertical (Z-axis, A) and horizontal (XY-axis, B) direction from groups of rats with stab wounds, either wrapped with PolyMem Plus® dressing or gauze dressing compared to sham-operated rats. The average activity per 15-minute increment was calculated for a continuous 18-hour period before stab wound placement (baseline) and for a continuous 18-hour period after stab wound placement. Rats with stab wounds alone or with stab wounds wrapped with gauze dressing, but not sham-operated rats or rats with stab wounds wrapped with PolyMem Plus® dressing, had statistically significantly fewer horizontal and vertical movements compared to baseline values and to values obtained from naïve animals placed in the activity box for 36 hours (data not shown). *Statistically significant differences compared to baseline; †significant difference from sham-operated animals.

by macrophages and neutrophils. Neutrophils were especially prominent in the subcutaneous tissue under the epidermis, whereas macrophages were more prevalent within the muscle at the stab wound site. Muscle fiber necrosis was evident within the stab wound area. Quantification of the number of inflammatory cells per unit area of tissue showed a significant decrease in the number of inflammatory cells at the stab wound site in animals wrapped with polymeric or gauze dressing compared with no dressing (Table 3). Histologic observations indicated that the PolyMem Plus® dressing reduced the spread of inflammatory cells from the stab wound site into the surrounding muscle (Fig 5). Quantification of the mean cross-sectional area of inflammation along the incisional stab wound site verified this finding, showing that the inflamed area was significantly smaller ($522,526 \mu\text{m}^2$) in the animals wrapped with PolyMem Plus® dress-

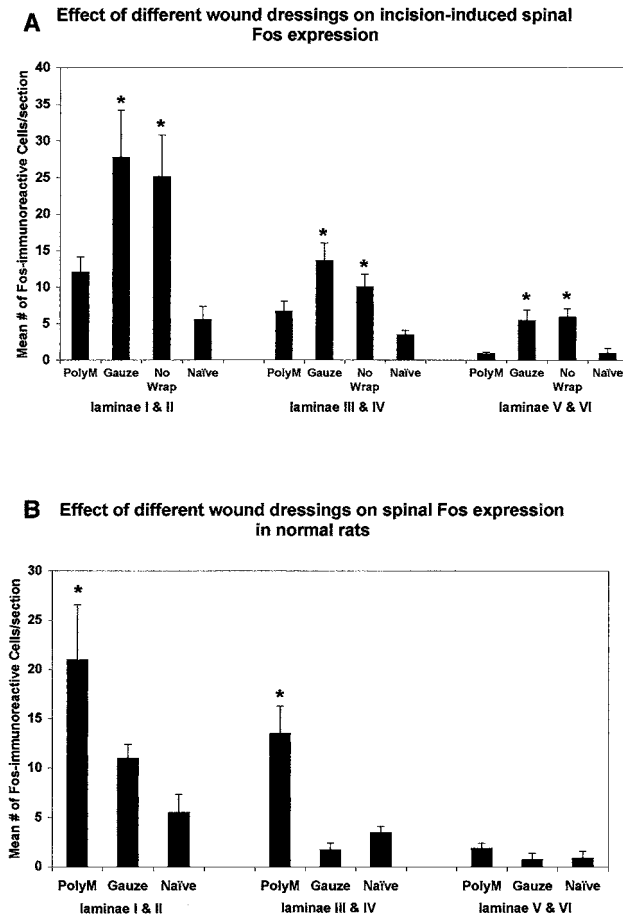


Figure 4. Mean number of Fos-immunoreactive cells per section in the ipsilateral spinal cord dorsal horn from animals with unilateral incisional stab wounds (A) wrapped with gauze dressing (group 6), PolyMem Plus® dressing (PolyM, group 5), or no dressing (No Wrap, group 4), and in naïve animals (B) with (groups 2 and 3) or without (group 1) dressing. Note that the incisions caused a significant increase in Fos expression in the laminae I and II (A). This increase was significantly reduced by application of the PolyMem Plus®, but not the gauze, dressing. Note also that application of the PolyMem Plus® dressing alone to naïve animals produced a significant increase in Fos expression (B). *Significant difference from naïve animals; †significant difference from the contralateral spinal cord (data not shown).

ing as compared to animals wrapped with gauze ($699,032 \mu\text{m}^2$) or with no wrap ($691,152 \mu\text{m}^2$; Fig 5).

Discussion

Antinociceptive Effect of the Polymeric Membrane Dressing

Although there is a large literature on wound and surgical dressings, most of the articles focus on the wound healing effects of different dressings or on the amount of pain induced during dressing removal and reapplication.^{16,17} A few studies have examined both the wound healing and pain relieving effects of different surgical/wound dressings.^{6,9} For example, Cannavo et al⁹ showed that patients with abdominal incisions covered with gauze dressing moistened with sodium hypochlorite (0.05%) experienced greater pain than those patients whose incisions

Table 3. Effect of Dressings on Incision Stab Wound-Induced Inflammation

	AVERAGE NUMBER OF INFLAMMATORY CELLS/ $625 \mu\text{m}^2$	AVERAGE AREA OF INFLAMMATION (μm^2)
Incision only	480 ± 18	$691,153 \pm 12,137$
Incision with gauze dressing	$270 \pm 14^*$	$699,032 \pm 12,780$
Incision with PolyMem Plus® dressing	$256 \pm 11^*$	$553,527 \pm 15,724^*$

*Significant difference from incision only.

were covered with standard alginate or a combined dressing pad. However, there have been very few carefully controlled studies of wound dressing effects on pain in humans and to our knowledge no studies examining the effects of surgical dressings on pain in an animal stab wound or incision model. One recent clinical study has evaluated the use of PolyMem Plus® versus standard dressing on pain levels in human patients after routine knee arthroscopy.²¹ This study found that the PolyMem Plus® group had lower pain ratings and less postoperative swelling than patients who received standard gauze dressing.

In the present study we demonstrate that stab wounds on one or both hind limbs of a rat produce a significant increase in pain behavioral responses at all post-stab wound time points examined. Wrapping the hind limb stab wound area with gauze dressing did not significantly alter these pain behaviors. In contrast, our results show that there is a robust, reproducible, and statistically significant decrease in both secondary mechanical and thermal hyperalgesia when the stab wounds were wrapped with PolyMem Plus® dressing. Although we did not examine the effect of the PolyMem Plus® dressing on primary hyperalgesia because of the complication of removing and then replacing the dressing at each post-stab wound time point, we did find that application of the dressing reversed the decrease in motor activity (activity cage behavior) caused by the stab wound. This would imply that the dressing reduces the pain associated with movement of the hind limb after deep tissue injury produced by the stab wound, and this further suggests that the dressing has a potent antinociceptive effect at the site of injury.

Interestingly, application of the PolyMem Plus® dressing in naïve and sham animals showed that this dressing increased baseline thermal latencies and produced a decrease in percent withdrawal compared to baseline values for the 2 different von Frey filaments tested. Because the gauze dressing did not mimic this effect, this suggests that the PolyMem Plus® dressing produces a local analgesic effect when applied to the skin. Coupled with previous results showing the wound healing capacity of this dressing^{4,10} and the effectiveness of the dressing in reducing postoperative morbidity after knee arthroscopy,²¹ this would argue that the PolyMem Plus® dressing might be useful for both increasing wound healing and reducing

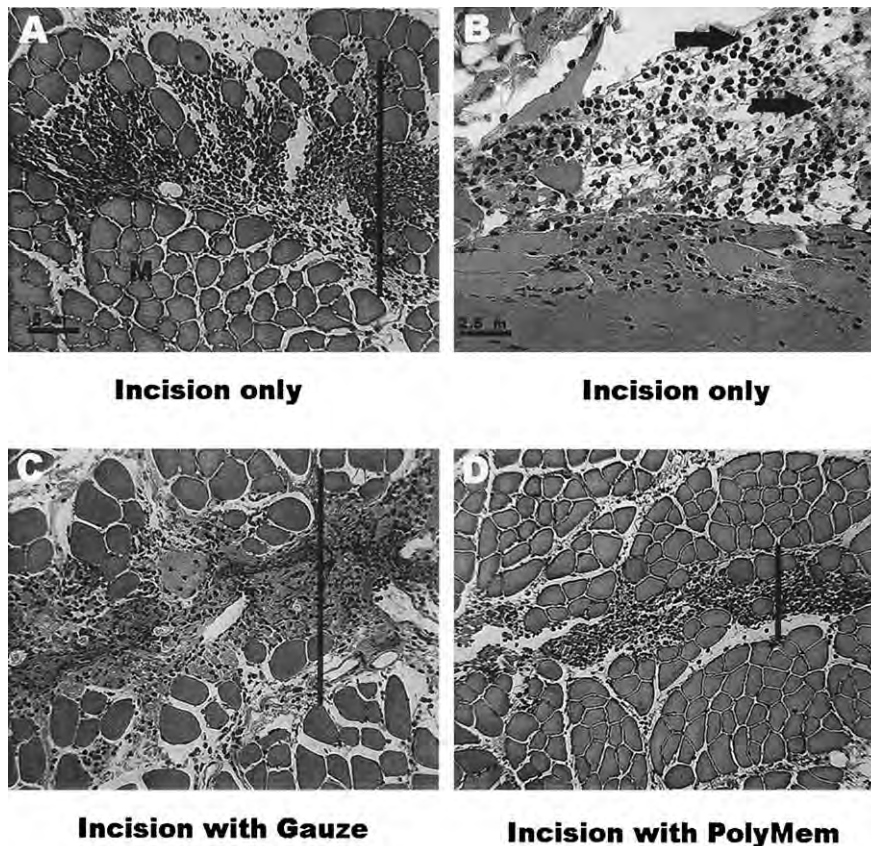


Figure 5. Photomicrographs of hematoxylin-eosin stained sections illustrating typical examples of the extent of inflammation around the incision site in animals with incisions (A and B) compared to animals with incisions wrapped with gauze (C) or PolyMem Plus® dressing (D). Individual muscle cells are indicated by an “M” in 11A. The vertical black bar in (A), (C), and (D) indicates the extent of the inflammatory area at the incision site. An example of the large infiltration of inflammatory cells (arrows) is illustrated at higher magnification in (B). Note that the spread of inflammatory cells from the incision site into the surrounding muscle is much more restricted in the rats with incisions that were wrapped with PolyMem Plus® dressing. In animals with stab wounds alone or stab wounds wrapped with gauze dressing, the inflammation spread into the periosteum of the tibia or fibula. Periosteal involvement did not occur in animals with stab wounds wrapped with PolyMem Plus® (not shown).

pain associated with stab wounds, deep lacerations, surgical incisions, or other types of penetrating injuries.

Antinociceptive Mechanisms Associated With the Polymeric Dressing

It is currently unclear how the PolyMem Plus® dressing produces its antinociceptive effect. In the context of the incisional stab wound model used here it is possible that the polymeric membrane dressing produced antinociception by limiting the extent of the inflammatory response at the stab wound site. This reduction in inflammation could produce a decrease in stab wound-induced nociception similar to that observed after the reduction of inflammation induced by acupuncture treatment^{24,25} or by nitrous oxide-naproxen treatment in rat models of arthritis.¹¹ However, a reduction in the amount and spread of inflammation is probably not the only explanation for the antinociceptive effect produced by the PolyMem Plus® wrap, because the dressing was found to exhibit analgesic qualities in naïve animals in which increased thermal latencies and decreased percent withdrawal to von Frey filaments were observed compared to baseline

values. There is recent evidence suggesting that the dressing might absorb sodium ions from the skin and subcutaneous tissue.²¹ If this is true, then a decrease in sodium ion concentration would result in reduced nerve conductance, and this could cause the analgesic effect of the dressing observed in normal animals and could contribute to the reduction in secondary hyperalgesia observed after peripheral stab wounds.

It is likely that the antinociceptive effect observed in this study, particularly the reduction in secondary hyperalgesia resulting from stab wound placement, is mediated both peripherally and centrally. The following 2 lines of evidence support this hypothesis. First, it is known that secondary hyperalgesia is related to central sensitization^{27,29,33}; thus, it seems likely that the reduction of secondary hyperalgesia observed here might be due in part to a peripheral effect in which the dressing prevents or reduces the development of secondary hyperalgesia via a local effect at the site of injury. If the dressing absorbs sodium ions from the skin and subcutaneous tissue as hypothesized above, it might produce a local analgesic effect that reduces the development of secondary hyperalgesia.

However, the dressing might also have a central effect as suggested by the second line of evidence showing that the PolyMem Plus®, but not gauze, dressing induces spinal cord Fos expression in laminae III and IV of the dorsal horn of naïve animals. The deep dorsal horn, including laminae III to VI, is a major termination zone for hair, tactile and muscle receptors with rapidly conducting myelinated axons^{5,28} and is therefore important for initial transformation of mechanical sensory information from the body. In particular, it is thought that laminae III and IV participate in the processing of weak mechanical stimuli applied cutaneously^{30,37}; thus, it seems feasible that the polymeric dressing might stimulate peripheral mechanoreceptors to activate neurons in these spinal laminae. In this regard, activation of laminae III and IV has previously been linked to analgesic mechanisms associated with acupuncture treatment²⁶ and nitrous oxide admin-

istration,¹⁹ and it is feasible that the polymeric dressing might also activate these laminae to reduce pain. It should be noted that the PolyMem Plus® and to some extent the gauze dressing also produced activation of laminae I and II in naïve animals. It is not clear whether activation of these laminae is also involved in the antinociceptive effect of the polymeric dressing, but these laminae are also known to be activated during acupuncture treatment¹³ and thus might contribute to the observed antinociception.

Acknowledgments

The authors would like to thank Dr George Wilcox for his helpful suggestions during the development of this study and Dr Robert Addison and Mr John Newton for their helpful comments during the writing of this manuscript.

References

- Alley J, Khasabov S, Simone D, Beitz AJ, Rodriguez M, Njenga MK: More severe neurologic deficits in male SJL/J mice than female mice following Theiler's virus-induced CNS demyelination. *Exp Neurol* 180:14-24, 2003
- Arpey CJ, Whitaker DC: Postsurgical wound management. *Dermatol Clin* 19:787-797, 2001
- Beitz AJ, Newman A, Shepard M, Ruggles TR, Eikmeier LJ. A new rodent model of hindlimb penetrating wound injury characterized by continuous primary and secondary hyperalgesia. *J Pain* 5:
- Blackman JD, Senseng D, Quinn L, Mazzone T: Clinical evaluation of a semipermeable polymeric membrane dressing for the treatment of chronic diabetic foot ulcers. *Diabetes Care* 17:4-7, 1994
- Brown, AG. *Organization of the Spinal Cord*. New York, Springer-Verlag, 1981
- Briggs M: Surgical wound pain: A trial of two treatments. *J Wound Care* 5:456-60, 1996
- Bruster S, Jarman B, Bosanquet N, Weston D, Erens R, Delbanco TL: National survey of hospital patients. *BMJ* 309: 1542-1546, 1994
- Bux M, Malhi JS: Assessing the use of dressings in practice. *J Wound Care* 5:305-308, 1996
- Cannavo M, Fairbrother G, Owen D, Ingle J, Lumley T: A comparison of dressings in the management of surgical abdominal wounds. *J Wound Care* 7:57-62, 1998
- Carr RD, Lalagos DE, Upman PJ: Comparative study of occlusive wound dressings on full thickness wounds in domestic pigs. *Wounds* 1:53-61, 1989
- Cicala C, Ianaro A, Fiorucci S, Calignano A, Bucci M, Gerli R, Santucci L, Wallace JL, Cirino G: NO-naproxen modulates inflammation, nociception and downregulates T cell response in rat Freund's adjuvant arthritis. *Br J Pharmacol* 130: 1399-1405, 2000
- Collier M, Hollinworth H: Pain and tissue trauma during dressing change. *Nurs Stand* 14:71-73, 2000
- Dai JL, Zhu YH, Li XY, Huang DK, Xu SF: C-fos expression during electroacupuncture analgesia in rats: An immunohistochemical study. *Acupunct Electrother Res* 17: 165-76, 1992
- Davey B: Pediatric lacerations and burns. *Aust Fam Physician* 28:117-123, 1999
- Eaglstein WH, Sullivan TP, Giordano PA, Miskin BM: A liquid adhesive bandage for the treatment of minor cuts and abrasions. *Dermatol Surg* 28:263-267, 2002
- Foster L, Moore P: The application of a cellulose-based fibre dressing in surgical wounds. *J Wound Care* 6:469-473, 1997
- Foster L, Moore P, Clark S: A comparison of hydrofibre and alginate dressings on open acute surgical wounds. *J Wound Care* 9:442-445, 2000
- Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32:77-88, 1988
- Hashimoto T, Maze M, Ohashi Y, Fujinaga M: Nitrous oxide activates GABAergic neurons in the spinal cord in Fischer rats. *Anesthesiology* 95:463-469, 2001
- Hartford JM, Gorczyca JT: Late arthroscopic debridement of metal fragments and synovectomy after penetrating knee joint injury by low-velocity missile: A report of two cases. *J Orthop Trauma* 15:222-224, 2001
- Hayden JK, Cole BJ: The effectiveness of a pain wrap compared to a standard dressing on the reduction of post-operative morbidity following routine knee arthroscopy: A prospective randomized single-blind study. *Orthopedics* 26: 59-63, 2003
- Huang N, Cunningham F, Laurito CE, Chen C: Can we do better with postoperative pain management? *Am J Surg* 182:440-448, 2001
- Kaplan JJ, Schwen SA: Clinical pearls: Stab wound to the left forearm. *Acad Emerg Med* 4:810, 1997 844-846
- Kwon YB, Lee HJ, Han HJ, Mar WC, Kang SK, Yoon OB, Beitz AJ, Lee JH: The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. *Life Sci* 71:191-204, 2002
- Kwon YB, Lee JD, Lee HJ, Han HJ, Mar WC, Beitz AJ, Lee

JH: Bee venom injection directly into an acupoint reduces arthritis associated edema and nociceptive responses. *Pain* 90:271-280, 2001

26. Lee J-H, Beitz AJ: Electroacupuncture modifies the expression of c-fos in the spinal cord induced by noxious stimulation. *Brain Res* 577:80-91, 1992

27. Li J, Simone DA, Larson AA: Windup leads to characteristics of central sensitization. *Pain* 79:75-82, 1999

28. Ling LJ, Honda T, Shimada Y, Ozaki N, Shiraishi Y, Sugiura Y: Central projection of unmyelinated (C) primary afferent fibers from gastrocnemius muscle in the guinea pig. *J Comp Neurol* 461:140-150, 2003

29. Raja SN, Cambell JN, Meyer RA: Evidence for different mechanisms of primary and secondary hyperalgesia following heat injury to the glabrous skin. *Brain* 107:1179-1188, 1984

30. Schneider SP: Functional properties and axon terminations of interneurons in laminae III-V of the mammalian spinal dorsal horn in vitro. *J Neurophysiol* 68:1746-1759, 1992

31. Stewart MP, Birch R: Penetrating missile injuries of the brachial plexus. *J Bone Joint Surg Br* 83:517-524, 2001

32. Terrill PJ, Varughese G: A comparison of three primary non-adherent dressings applied to hand surgery wounds. *J Wound Care* 9:359-363, 2000

33. Treede RD, Magerl W: Multiple mechanisms of secondary hyperalgesia. *Prog Brain Res* 129:331-341, 2000

34. Vitale GC, Richardson JD, George SM Jr, Miller FB: Fasciotomy for severe, blunt and penetrating trauma of the extremity. *Surg Gynecol Obstet* 166:397-401, 1988

35. Wacnik PW, Eikmeier LJ, Ruggles TR, Ramnaraine ML, Walcheck BK, Beitz AJ, Wilcox G: Functional interactions between tumor and peripheral nerve: Morphology, algogen identification and behavioral characterization of a new murine model of cancer pain. *J Neurosci* 21:9355-9366, 2001

36. Wiechula R: The use of moist wound-healing dressings in the management of split-thickness skin graft donor sites: A systematic review. *Int J Nurs Pract* 9:59-517, 2003

37. Willis WD, Coggeshall RE: Sensory Mechanisms of the Spinal Cord. New York, NY, Plenum Press, 1991, 153-212