Prevention of peridural fibrosis using NSAIDs soaked cellulose membrane: an experimental comparative study in animal model

Presenter: Dr. Surachai Sae-Jung

Faculty of Medicine KhonKaen University, Thailand
2011
Presentation on
Research Proposal

Title

Prevention of peridural fibrosis using NSAIDs soaked cellulose membrane: an experimental comparative study in animal model

Presenter: Surachai Sae-Jung, MD
Clinical Epidemiology Unit
Faculty of Medicine
Khon Kaen University

International Short Course Training
in
Research Methodology and Biostatistics
2011
## Content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>4</td>
</tr>
<tr>
<td>Background and Rationale</td>
<td>5</td>
</tr>
<tr>
<td>Literature review</td>
<td>6</td>
</tr>
<tr>
<td>Justification</td>
<td>12</td>
</tr>
<tr>
<td>Research question</td>
<td>12</td>
</tr>
<tr>
<td>Objective</td>
<td>12</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>13</td>
</tr>
<tr>
<td>Conceptual framework</td>
<td>14</td>
</tr>
<tr>
<td>Keywords and operation definitions</td>
<td>15</td>
</tr>
<tr>
<td>Research design</td>
<td>16</td>
</tr>
<tr>
<td>Research methodology</td>
<td>16</td>
</tr>
<tr>
<td>Population</td>
<td>17</td>
</tr>
<tr>
<td>Intervention</td>
<td>17</td>
</tr>
<tr>
<td>Study flow chart</td>
<td>18</td>
</tr>
<tr>
<td>Outcome variable</td>
<td>19</td>
</tr>
<tr>
<td>Measurement of outcomes</td>
<td>19</td>
</tr>
<tr>
<td>Data/variables</td>
<td>20</td>
</tr>
<tr>
<td>Outcome measurement &amp; data collection</td>
<td>20</td>
</tr>
</tbody>
</table>
Abstract

Introduction: Peridural fibrosis is one of the more frequent complications of spine surgery. Nonsteroidal antiinflammatory drugs inhibit the inflammatory and fibroblastic response while surgical cellulose membrane interposes between dura & paraspinal muscle can stop the bleeding from surgical site.

Purpose: To study the efficacy and safety of peridural NSAIDs (parecoxib) soaked cellulose membrane and epidural cellulose membrane for prevention of peridural fibrosis in animal model.

Material & Method: All adult Spraque-Dawley rats will be laminectomized at L4-5 via posterior midline approach and randomly divided into one of three groups. The first group will receive parecoxib soaked cellulose membrane place over dura, the second group will be placed over the dura with cellulose membrane and the third or control group will receive physiological saline dripping over the dura before surgical wound closure. All rats in each group will be sacrificed at 4 weeks for histopathological assessments. The sample specimens will be stained using hematoxylin & eosin and Masson trichrome methods. The cellular populations in the fibroblast, inflammatory reaction and the thickness of the fibrous membrane will be quantified by 2 blinded outcome assessors. The adverse event will also be recorded.

Statistical analysis: The fibroblast density and inflammatory cell density will be compared between 3 groups using ANOVA and compared between the two groups with the modified t-test & Bonferroni correction while the fibrous adherence will be compared using the Kruskal-Wallis ANOVA test and Dunn method of multiple comparisons.

Results: The primary outcomes, fibroblast density, will be reported in mean ± standard deviation, compared between 2 groups and will be reported in mean difference and 95% confidence interval. The secondary outcomes, the inflammatory cell densities, will be reported in mean ± standard deviation, mean difference and 95% confidence interval. The peridural fibrous adherence grading, will be reported in term of experimental event rate, control event rate, absolute risk reduction, relative risk, relative risk reduction, number needed to treat and 95% confidence interval.

Conclusion: The study will be concluded in the safety and efficacy of NSAIDs (parecoxib) and cellulose membrane in peridural fibrosis prevention comparing between groups.
Failed back surgery syndrome (FBSS) is a diagnosis given to patients who complain of recurrent back or leg pain with or without neurological symptoms after unsuccessful lumbar surgery. The incidence of FBSS is up to 40%. Many FBSS patients present for more surgery. The incidence of reoperation following lumbar spine surgery ranges from 4 to 19% \(^\text{[1], [2]}\). While the success rate of reoperation fall to 30, 15 and 5 % after the 2\(^{\text{nd}}\), 3\(^{\text{rd}}\) and 4\(^{\text{th}}\) operations \(^\text{[3]}\). An understanding of the common causes of FBSS can help prevent inappropriate surgery whenever possible. Common identifiable causes for FBSS include poor patient selection, incorrect initial diagnosis, incorrect or inadequate surgery, peridural fibrosis, infection, and progressive disease \(^\text{[4]}\).

Peridural fibrosis or scarring is one of the common causes of FBSS and it is the causative factor to persistent pain in 20-36 % of FBSS patients \(^\text{[5]}\). Fibrosis is a part of healing process, so the peridural fibrosis is inevitable when the epidural space is exposed such as peridural fibrosis following the lumbar laminectomy procedure. The victims of peridural fibrosis are dural sac compression, nerve root tethering, interfere cerebrospinal fluid flow or compromise the nerve root vascular supply \(^\text{[6]}\). These cause a new onset of pain or neurological deficit. The symptomatic peridural fibrosis occurs in 13 to 61% of patients who undergo back surgery \(^\text{[7]}\). Once the scar forms, there is no effective treatment and the scar revision surgery can also make a new scar \(^\text{[8]}\). The reoperation for scar had significantly poor result due to difficulty in operating the scar tissue, increase risk of adhesive arachnoiditis and dural tears. One of the modalities for peridural fibrosis is prevention or reduction of fibrosis formation. Different substances that used to minimize the hematoma, minimize inflammatory changes or interpose the dura & paraspinal muscle by the barrier for peridural fibrosis prevention perioperatively are currently under development. We combine these concepts using the NSAIDs soaked cellulose membrane place over the dura before surgical wound closure in the laminectomized animal model.
The wound healing process that contributes to fibrosis formation consists of 4 phases including the hemostasis after soft tissue injury or surgical operation, inflammatory phase, proliferative phase and maturation phase \(^9\). Once the hemostasis take place, the inflammatory phase is induced. The cellular and cytokine responses induce the fibroblast proliferation and reestablish tissue continuity due to matrix and collagen synthesis that the fibrosis or scar is found in this phase. Finally the collagen is organized and maintained its nearly normal strength while the fibrosis remained present. The pathophysiology staging of peridural fibrosis also resembled with the wound healing process based on the classic study of LaRocca H 1974 \(^10\), the study was conducted on the 18 L5 laminectomized dogs. Each 3 dogs were killed at 3 days, 1 week, 3 weeks, 6 weeks, 9 weeks and 12 weeks. The histological examination was performed and found that at the third day, the hematoma completely filled the epidural space and in contact with the surface of erector spinae muscles. At first week, the fibroblasts could be seen to follow the hematoma extension and fibroblastic activity was found mostly at the deep layer of erector spinae muscles and extended over the dura & nerve root (if the surgery deep to foramen, then the fibrous tissue also found in the foramen). At third week, the hematoma resolved. No fibroblastic activity was found after the third week.

The factors influences the peridural fibrosis are hematoma, inflammation and the paraspinal muscle fiber invasion to the dura \(^11\). Many researches tried to work out by the decreasing of these risk factors (Table 1, 2 and 3) such as drainage of hematoma using the closed-suction drainage \(^12\). Prevention of paraspinal muscle invasion using several types of barriers such as free fat graft, cellulose membrane \(^13\), human amniotic membrane \(^14\), dacron, hyaluronate sheath \(^15\), oxiplex or Gore-Tex \(^16\), preservation of ligamentum flavum \(^17\). Reduction of the inflammation or fibroblast inhibitors using steroid \(^18\), honey \(^19\), free radical scavengers(melatonin and octreotide) \(^20\), chemotherapeutic (mitomicin C, 5-fluorouracil and cyclosporine A) \(^21\). Most of the studies were conducted in animal models to study the efficacy and safety of these active ingredients or materials. The results of all these medications or
barriers have been mildly effective or ineffective and, until up to date, there is no drug or material routinely used to prevent or reduce the peridural fibrosis following spinal surgery\(^{(21)}\) and because of their adverse events such as free fat graft had been reported for their compression neuropathy\(^{(22)}\). Recently, Adcon-L, an adhesion-reduction device in laminectomy, was also withdrawn due to serious adverse events\(^{(23)}, (24)\). So the development of materials or chemicals concerning the efficacy and safety are required.

The nonsteroidal anti-inflammatory drugs (NSAIDs) are the examples of systemic chemical substances. They present an advantage over the physical barriers of not introducing foreign bodies, which may increase the inflammatory reaction. Moreover, NSAIDs inhibit cyclooxygenase, an enzyme essential for metabolism of arachidonic acid, which is responsible for synthesis of prostaglandins. The end products of arachidonic acid metabolism are involved in inflammatory process, ultimately leading to adhesion formation. The beneficial effect of the NSAIDs in the prevention of calcifications of the soft tissue, heterotopic ossifications and adherences is well known\(^{(25)}, (26)\). The NSAIDs such as ibuprofen, showed significant postoperative adhesion inhibition while the ibuprofen treatment did not appear to inhibit normal wound healing nor abnormal bleeding at the operative site in hand flexor tendon repairs\(^{(27)}\).

The cellulose membrane (oxidized regenerated cellulose) is the hemostat using in operative fields. The agents have acidic properties, due to their low pH level, and achieve hemostasis via denaturation of blood proteins, mechanical activation of the clotting cascade, and local vasoconstriction. Because of its low pH, the cellulose membrane is bactericidal against many common pathogens of the reproductive tract\(^{(28)}\). A few studies have explored laparoscopic application of the membrane to achieve hemostasis at sites of uterine perforation and for tubal bleeding secondary to sterilization\(^{(29)}, (30)\). Successful hemostasis of moderate bleeding was achieved without the need for suture or conversion to laparotomy in all cases without brisk arterial bleeding. The cellulose membrane also use in prevention of peridural fibrosis following laminectomy safely with good results according the recent consecutive case-series study\(^{(13)}\).
Table 1: The prevention of peridural fibrosis studies based on the hemostasis phase

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>Study groups</th>
<th>Measurement</th>
<th>results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sen O, 2005 (12)</td>
<td>79 discectomy patients</td>
<td>1. closed-suction drainage</td>
<td>MRI for fibrosis clinical (VAS)</td>
<td>less fibrosis on MRI, better VAS (0.32 vs 2.16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. no drainage</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The prevention of peridural fibrosis studies based on the inflammatory, cellular adhesion & fibroblast proliferative phases

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>Study groups</th>
<th>Measurement</th>
<th>results</th>
</tr>
</thead>
</table>
| Cekinmez M, 2005 (18) | 100 Spraque-Dawley rats L4-5 laminectomy | 1. fibrin glue  
2. methylprednisolone  
3. methylprednisolone+fibrin glue  
4. control | Histopathology | No significance differences in all 4 groups |
| Farrokhis MR, 2011 (19) | 45 Spraque-Dawley rats L5-6 laminectomy | 1. honey  
2. saline  
3. control | Histopathology | No significance differences in all 3 groups |
| Yildiz KH, 2007 (21) | 32 Wistar rats L5-6 laminectomy | 1. MMC  
2. 5-FU  
3. CsA  
4. control | Histopathology | * Significant differences in MMC & 5-FU  
* further studies are needed to determine side effects of agents |
| Erol FS, 2010 (20) | 36 Wistar rats T8-L3 laminectomy | 1. melatonin  
2. octreotide  
3. control | Histopathology TGF-β1 level | * sig. lower TGF-β1 level in melatonin & octreotide comparing the control  
* histopathology improvement was sig. only in melatonin group |
<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Animals</th>
<th>Laminectomy Level</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Histology Measure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emmez H, 2011 (31)</td>
<td>24 Wistar rats</td>
<td>Not defined</td>
<td>1. Azithromycin (20 mg/kg intraperitoneal)</td>
<td>2. Azithromycin (80 mg/kg intraperitoneal)</td>
<td>3. Saline</td>
<td>Amount fibrosis, Fibroblast density, Inflammatory cell density</td>
<td>*sig. lower of fibrosis in azithromycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Laminectomy level</td>
<td></td>
<td></td>
<td></td>
<td>* not sig. between treatment groups</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*no difference in inflam. Cells</td>
</tr>
<tr>
<td>He Y, 1995 (33)</td>
<td>32 rats</td>
<td>L5 laminectomy</td>
<td>1. Systemic ketoprofen</td>
<td>2. Control</td>
<td></td>
<td>Amount fibrosis, Fibrous adherence</td>
<td>*Less amount of fibrosis in ketoprofen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*no difference in fibrous adherence</td>
</tr>
<tr>
<td>Sabuncuoglu H, 2007 (34)</td>
<td>30 rats</td>
<td>L4 laminectomy</td>
<td>1. anti-ICAM and CD-18</td>
<td>2. monoclonal anti human IgG</td>
<td>3. control</td>
<td>Fibrous adherence, Adhesion degree, New bone formation</td>
<td>*sig. less amount of fibroblast in anti-ICAM and CD-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*anti IgG show no sig. diff. to control and anti-ICAM and CD-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*sig. less of adhesion degree in anti-ICAM and CD-18 group compare to control group</td>
</tr>
<tr>
<td>Author</td>
<td>Sample</td>
<td>Study groups</td>
<td>Measurement</td>
<td>results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Su WR, 2009 (35)   | 24 rats L5 laminotomy         | 1. low dose irradiation (700 cGy)  
2. high dose irradiation (9-MeV)  
3. sham operation | Histology Neurological status | *Sig. less amount of fibrosis in low dose irradiation group  
*no sig. diff. in pre&postoperative neurological status  
*sig. reduced amount of scar tissue and fibroblast density in the low-dose IFN-gamma group compared with control and high-dose IFN-gamma groups  
*sig. increase was detected in inflammatory cell density in the high-dose IFN-gamma group compared with control and low-dose IFN-gamma groups |
| Emmez H, 2008 (36) | 30 rats laminectomy           | 1. epidural 2000 U/d IFN-gamma x3days  
2. epidural 20,000 U/d IFN-gamma x3days  
3. epidural 0.2 ml/d saline x 3days | Histology -fibroblast number  
-adhesion degree  
-new bone formation | *Sig. less amount of fibrosis in low dose irradiation group  
*no sig. diff. in pre&postoperative neurological status  
*sig. reduced amount of scar tissue and fibroblast density in the low-dose IFN-gamma group compared with control and high-dose IFN-gamma groups  
*sig. increase was detected in inflammatory cell density in the high-dose IFN-gamma group compared with control and low-dose IFN-gamma groups |
| Da Costa RC, 2006 (37) | 18 mixed breed dogs T13-L1 laminectomy | 1. FFG  
2. cellulose membrane  
3. control | Clinical & histopathology | *Cellulose membrane & FFG partially effective in peridural fibrosis prevention  
*FFG causing spinal cord compression & neurodeficit significantly |
| Tao H, 2009 (14)   | 24 mongrel dogs L1,3,5,7 laminectomy | 1. FAM  
2. CAM  
3. FFG  
4. control | Histopathology | CAM & FFG reduces fibroblast infiltration better than FAM & control |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals</th>
<th>Surgery</th>
<th>Conditions</th>
<th>Histology/Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee HM, 1990 (15)</td>
<td>45 white rabbits</td>
<td>L4 laminectomy</td>
<td>1. dacron sheet 2. hyaluronate gel 3. Control</td>
<td>Histopathology</td>
</tr>
<tr>
<td>Kurt G, 2009 (16)</td>
<td>30 Wistar rats</td>
<td>L4 laminectomy</td>
<td>1. oxiplex 2. Gore-Tex 3. control</td>
<td>Histopathology</td>
</tr>
<tr>
<td>Lin HB, 2009 (17)</td>
<td>45 Rabbits</td>
<td>Laminectomy</td>
<td>1. complete ligamentum flavum preservation 2. partial ligamentum flavum preservation 3. no ligamentum flavum preservation</td>
<td>Gross &amp; histology</td>
</tr>
</tbody>
</table>
| Shih HN, 2004 (38) | 48 rabbits | L6 laminectomy | 1. HA/collagen (60/40) membrane 2. HA/collagen (40/60) membrane 3. control | Histology | *amount of fibrosis decrease with time in all groups  
* HA/collagen (40/60) membrane appeared to reduce peridural scar adhesion |
| Quist JJ, 1998 (39) | 16 dogs | L2,4,6 laminectomy | 1. free fat graft 2. polyethylene oxide (PEO)/polybutylene terephthalate (PBT) copolymer 3. control | Histology | * Fat grafts produced significantly less fibrous tissue, but the presence of the fat graft in the bony defect prevented closure  
* extensive and consistent peridural fibrosis in control and PEO/PBT groups |
| Klopp LS, 2008 (40) | 11 sheeps | Laminectomy & dural puncture | 1. bioresorbable Mesofol film 2. bioresorbable Lactosorb film 3. control | Histology | *sig. reduction of fibrosis in both types of bioresorbable films  
* Impairment to healing of dural tears or active inflammation was not identified with any product |
Justification to do the study

Peridural fibrosis occurs inevitably when the epidural space is exposed. The harmful effect of this fibrosis is it adheres and compress to dura, spinal cord or nerve root causing the recurrent symptom of back pain, leg pain or neurological deficit following the index spine surgery necessitate the reoperation for adhesiolysis to remove the peridural fibrosis. The success rate of reoperation is fall to 30, 15 and 5% following the 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} operation respectively \(^{(3)}\). These outcomes are from difficulty in operating the fibrotic tissue and extensive peridural fibrosis increase the likelihood of accidental durotomy or nerve root injury (3-8\%) \(^{(41)}\). So prevention of peridural fibrosis is better. The prevention of peridural fibrosis using the anti-inflammation is feasible. The NSAIDs is one of anti-inflammator agents that may have a role in peridural fibrosis prevention.

This study evaluates the efficacy and safety of the NSAIDs and cellulose membrane in peridural fibrosis prevention.

Research question

Can the NSAIDs and cellulose membranes prevent peridural fibrosis?

Objectives of the study

- Primary objective: to evaluate the efficacy of NSAIDs and cellulose membrane on peridural fibrosis prevention
- Secondary objective: to evaluate the safety of peridural NSAIDs and cellulose membrane in animal model
Hypothesis

Ho: NSAIDs and cellulose membrane have no beneficial effect on peridural fibrosis prevention (no difference in peridural fibrosis in all treatment groups)

(Peridural fibrosis in NSAIDs soaked cellulose membrane group = epidural cellulose membrane group = control group)

or \( \mu_1 = \mu_2 = \mu_3 \)

Ha: NSAIDs or cellulose membrane have beneficial effect on peridural fibrosis prevention (difference in peridural fibrosis in at least two groups of all three treatment groups)

(Peridural fibrosis in NSAIDs soaked cellulose membrane group \( \neq \) cellulose membrane group \( \neq \) control group)

or \( \mu_1 \neq \mu_2 \neq \mu_3 \) (at least two of the population means are not equal)
Failed back surgery syndrome

Preoperative factor
- Wrong diagnosis
- Wrong patient

Intraoperative factor
- Wrong level surgery
- Inadequate decompression
- Misplaced instrumentation
- Iatrogenic instability

Postoperative factor
- Peridural fibrosis

Others
- Pseudarthrosis
- Recurrent HNP

Remodeling phase
- Maturation of collagen

Fibrosis from paraspinal muscle adhere to dura & nerve roots

Cellulose membrane as a barrier
- Interpose between muscle & dura

Proliferative phase
- Fibroblast proliferation, collagen deposition, angiogenesis

Inflammatory phase: chemotaxis
- Inflammatory cell response

Hemostasis phase: hematoma
- Platelet aggregation
- Fibrin clot

NSAIDs: Parecoxib
- Inhibit inflammation
- Finally peridural fibrosis prevention

Cellulose membrane as a hemostatic material

Surgical wound with epidural space exposure

Reoperations: With poor results
Keywords and operational definitions

Laminectomy procedure

Under adequate anesthesia and antibiotic protection, a longitudinal incision of 4 cm was made in a posterior line between L4 and L5; the fascia was incised in order to expose the extreme of the spinous processes. The paraspinal muscles were detached subperiosteally from the spinous processes and the laminas, and retracted with an autostatic separator. Following a meticulous technique in order not to damage the spinal cord, the spinous processes of the caudal vertebra, the ligamentum flavum, inferior articular processes and the third distal part of the lamina of the cranial vertebra were resected until achieving an exposure of the dura of 4 x 8 mm. A drain was used for 24 h.

Peridural fibrosis

The synonyms are laminectomy membrane, epidural fibrosis or scarring. These are characterized by the fibrosis contain in epidural space, adhere or compress to dural sac and/or nerve root.

Parecoxib

Parecoxib is a water soluble and injectable prodrug of valdecoxib. Parecoxib is a COX2 selective inhibitor NSAIDs. It has a very high selectivity for inhibiting cyclo-oxygenase-2 (COX-2) mediated prostaglandin synthesis to reduce mediators of pain and inflammation. As it is injectable, it can be used perioperatively when patients are unable to take oral medications. It is approved through much of Europe for short term perioperative pain control much in the same way ketorolac is used in the United States. However, unlike ketorolac or aceclofenac, parecoxib has no effect on platelet function and therefore does not promote bleeding during or after surgery. Half life of parecoxib is 9.9 hours\(^{(42)}\).

Surgical cellulose membrane

The surgical cellulose is an absorbable membrane made of an oxydized cellulose polymer. This membrane is sheer weave, bactericidal and hemostat properties.
**Sprague-Dawley rat**

The laboratory rat most commonly uses in wound healing studies\(^{(43)}\). The majority of the studies used rats weighting from 250-300 grams. The Harlan Laboratory Animal Company reports that weights of 150-299 grams correspond to 41-69 days old\(^{(44)}\) male Spraque-Dawley rats that are compatible to young humans\(^{(45)}\).

**Research design**

Randomized controlled experimental study in animal model

**Research methodology**

The adult Spraque-Dawley rats weighted between 250 and 300 grams\(^{(43)}\) are included in the study. All rats will be laminectomized on their L4-5 laminas. The rats with any complication from laminectomy procedure such as dural tear or cerebrospinal fluid leakage will be classified as non-eligible and excluded from the study. All eligible rats will be randomized and allocated into control or experimental groups using the computer generated randomization in the opaque sealed envelopes. The control group will be placed the epidural space with normal saline solution before surgical wound closure, whereas experimental groups will be filled the epidural space with the cellulose membrane or NSAIDs soaked surgical cellulose membrane before surgical wound closure.

The rats will be sacrificed at 4 weeks after the operation. The specimens will be prepared for histopathologic studies by 2 blinded outcome assessors.

The primary outcome is fibroblast density. This will count in term of mean, standard deviation and 95% confidence interval.

The secondary outcomes are inflammatory cell density and fibrous adherence grading. The inflammatory cell densities are calculated in term of mean, standard deviation and 95% confidence interval. The fibrous adherences are classified in categorical data (grade 0 or 1 fibrosis is considered negative for
disease and grade 2 or 3 is positive for disease) and collect the data in 2x2 table as the following.

**Table 4: 2x2 table for fibrous adherence grading**

<table>
<thead>
<tr>
<th></th>
<th>Fibrosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>disease</td>
<td>Not disease</td>
</tr>
<tr>
<td>Experimental groups</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control group</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td>total</td>
<td>a+c</td>
<td>b+d</td>
</tr>
</tbody>
</table>

Then calculate the experimental event rate (EER), control event rate (CER), absolute risk reduction (ARR), relative risk (RR), relative risk reduction (RRR) and number needed to treat (NNT).

The adverse events are also recorded and report in term of incidence.

**Population**

L4-5 laminectomized adult Spraque-Dawley rats will be randomized and allocated into NSAIDs soaked cellulose membrane, epidural cellulose membrane or control groups before surgical wound closure.

We need the randomization to ensure that underlying variables do not result in skewed data in all groups of the animals \(^{(46)}\). So the permuted block randomization number is generated by computer using the Microsoft Excel 2007 software. These randomization codes will be kept in opaque sealed envelopes. Each envelope will be open and assign the treatment to the rat after the L4-L5 spines are completely laminectomized.

**Intervention**

Peridural NSAIDs soaked cellulose membrane group: the peridural space of lumbar laminectomized rats is placed with NSAIDs soaked surgical cellulose membrane before wound closure.
Epidural cellulose membrane group: the peridural space of lumbar laminectomized rats is placed with cellulose membrane before wound closure.

Control group: peridural space of lumbar laminectomized rats is filled with normal saline before wound closure.

**Study flow chart**

Sprague – Dawley rats

L4-5 laminectomy

Randomization & Allocation

Study group (peridural NSAIDs soaked cellulose)

Control group (peridural saline dripping)

Study group (epidural cellulose membrane)

All rats are sacrificial at 4 weeks

All rats are sacrificial at 4 weeks

Histopathology (Outcomes)

2 blinded outcome assessors

Statistical analysis
Outcome variable

The outcome of histopathology is the grading of peridural fibrosis, that is grade 0, 1, 2 or 3, and reports in experimental event rate, control event rate, absolute risk reduction, relative risk, relative risk reduction and number needed to treat. The fibroblast or inflammatory cell densities are reported in mean ± standard deviation and 95% confidence interval.

Measurement of the outcome

The spine tissue will be fixed in phosphate buffered 10% formaldehyde for 2 days, decalcified in De Castro’s fluid, dehydrated in alcohol and embedded in paraffin. Cross-sectional serial section 5 µm thick will be stained with hematoxylin and eosin and Masson’s trichrome stains. The section will be evaluated for fibroblast density, fibrous adherence degree and inflammatory cell density.

1. Fibroblast density : as a unit of density of fibroblasts per square millimetre
2. Inflammatory cell density: as a unit of density of the inflammatory cells per square millimetre
3. Histopathologic grading of fibrous adherence $^{(47)}$

Grade 0: no scar tissue on the dura mater

Grade 1: only thin fibrous band were observed

Grade 2: continuous adherence was observed for less than two thirds of laminectomy area

Grade 3: scar tissue was large, more than two third of laminectomy area, and/or extended to spinal nerve roots

All rats will be sacrificed at 4th weeks for histopathological study and the outcomes will be assessed by 2 blinded pathologists using the microscope for histologic grading and computer software (Image Tool version 3.0) for fibroblast or inflammatory cell density.
Data/Variables

1. Baseline variables
   - Gender
   - Age
   - Weight

2. Outcome Variables
   2.1 Primary outcome variables: fibroblast density as a unit of cells/mm²
   2.2 Secondary outcome variables
      - Inflammatory cell density as a unit of cells/mm²
      - Fibrous adherence grading or histopathologic grading (0, 1, 2 or 3)

Outcome measurement and data collection

1. How do you measure the outcome?

   The spine tissue will be fixed in phosphate buffered 10% formaldehyde for 2 days, decalcified in De Castro’s fluid, dehydrated in alcohol and embedded in paraffin. Cross-sectional serial section 5 µm thick will be stained with hematoxylin and eosin and Masson’s trichrome stains. The section will be evaluated for fibroblast density, fibrous adherence degree and inflammatory cell density.

   - Fibroblast density: as a unit of density of fibroblasts per square millimetre
   - Inflammatory cell density: as a unit of density of the inflammatory cells per square millimetre
   - Histopathologic grading of fibrous adherence\(^{(47)}\)

     Grade 0: no scar tissue on the dura mater

     Grade 1: only thin fibrous band were observed

     Grade 2: continuous adherence was observed for less than two thirds of laminectomy area

     Grade 3: scar tissue was large, more than two third of laminectomy area, and/or extended to spinal nerve roots
2. **Which tool will be used?** and
3. **Who will do these jobs?**

The fibroblast density & inflammatory cell density will be count and calculated per area (mm²) using the computer software (Image Tool version 3.0) that can give a result as mean± standard deviation automatically.

The fibrous adherence grading will be classified by 2 blinded pathologists. The grading will be calculated and reported as inter-rater agreement using the Kappa statistics.

Table 5: The 2x2 table for Kappa calculation

<table>
<thead>
<tr>
<th>Pathologist B</th>
<th>Pathologist A</th>
<th></th>
<th></th>
<th></th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 0</td>
<td>Grade 1</td>
<td>Grade 2</td>
<td>Grade 3</td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>a</td>
<td>e</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>b</td>
<td>f</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>c</td>
<td>g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>d</td>
<td>h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>i</td>
<td>j</td>
<td>k</td>
<td>l</td>
<td>n</td>
</tr>
</tbody>
</table>

\[
K = \frac{nD - \sum t_i U_i}{n^2 - \sum t_i U_i}
\]

\[
Kappa = \frac{n(a+b+c+d)-[(e*i)+(f*j)+(g*k)+(h*l)]}{n^2-[(e*i)+(f*j)+(g*k)+(h*l)]}
\]

For unconsensus opinion, the result will be discussed together and given the final results by the 2 blinded pathologists.

4. **When do you collect the data?**

The data will be collected at 4 weeks postoperatively. All of the rats will be sacrificed using euthanasia (lethal doses of CO₂). The specimens will be prepared for histopathological examination.
5. Method of handling the potential bias?
   The potential bias in the study may be:
   1. Selection bias
      The age and weight of the rats may influence the fibrous tissue formation such as the younger age can heal rapidly and give a dense fibrous tissue formation more than the older age.
      Correction:
      1.1 The age and the weight of the rats will be controlled, so the Sprague-Dawley rats weight between 150-300 grams that compatible to 41-69 days old rats or young adult human$^{(43)}$ will be used in the study.
      1.2 Due to the other unknown confounders, the randomization and allocation concealment will be used to balance these factors and to ensure the baseline characteristics of the rats in all groups.
   2. Performance bias
      The surgeon’s soft tissue dissection may associate with the degree of fibrous tissue proliferation, example, more soft tissue dissection result in more fibrous tissue proliferation.
      Correction:
      2.1 The surgeon operates the laminectomy before the randomization process take place.
      2.2 The surgeon must familiar and experiences the surgical operation well.
   3. Interviewer (outcome assessment) bias
      The outcome assessment especially the fibrous adherence grading may be biased by the outcome assessors.
      Correction:
      3.1 The outcome assessors will be masked to all of processes in the study except only the histopathological examination.
Sample size calculation

The hypothesis is detection the mean difference (continuous data) between the two independent groups. The formula for sample size calculation is based on Z distribution as follow.

\[
n = \frac{(Z\alpha/2 + Z\beta)^2 \cdot 2\delta^2}{(\mu_1 - \mu_2)}
\]

Shah H (48) derived this formula to simplify the sample size estimation in animal study by the following formula (with changing the \(Z\alpha/2 + Z\beta\) to \(C\), \(\delta\) to \(S\) and \(\mu_1 - \mu_2\) to \(d\)).

\[
n = 1 + 2C \left(\frac{s}{d}\right)^2
\]

The prerequisite for sample size calculation are as follow.

1. Standard deviation (S): It can be calculated by previous studies or pilot study
2. Power of the study (1- \(\beta\)) (\(\beta\) is type II error) (Usually 1- \(\beta\) is fixed at \(>\) 80%)
3. Significance level (\(\alpha\)) (\(\alpha\) is type I error) (Usually \(\alpha\) is fixed at 5% i.e \(p < 0.05\))
4. Difference of outcome between two group, researcher wishes to detect.
5. The \(Z\alpha/2\) and \(Z\beta\) can point to the \(C\) (using the table for \(C\)) to calculate the sample size per group finally.

<table>
<thead>
<tr>
<th>(\alpha)</th>
<th>Power(1-(\beta))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>0.8</td>
<td>7.85</td>
</tr>
<tr>
<td></td>
<td>10.51</td>
</tr>
<tr>
<td></td>
<td>11.68</td>
</tr>
<tr>
<td>0.9</td>
<td>10.51</td>
</tr>
<tr>
<td></td>
<td>13.56</td>
</tr>
<tr>
<td></td>
<td>14.88</td>
</tr>
</tbody>
</table>
The study design is to compare the mean of fibroblast densities in each two groups of all these three groups, so the Bonferroni method is needed for level of significance adjusting. In comparing mean of three groups, the comparison can be NSAIDs soaked cellulose membrane to epidural cellulose membrane, epidural cellulose membrane to control or NSAIDs soaked cellulose membrane to control (3 possibilities of comparison) therefore the adjusted significance level should be 0.05/3 or 0.016 instead of 0.05.

Example for sample size calculation

s- Standard deviation from the previous study by Sabuncuoglu H (34) is 338.6 cells/mm² and mean of fibroblast density is 1,627 cell/mm²

d- Expected difference (effect size) between two means is 400 cells/mm²

C- Constant

The study sets the power to 80% while 𝛼 is 0.05 or the adjusted 𝛼 according Bonferroni method is 0.016, so the constant (C) will be 10.51.

So \( n = 1 + 2(10.51)(338.6/400)^2 = 16.06 \)

The sample size (rats) should be 17 in each group.

Table 7: The sample size calculation based on the varied power and effect size

<table>
<thead>
<tr>
<th>α</th>
<th>Power</th>
<th>SD</th>
<th>Effect size</th>
<th>C</th>
<th>n/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>100</td>
<td>10.51</td>
<td>190.18</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>200</td>
<td>10.51</td>
<td>48.295</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>300</td>
<td>10.51</td>
<td>22.02</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>400</td>
<td>10.51</td>
<td>12.82375</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>500</td>
<td>10.51</td>
<td>8.5672</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>600</td>
<td>10.51</td>
<td>6.255</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>700</td>
<td>10.51</td>
<td>4.860816</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>800</td>
<td>10.51</td>
<td>3.955938</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>900</td>
<td>10.51</td>
<td>3.335556</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>300</td>
<td>1000</td>
<td>10.51</td>
<td>2.8918</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>100</td>
<td>10.51</td>
<td>241.9942</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>200</td>
<td>10.51</td>
<td>61.24855</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>300</td>
<td>10.51</td>
<td>27.77714</td>
</tr>
<tr>
<td>Temperature</td>
<td>Velocity</td>
<td>Load</td>
<td>Power Density</td>
<td>Efficiency</td>
<td>Capacity</td>
</tr>
<tr>
<td>-------------</td>
<td>----------</td>
<td>------</td>
<td>--------------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>400</td>
<td>10.51</td>
<td>16.06214</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>500</td>
<td>10.51</td>
<td>10.63977</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>600</td>
<td>10.51</td>
<td>7.694284</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>700</td>
<td>10.51</td>
<td>5.918249</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>800</td>
<td>10.51</td>
<td>4.765535</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>900</td>
<td>10.51</td>
<td>3.975237</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>338.6</td>
<td>1000</td>
<td>10.51</td>
<td>3.409942</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>100</td>
<td>10.51</td>
<td>258.495</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>200</td>
<td>10.51</td>
<td>65.37375</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>300</td>
<td>10.51</td>
<td>29.61056</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>400</td>
<td>10.51</td>
<td>17.09344</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>500</td>
<td>10.51</td>
<td>11.2998</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>600</td>
<td>10.51</td>
<td>8.152639</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>700</td>
<td>10.51</td>
<td>6.255</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>800</td>
<td>10.51</td>
<td>5.023359</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>900</td>
<td>10.51</td>
<td>4.178951</td>
</tr>
<tr>
<td>0.05</td>
<td>80</td>
<td>350</td>
<td>1000</td>
<td>10.51</td>
<td>3.57495</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>100</td>
<td>13.56</td>
<td>245.08</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>200</td>
<td>13.56</td>
<td>62.02</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>300</td>
<td>13.56</td>
<td>28.12</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>400</td>
<td>13.56</td>
<td>16.255</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>500</td>
<td>13.56</td>
<td>10.7632</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>600</td>
<td>13.56</td>
<td>7.78</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>700</td>
<td>13.56</td>
<td>5.981224</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>800</td>
<td>13.56</td>
<td>4.81375</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>900</td>
<td>13.56</td>
<td>4.013333</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>300</td>
<td>1000</td>
<td>13.56</td>
<td>3.4408</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>100</td>
<td>13.56</td>
<td>311.9307</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>200</td>
<td>13.56</td>
<td>78.73267</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>300</td>
<td>13.56</td>
<td>35.54785</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>400</td>
<td>13.56</td>
<td>20.43317</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>500</td>
<td>13.56</td>
<td>13.43723</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>600</td>
<td>13.56</td>
<td>9.636964</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>700</td>
<td>13.56</td>
<td>7.345524</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>800</td>
<td>13.56</td>
<td>5.858292</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>900</td>
<td>13.56</td>
<td>4.838651</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>338.6</td>
<td>1000</td>
<td>13.56</td>
<td>4.109307</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>350</td>
<td>100</td>
<td>13.56</td>
<td>333.22</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>350</td>
<td>200</td>
<td>13.56</td>
<td>84.055</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>350</td>
<td>300</td>
<td>13.56</td>
<td>37.91333</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>350</td>
<td>400</td>
<td>13.56</td>
<td>21.76375</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>350</td>
<td>500</td>
<td>13.56</td>
<td>14.2888</td>
</tr>
</tbody>
</table>
Based on the $\alpha = 0.05$, 80% power and the effect size for 400 cell/mm$^2$ (from previous study (34) the effect size for fibroblast density was 504 cell/mm$^2$, but this study needs to be detect the better effect size, so we set the effect size to 400 cell/mm$^2$), the sample size in each group will be 17 rats.

When calculate back to pretest the prediction for precision using the STATA (version 11, command: ttesti 17 1627 338.6 17 1227 400), the 95% confidence interval of study group is from 1021.339 to 1432.661 cell/mm$^2$ comparing the control group that is 1452.908 to 1801.092 cell/mm$^2$.

Table 8: The prediction of 95% confidence interval using STATA

<table>
<thead>
<tr>
<th>diff</th>
<th>x</th>
<th>y</th>
<th>combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400</td>
<td>127.1058</td>
<td>141.094</td>
</tr>
<tr>
<td></td>
<td>338.6</td>
<td>417.5832</td>
<td>1281.298</td>
</tr>
<tr>
<td></td>
<td>1452.908</td>
<td>1572.702</td>
<td></td>
</tr>
</tbody>
</table>

Two-sample t test with equal variances

. ttesti 17 1627 338.6 17 1227 400

<table>
<thead>
<tr>
<th></th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Err.</th>
<th>Std. Dev.</th>
<th>[95% Conf. Interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>diff</td>
<td>17</td>
<td>1627</td>
<td>82.12256</td>
<td>338.6</td>
<td>1452.908</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1227</td>
<td>97.01425</td>
<td>400</td>
<td>1021.339</td>
</tr>
<tr>
<td>combined</td>
<td>34</td>
<td>1427</td>
<td>71.61493</td>
<td>417.5832</td>
<td>1281.298</td>
</tr>
</tbody>
</table>

$\text{diff} = \text{mean}(x) - \text{mean}(y)$

$t = 3.1470$

degrees of freedom = 32

Ha: diff $< 0$

Pr($T < t$) = 0.9982

Ha: diff $! = 0$

Pr($|T| > |t|$) = 0.0036

Ha: diff $> 0$

Pr($T > t$) = 0.0018
Summary statements

An unpaired modified t-test with Bonferroni correction on continuous data (fibroblast density) with a sample size of 17 rats per group achieves 80% power at 0.05 significance level to detect a difference in mean at least 400 fibroblast cells/mm². The dropout rate of animal does not concern. Therefore this study needs the sample size for 17 rats per group.

Plan for data analysis and data presentation

Baseline characteristics of the animals in this study are gender, age and weight.

- The gender will be presented in form of frequency as percent between male and female.
- The age and weight are continuous data, will be presented as mean ± sd.

Table 9: Baseline characteristics of the Spraque-Dawley rats

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NSAIDs+cellulose membrane group</th>
<th>Epidural cellulose membrane group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
</tr>
<tr>
<td>Gender</td>
<td>male : female (%)</td>
<td>male : female (%)</td>
<td>male : female (%)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
<td>Mean ± sd</td>
</tr>
</tbody>
</table>

Primary outcome analysis: fibroblast density

- The fibroblast density at 4 weeks postoperative period will be presented as mean ± sd in both study and control group.
- The fibroblast density in study and control groups will be test for the difference using the t-test.
Statistical methods

Sample

- NSAIDs soaked cellulose membrane
- Epidural cellulose membrane
- control

Test for normality

Normal

ANOVA

Significance difference:
Comparison between each 2 groups

Modified t-test
With Bonferroni method

Non-normal

Kruskal–Wallis ANOVA

Significance difference:
Comparison between each 2 groups

Multiple comparisons using Dunn method

The data will be firstly tested for normality.

The normal distribution of data will be further tested by ANOVA. If the ANOVA shows significant differences, then the modified t-test with Bonferroni method for each of 2 group comparisons will be used.

The non-normal distribution of data or categorical data will be tested by Kruskal-Wallis ANOVA. If the Kruskal-Wallis ANOVA shows significant differences, then the Dunn multiple comparisons will be used to test the differences between each 2 groups.
Statistics for primary outcomes: Fibroblast density

The fibroblast density (cells/mm²) will be counted and calculated by computer software.

The comparison of mean in three groups will be tested using the ANOVA to test null hypothesis that all groups have the same means. If the null hypothesis is rejected, then the modified t-test will be used to compare mean differences in each independent two groups (peridural NSAIDs soaked cellulose membrane group to epidural cellulose membrane group, peridural NSAIDs soaked cellulose membrane group to control group, and epidural cellulose membrane group to control group).

Table 10: Summarized statistics for primary outcome

<table>
<thead>
<tr>
<th>outcome</th>
<th>scale</th>
<th>Report</th>
<th>Hypothesis</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast density</td>
<td>ratio</td>
<td>Mean+sd 95%CI</td>
<td>Ho: µ1=µ2=µ3</td>
<td>ANOVA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ha: µ1≠µ2=µ3</td>
<td></td>
</tr>
<tr>
<td>If ANOVA show significance difference in mean of at least one group, then the comparison between two groups will be tested using the modified t-test with Bonferroni adjustment.</td>
<td>Ho: µ1=µ2</td>
<td>Modified t-test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ha: µ1≠µ2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ho: µ1=µ3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ha: µ1≠µ3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ho: µ2=µ3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ha: µ2≠µ3</td>
<td></td>
</tr>
</tbody>
</table>

Explanation for statistical analysis of primary outcome (fibroblast density)

1. Test for normal distribution of the fibroblast density for study and control groups using the histogram or Shapiro Wilk test.
2. Test for equality of variances among the study and control groups using F distribution.
3. If the ANOVA show significance difference in variance among the groups, then the modified t-test with Bonferroni correction will be used to compare the mean between each two groups.
   - If all independent random samples are normally distribute, then the ANOVA will be used to analyze the variance by the F equation.
F = BMS
\[ \frac{WMS}{WMS} \]

- If ANOVA show significance difference of mean at least in one group, then the two out of three groups will be compared using the modified t-test and Bonferroni adjustment.

\[ T \left( N-k \right) = \frac{1^2 - \frac{2}{n_1 + WMS/n_2}}{\sqrt{WMS/n_1 + WMS/n_2}} \]

Comparing the two out of three groups can be 3 comparisons (peridural NSAIDs soaked cellulose membrane group to epidural cellulose membrane group, peridural NSAIDs soaked cellulose membrane group to control group, and epidural cellulose membrane group to control group). So adjustment of \( \alpha \) according Bonferroni method is \( \alpha/3 \) or \( 0.05/3 \) (\( =0.016 \)). The null hypothesis will be rejected when the p value less than 0.016.

The magnitude of effect will be calculated in 95% confidence interval depend on the chosen statistical test, such as the 95%CI of mean difference from the t test with pooled variance is \( (1 - 2) + t_{\alpha/2,df} \text{S.E.} \).

**Secondary outcome analysis: inflammatory cell density**

- The postoperative inflammatory cell density at 4 weeks will be presented as mean\( \pm \) sd in both studies and control groups.
- The means of inflammatory cell density in studies and control groups will be tested for the difference using the ANOVA and modified t-test respectively as same as the fibroblast cell density.

Statistics for inflammatory cell density

The inflammatory cell density(cells/mm\(^2\)) that will be counted and calculated by computer software.

Table 11: Summarized statistics for inflammatory cell density

<table>
<thead>
<tr>
<th>outcome</th>
<th>scale</th>
<th>Report</th>
<th>Hypothesis</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>inflammatory</td>
<td>ratio</td>
<td>Mean(\pm)sd</td>
<td>Ho : (\mu_1=\mu_2=\mu_3)</td>
<td>ANOVA</td>
</tr>
</tbody>
</table>
If ANOVA show significance difference in mean of at least one group, then the comparison between two groups will be tested using the modified t-test with Bonferroni adjustment.

<table>
<thead>
<tr>
<th>cell density</th>
<th>95%CI</th>
<th>Ha : µ1≠µ2≠µ3</th>
<th>Modified t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>If ANOVA show significance difference in mean of at least one group, then the comparison between two groups will be tested using the modified t-test with Bonferroni adjustment.</td>
<td>Ho : µ1=µ2</td>
<td>Ha : µ1≠µ2</td>
<td>Modified t-test</td>
</tr>
<tr>
<td>Ho : µ1=µ3</td>
<td>Ha : µ1≠µ3</td>
<td>Modified t-test</td>
<td></td>
</tr>
<tr>
<td>Ho : µ2=µ3</td>
<td>Ha : µ2≠µ3</td>
<td>Modified t-test</td>
<td></td>
</tr>
</tbody>
</table>

The steps of calculation are the same as fibroblast density.

Table 12: The presentation for outcomes: fibroblast density & inflammatory cell density

<table>
<thead>
<tr>
<th>Densities</th>
<th>NSAIDs + cellulose membrane group</th>
<th>Epidural cellulose membrane group</th>
<th>Control group</th>
<th>95% CI mean difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast Density</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
</tr>
<tr>
<td>Inflammatory cell density</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
<td>Mean + sd</td>
</tr>
</tbody>
</table>

Statistics: unpaired t-test

Fibrous adherence

- The Kruskal-Wallis one way analysis of variance will be used for test the median of fibrous adherence grading between the groups.
- Ho : Population in all groups have the same median of fibrous adherence grading.
- Ha : Population in all groups have different median of fibrous adherence grading.
- The table of Kruskal-Wallis test will be made as follow.
Table 14: The table for Kruskal-Wallis test

<table>
<thead>
<tr>
<th></th>
<th>NSAIDs +cellulose membrane</th>
<th>Epidural cellulose membrane</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranking of median in the groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
</tbody>
</table>

Calculate the \( H = \frac{12}{N(N+1)} \sum_{i=1}^{K} \left( \frac{T_i^2}{n_i} \right) - 3(N+1) \)

Using the \( X^2 \) table (\( X^2 \alpha 0.05, \text{df k-1}=2 \)), the critical value will be 5.99. The calculated \( H \) value will be compared with the critical \( X^2 \) value of 5.99. If the calculated \( H \) value more than critical \( X^2 \) value, then the null hypothesis will be rejected and there is one group has different in fibrous adherence grading will be concluded.

The multiple comparison test will be used follow Kruskal-Wallis test that reject the null hypothesis about the variance. The Dunn method of multiple comparisons will be used to compare the NSAIDs soaked cellulose membrane to control, epidural cellulose membrane to control and NSAIDs soaked cellulose membrane to epidural cellulose membrane. If the null hypothesis can be rejected, then the conclusion is the statistical significant in different between the paired of comparison.

- The fibrous adherence grading: The fibrous adherence grade 0 and 1 will be classified as non-disease whereas grade 2 and 3 will be classified as disease and the data will be presented in form of frequency per categories in 2x2 table.
- The Dunn multiple comparisons will be used to test the difference between the study and control groups. (The same test for comparing the NSAIDs soaked cellulose membrane to control and epidural cellulose membrane to control)
Table 15: Summarize statistics for fibrous adherence

<table>
<thead>
<tr>
<th>outcome</th>
<th>scale</th>
<th>Report</th>
<th>Hypothesis</th>
<th>test</th>
</tr>
</thead>
<tbody>
<tr>
<td>fibrous adherence</td>
<td>ordinal</td>
<td>frequency, rank</td>
<td>Ho: $\prod_1=\prod_2=\prod_3$ Ha: $\prod_1 \neq \prod_2 \neq \prod_3$</td>
<td>Kruskal-Wallis ANOVA</td>
</tr>
</tbody>
</table>

If Kruskal-Wallis ANOVA show significance difference in proportion of at least one group, then the comparison between two groups will be tested using the multiple comparison according Dunn method.

$Ho: \prod_1=\prod_2$
$Ha: \prod_1 \neq \prod_2$
$Ho: \prod_1=\prod_3$
$Ha: \prod_1 \neq \prod_3$
$Ho: \prod_2=\prod_3$
$Ha: \prod_2 \neq \prod_3$

Note: $\prod_1$ is the proportion of moderate to severe peridural fibrosis (disease) in study group

$\prod_2$ is the proportion of moderate to severe peridural fibrosis in control group

The magnitude of effect will be calculated using the 2x2 table.

Table 16: The 2x2 table and important magnitude of effects calculation

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Peridural fibrosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease (moderate to severe)</td>
<td>No disease (none to mild)</td>
</tr>
<tr>
<td>Study group</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control group</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+d</td>
</tr>
</tbody>
</table>

$EER = a/(a+b)$, $CER=c/(c+d)$, $ARR=EER-CER$

$RR=EER/CER$, $RRR=1-RR$, $NNT=1/ARR$

The 95% confidence interval will also be calculated and presented especially the 95%CI of ARR and 95% of NNT.
### Adverse events

The adverse events, such as wound infection or wound dehiscence will be recorded and presented as incidence.

The adverse event will be compared between the treatment and control groups using the Pearson’s chi square test for independence will be used for test the association between the experimental group and adverse events.

- **H₀**: There is no association between the treatment group and adverse events.
- **Hₐ**: There is association between the treatment group and adverse events.
- The table of $X^2$ test for independence will be made as follow.

#### Table 17: The $X^2$ calculation

<table>
<thead>
<tr>
<th></th>
<th>NSAIDs +cellulose membrane</th>
<th>Epidural cellulose membrane</th>
<th>control</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event 1</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A+B+C</td>
</tr>
<tr>
<td>Event 2</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>D+E+F</td>
</tr>
<tr>
<td>total</td>
<td>A+D</td>
<td>B+E</td>
<td>C+F</td>
<td>A+B+C+D+E+F</td>
</tr>
</tbody>
</table>

\[
EA = \frac{(A+B+C)(A+D)}{(A+B+C+D+E+F)}
\]

\[
EB = \frac{(A+B+C)(B+E)}{(A+B+C+D+E+F)}
\]

\[
EC = \frac{(A+B+C)(C+F)}{(A+B+C+D+E+F)}
\]

\[
ED = \frac{(D+E+F)(A+D)}{(A+B+C+D+E+F)}
\]

\[
EE = \frac{(D+E+F)(B+E)}{(A+B+C+D+E+F)}
\]

\[
EF = \frac{(D+E+F)(C+F)}{(A+B+C+D+E+F)}
\]
The calculated $X^2$ value will be compared with the critical $X^2$ value of 5.99 (that based on 0.05 significance level and $df = (r-1)(c-1) = (2-1)(3-1) = 2$). If the calculated $X^2$ value more than critical $X^2$ value, then the null hypothesis will be rejected and there is association between the type of treatment and adverse events.

The calculation for the comparison can summarize in these steps.

From the formular of $a X^2 = \sum (O-E)^2$ 

\[ E = np = 0.5 \times 34 = 17 \text{ in both groups} \]

1. The data will be presented in 2x2 table.

Table 18: The 2x2 table for $X^2$ calculation

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Adverse event</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wound infection</td>
<td>No wound infection</td>
</tr>
<tr>
<td>Study group</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control group</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+d</td>
</tr>
</tbody>
</table>

2. The expected value can be calculated as the formula.

\[ Ea = \frac{(a+b)(a+c)}{(a+b+c+d)} \]
\[ Eb = \frac{(a+b)(b+d)}{(a+b+c+d)} \]
\[ Ec = \frac{(a+c)(c+d)}{(a+b+c+d)} \]
\[ Ed = \frac{(c+d)(b+d)}{(a+b+c+d)} \]
3. Degree of freedom (df) = (r-1)(c-1)=(2-1)(2-1)=1

4. Calculation the $x^2$ value from the table

Table 19: showed the $X^2$ value

<table>
<thead>
<tr>
<th>Cell</th>
<th>observed</th>
<th>expected</th>
<th>(O-E)</th>
<th>(O-E)^2</th>
<th>(O-E)^2/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. The $X^2$ value and df=1, the p-value can predict.

If the p-value less than 0.05, the null hypothesis will be rejected and the results can conclude that there is significance difference in proportion of adverse event (e.g. Wound infection) in treatment group compare to the control group.

The magnitude of effect will be calculated using the 2x2 table.

Table 20: The 2x2 table and important magnitude of effects calculation

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Adverse event</th>
<th>Adverse event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wound infection</td>
<td>Wound infection</td>
</tr>
<tr>
<td>Study group</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control group</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+d</td>
</tr>
</tbody>
</table>

EER = a/(a+b), CER=c/(c+d), ARR=EER-CER

RR=EER/CER, RRR=1-RR, NNT=1/ARR

The 95% confidence interval will also be calculated and presented especially the 95%CI of ARR and 95% of NNT.

6. If the assumption of $X^2$ is break such as in a case of the value of less than 5 in any space of 2x2 table, then the Fisher’s exact test will be used to test the hypothesis instead of $X^2$ test.
6.1 Hypothesis
Ho : Proportion of adverse event in study group = control group (P1=P2)
Ha : P1≠P2

6.2 Make a 2x2 table

Table 21: The 2x2 table for adverse events

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Adverse event (eg. Wound infection)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection</td>
<td>No infection</td>
</tr>
<tr>
<td>Study group</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Control group</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Total</td>
<td>A+C</td>
<td>B+D</td>
</tr>
</tbody>
</table>

7. Calculate the exact probability (P)

\[
P = \frac{(A+B)!(C+D)!(A+C)!(B+D)!}{N!A!B!C!D!}
\]

8. The P-value will be compared to the \( \alpha \) (0.05).
9. If P-value < 0.05, then the Ho is rejected. The conclusion will be “there is significance difference in proportion of adverse event between study and control groups”.

Table 22: Presentation for the adverse events

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>NSAIDs + cellulose membrane group</th>
<th>Epidural cellulose membrane group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound infection</td>
<td>frequency</td>
<td>frequency</td>
<td>frequency</td>
<td></td>
</tr>
<tr>
<td>Wound dehiscence</td>
<td>frequency</td>
<td>frequency</td>
<td>frequency</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>frequency</td>
<td>frequency</td>
<td>frequency</td>
<td></td>
</tr>
</tbody>
</table>

*Statistics: Fisher’s Exact test*
Limitation

This study is the first study to use the peridural NSAIDs soaked cellulose membrane for peridural fibrosis prevention. So, we need to test the safety and efficacy in animal model before apply this technique into human and also need further clinical study in human.

Expecting benefits

The use of animal model in biomedical research is accepted by most of scientists and researchers to the advancement of useful knowledge that bring about relieve from suffering. Animals and human are symbiotic in many ways, they make much sense as whether clinical trials are necessary before new medical therapies are allowed to be widely used in general population. A recent review by the Nuffield Council on Bioethics concluded that “animal research has been, and can potentially be, scientifically valid, in that it is possible to extrapolate from animal models to human”. The Council further cautioned that data on the validity of animal experiments have been interpreted and used in different ways by both opponents and proponents of the scientific validity of using animal models.

So the benefit from this study is the new basic concept and treatment modality for peridural fibrosis prevention. This technique can generalize to use safely in further human study to test the actual effectiveness in human and finally give a new surgical technique in clinical practice. Prevention of peridural fibrosis can reduce FBSS up to 36% of patients.

Ethical consideration

The study protocol needs to be approved by Animal Ethic Committee of Khon Kaen University before the study begins. However, the researchers keep the 3 R’s principle \(^{(49), (50)}\) for animal uses in this study.

Reduction: Reducing the number of animal uses in the study by the sample size calculation to use the least number of animals while the outcomes from the study are most robust.
Refinement: The animals will be cared to minimize their suffering by the sterile technique in surgical operation for postoperative infection prevention, analgesics given for animal postoperatively, the animal will be sacrificed by euthanasia.

Replacement: The animal uses in this study are replaced by small animals instead of large animals. The small animal uses here are Sprague-Dawley rats, the widely used animal in spine surgery and wound healing studies.

We also realize the needs for good animal caring and housing to keep all animals can freedom from thirst, hunger, pain, discomfort, fear, distress while freedom to express normal behavior.

**Time schedule**

The activities including proposal development, ethical consideration, operation & data collection, statistical analysis, full reporting and the manuscript writing for publication will be taken as the table of time schedule.

**Table 23: The time schedule**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposal development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethical consideration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operation &amp; data collection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full report and manuscript writing for publication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Table 24: The budgets

<table>
<thead>
<tr>
<th>Details</th>
<th>Cost (Baht)</th>
<th>Units</th>
<th>For study course</th>
<th>Baht</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Salary for personnel</td>
<td>(per month)</td>
<td>(persons)</td>
<td>10,000x2x2</td>
<td>40,000</td>
</tr>
<tr>
<td>1.1 data collectors</td>
<td>10,000</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 Spraque-Dawley rats</td>
<td>240/rat</td>
<td>51</td>
<td>240x51</td>
<td>12,240</td>
</tr>
<tr>
<td>2.2 Transportation</td>
<td>500</td>
<td>10</td>
<td>500x10</td>
<td>5,000</td>
</tr>
<tr>
<td>2.3 Box for transportation</td>
<td>150</td>
<td>10</td>
<td>150x10</td>
<td>1,500</td>
</tr>
<tr>
<td>2.4 Foods</td>
<td>50/kg</td>
<td>15</td>
<td>50x15</td>
<td>750</td>
</tr>
<tr>
<td>2.5 Housing</td>
<td>5.5/day</td>
<td>51</td>
<td>5.5x51x60</td>
<td>16,830</td>
</tr>
<tr>
<td>3. Laboratory cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 NSAIDs(Parecoxib)</td>
<td>500</td>
<td>17</td>
<td>500x17</td>
<td>8,500</td>
</tr>
<tr>
<td>3.2 Cellulose membrane</td>
<td>1,000</td>
<td>34</td>
<td>1,000x34</td>
<td>34,000</td>
</tr>
<tr>
<td>3.3 Normal saline</td>
<td>200</td>
<td>12</td>
<td>200x12</td>
<td>2,400</td>
</tr>
<tr>
<td>3.4 Analgesics</td>
<td>1,500</td>
<td>3</td>
<td>1,500x3</td>
<td>4,500</td>
</tr>
<tr>
<td>3.5 Euthanasia</td>
<td>20/rat</td>
<td>51</td>
<td>1,020</td>
<td>1,020</td>
</tr>
<tr>
<td>3.6 Ketamine</td>
<td>1,600</td>
<td>3</td>
<td>1,600x3</td>
<td>4,800</td>
</tr>
<tr>
<td>3.7 Betadine solution</td>
<td>1,500</td>
<td>2</td>
<td>1,500x2</td>
<td>3,000</td>
</tr>
<tr>
<td>3.8 Hibiscrub</td>
<td>1,500</td>
<td>2</td>
<td>1,500x2</td>
<td>3,000</td>
</tr>
<tr>
<td>4. Material cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.1 paraffin</td>
<td>5,000</td>
<td>1</td>
<td></td>
<td>5,000</td>
</tr>
<tr>
<td>4.2 10% formalin</td>
<td>3,500</td>
<td>1</td>
<td></td>
<td>3,500</td>
</tr>
<tr>
<td>4.3 Hematoxylin &amp; eosin</td>
<td>20,000</td>
<td>1</td>
<td></td>
<td>20,000</td>
</tr>
<tr>
<td>4.4 Masson trichrome</td>
<td>25,000</td>
<td>1</td>
<td></td>
<td>25,000</td>
</tr>
<tr>
<td>4.5 Glass slides</td>
<td>8,000</td>
<td>1</td>
<td></td>
<td>8,000</td>
</tr>
<tr>
<td>4.6 70% alcohol</td>
<td>3,000</td>
<td>1</td>
<td></td>
<td>3,000</td>
</tr>
<tr>
<td>4.7 95% alcohol</td>
<td>2,500</td>
<td>1</td>
<td></td>
<td>2,500</td>
</tr>
<tr>
<td>4.8 100% alcohol</td>
<td>2,500</td>
<td>1</td>
<td></td>
<td>2,500</td>
</tr>
<tr>
<td>5. Miscellaneous</td>
<td>30,000</td>
<td>1 unit</td>
<td>30,000x1</td>
<td>30,000</td>
</tr>
<tr>
<td>Total cost (include laboratory cost)</td>
<td></td>
<td></td>
<td></td>
<td>237,040</td>
</tr>
</tbody>
</table>


References

1. Martin BI, Mirza SK, Comstock BA, Gray DT, Kreuter W, Deyo RA. Reoperation rates following lumbar spine surgery and the influence of spinal fusion procedures. Spine. 2007; 32(3).


44. Harlan Laboratory Animals. Harlan product guide. 2003..


Appendix 1

_data recorded form_

ID code ______________ randomization no_________

Age ____ days sex male female

Weight____ grams

Adverse event no yes_____________________

File name no.

Fibroblast density = ______________ /mm²

Inflammatory cell density = ______________ /mm²

Slide no.

Fibrous adherence grading

Grade 0: no scar tissue on the dura mater

Grade 1: only thin fibrous band were observed

Grade 2: continuous adherence was observed for less than two thirds of laminectomy area

Grade 3: scar tissue was large, more than two third of laminectomy area, and/or extended to spinal nerve roots

(Pathologist’s signature)
## Appendix 2

**Dummy table and example of recorded data in spreadsheet (for data analysis)**

<table>
<thead>
<tr>
<th>age</th>
<th>sex</th>
<th>weight</th>
<th>fibroblast</th>
<th>inflam_cell</th>
<th>histo1</th>
<th>histo2</th>
<th>group</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
<td>199.00</td>
<td>130.80</td>
<td>123.37</td>
<td>3.00</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>240.00</td>
<td>123.57</td>
<td>250.30</td>
<td>2.00</td>
<td>3.00</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>236.00</td>
<td>151.45</td>
<td>231.20</td>
<td>3.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>229.00</td>
<td>147.98</td>
<td>139.80</td>
<td>1.00</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>215.00</td>
<td>260.00</td>
<td>123.37</td>
<td>2.00</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>229.00</td>
<td>231.20</td>
<td>131.45</td>
<td>2.00</td>
<td>3.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>201.00</td>
<td>130.80</td>
<td>147.98</td>
<td>3.00</td>
<td>3.00</td>
<td>1.00</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>249.00</td>
<td>123.37</td>
<td>250.30</td>
<td>3.00</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>244.00</td>
<td>131.45</td>
<td>231.20</td>
<td>2.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>237.00</td>
<td>147.98</td>
<td>139.80</td>
<td>1.00</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>242.00</td>
<td>130.80</td>
<td>123.37</td>
<td>2.00</td>
<td>3.00</td>
<td>1.00</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>220.00</td>
<td>123.37</td>
<td>131.45</td>
<td>3.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>231.00</td>
<td>260.30</td>
<td>147.98</td>
<td>2.00</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>199.00</td>
<td>231.20</td>
<td>130.80</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>240.00</td>
<td>130.80</td>
<td>123.37</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>236.00</td>
<td>123.37</td>
<td>250.30</td>
<td>3.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>229.00</td>
<td>131.45</td>
<td>231.20</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>215.00</td>
<td>147.98</td>
<td>130.80</td>
<td>3.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>229.00</td>
<td>130.80</td>
<td>123.37</td>
<td>1.00</td>
<td>1.00</td>
<td>2.00</td>
</tr>
<tr>
<td>23</td>
<td>1</td>
<td>201.00</td>
<td>123.37</td>
<td>131.45</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>249.00</td>
<td>260.30</td>
<td>147.98</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>244.00</td>
<td>231.20</td>
<td>250.00</td>
<td>3.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>237.00</td>
<td>130.80</td>
<td>231.20</td>
<td>3.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
<td>240.00</td>
<td>123.37</td>
<td>130.80</td>
<td>2.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
<td>220.00</td>
<td>123.37</td>
<td>130.80</td>
<td>2.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>
Appendix 3

Examples of data presentation (based on the exampled data in appendix 2)

1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NSAIDs + cellulose membrane group</th>
<th>Epidural cellulose membrane group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>42.94±2.51</td>
<td>41.76±2.84</td>
<td>42.35±2.85</td>
</tr>
<tr>
<td>Gender</td>
<td>7:10</td>
<td>8:9</td>
<td>8:9</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>224.65±16.99</td>
<td>227.29±14.55</td>
<td>226.82±15.47</td>
</tr>
</tbody>
</table>

2. Bar chart showed percentage of sex between the 2 groups
3. Box plot of age between 2 groups

![Box plot of age between 2 groups](image)

4. Box plot of weight between 2 groups

![Box plot of weight between 2 groups](image)
5. Presentation of results (fibroblast density & inflammatory cell density)

<table>
<thead>
<tr>
<th>Densities</th>
<th>NSAIDs+cellulose membrane group</th>
<th>Epidural cellulose membrane group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast density</td>
<td>169.94±54.65</td>
<td>163.56±50.78</td>
<td>170.52±51.88</td>
</tr>
<tr>
<td>Inflammatory cell density</td>
<td>163.56±50.78</td>
<td>169.14±55.08</td>
<td>169.14±55.08</td>
</tr>
</tbody>
</table>

6. Comparisons between the groups

95% confidence interval of mean differences

<table>
<thead>
<tr>
<th>Densities</th>
<th>NSAIDs+cellulose and control</th>
<th>Epidural cellulose membrane and control</th>
<th>NSAIDs+cellulose and epidural cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast density</td>
<td>-30.48 43.23</td>
<td>-42.82 28.91</td>
<td>-37.80 36.64</td>
</tr>
<tr>
<td>Inflammatory cell density</td>
<td>-49.32 24.95</td>
<td>-45.21 32.00</td>
<td>-42.59 31.43</td>
</tr>
</tbody>
</table>

Statistics: unpaired t-test

7. Presentation of mean of fibroblast density and 95% confidence interval between 2 groups
8. Presentation of mean of inflammatory cell density and 95% confidence interval between 2 groups

9. Presentation of adverse events

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>NSAIDs+cellulose Membrane group</th>
<th>Epidural cellulose membrane group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound dehiscence</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.60</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Test : Fisher’s exact test
Appendix 4

The ethical consideration submission form for Animal Ethic Committee of Khon Kaen University

แบบฟอร์มการขอรับการพิจารณาการอนุญาตการใช้สัตว์ทดลอง
สำหรับงานสอน งานวิจัย งานทดสอบและงานผลิตชีววัตถุ มหาวิทยาลัยขอนแก่น

ชื่อโครงการ

(ภาษาไทย) การป้องกันพื้นผิวชอบเอ็มูลัต้า ด้วยการใช้เยื่อเซลลูโลซในสัตว์ทดลอง
(ภาษาอังกฤษ) Prevention of peridural fibrosis using NSAIDs soaked cellulose membrane: an experimental comparative study in animal model

1. ข้อมูลเกี่ยวกับผู้ใช้สัตว์ (กรณีที่มีศึกษาเป็นหัวหน้าโครงการ ให้ระบุชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์)

1.1 หัวหน้าโครงการ
ชื่อ – นามสกุล (ภาษาไทย) นายแพทย์ สุรชัย แซ่จึง
(ภาษาอังกฤษ) Surachai Sae-Jung
d้านหน้าทางวิชาการ รองศาสตราจารย์ ตําแหน่งอื่นๆ..........................................

สถานภาพ □ อาจารย์/เจ้าหน้าที่ของคณะ
☑ นักศึกษา (□ ตรี/ □ โท/ □ เอก)
□ ชื่อๆ โปรดระบุ..........................................

สถานที่ติดต่อ (ภาษาไทย/ เนื้อหาหรือสถานที่)

(ภาษาไทย) ภาควิชานิพนธ์วิทยาศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
อาศัยจากข้อมูลที่ปรากฏในบัตรประจำตัวประชาชน (กรณีนักศึกษาเป็นหัวหน้าโครงการ) คร. เลข์ จ. ชัยรัตน์ จ. ชัย

1.2 ผู้ร่วมงาน

1.2.1 ชื่อ-นามสกุล……………………………………ตําแหน่ง………………………………
สถานที่ทำงาน………………………………………………………………………………
โทรศัพท์…………………………………… E-mail address…………………………

1.2.2 ชื่อ-นามสกุล……………………………………ตําแหน่ง………………………………
สถานที่ทำงาน………………………………………………………………………………
โทรศัพท์…………………………………… E-mail address…………………………

1.2.3 ชื่อ-นามสกุล……………………………………ตําแหน่ง………………………………
สถานที่ทำงาน………………………………………………………………………………
โทรศัพท์…………………………………… E-mail address…………………………

1.2.4 ชื่อ-นามสกุล……………………………………ตําแหน่ง………………………………
สถานที่ทำงาน………………………………………………………………………………
โทรศัพท์…………………………………… E-mail address…………………………
1.3 ผู้รับผิดชอบปฏิบัติงานกับสัตว์

✓ หัวหน้าโครงการ

☐ ผู้ร่วมงานคนที่ 1.2.1 ☐ 1.2.2 ☐ 1.2.3 ☐ 1.2.4

☐ อื่นๆ (โปรดระบุ ชื่อ – สถานที่ติดต่อ)

...........................................................................................................................

1.4 ประสบการณ์เกี่ยวกับการปฏิบัติงานด้านสัตว์ทดลอง

1.4.1 หัวหน้าโครงการ ✓ มีประสบการณ์ 1 ปี ☐ ไม่มีประสบการณ์

ประสบการณ์ด้าน ✓ การเรียนการสอน ☐ การทดลอง ☐ การวิจัย ☐ อื่นๆ

✓ ผ่านการอบรมจรรยาบรรณการใช้สัตว์ทดลอง เมื่อ (ระบุ) เอ็มจิตร์สันนิทิวิทยาศาสตร์ วิทยาศาสตร์ differ  เมื่อ 7 ก.ค. 2554 ณ ห้องบรรยายวิจัยพื้นที่ ภาควิชาวิทยาศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

☐ ไม่เคยผ่านการอบรมจรรยาบรรณการใช้สัตว์ทดลอง

ปัจจุบันผู้วิจัยมีจำนวนงานวิจัยในความรับผิดชอบจำนวน 1 โครงการ

1.4.2 ผู้ร่วมงาน คนที่ 1 ☐ มีประสบการณ์ …… ปี ☐ ไม่มีประสบการณ์

☐ ผ่านการอบรมจรรยาบรรณการใช้สัตว์ ☐ ไม่เคยผ่านการอบรมจรรยาบรรณการใช้สัตว์

ผู้ร่วมงาน คนที่ 2 ☐ มีประสบการณ์ …… ปี ☐ ไม่มีประสบการณ์

☐ ผ่านการอบรมจรรยาบรรณการใช้สัตว์ ☐ ไม่เคยผ่านการอบรมจรรยาบรรณการใช้สัตว์

ผู้ร่วมงาน คนที่ 3 ☐ มีประสบการณ์ …… ปี ☐ ไม่มีประสบการณ์

☐ ผ่านการอบรมจรรยาบรรณการใช้สัตว์ ☐ ไม่เคยผ่านการอบรมจรรยาบรรณการใช้สัตว์
2. ข้อมูลทั่วไปเกี่ยวกับโครงการ (General information on the project)

2.1 วัตถุประสงค์ของโครงการวิจัย

2.1.1 เพื่อศึกษาประสิทธิภาพของยาต้านการอักเสบชนิดไม่ใช่สเตียรอยด์ (parecoxib) และแผ่นเซลลูโลส ในการป้องกันการเกิดพังผืดรอบเยื่อดูราภายหลังการผ่าตัดกระดูกสันหลังในหนูทดลอง

2.1.2 เพื่อศึกษาความปลอดภัยของยาต้านการอักเสบชนิดไม่ใช่สเตียรอยด์ (parecoxib) และแผ่นเซลลูโลส ที่วางรอบเยื่อดูราภายหลังการผ่าตัดกระดูกสันหลังในหนูทดลอง

2.1.3 ..............................................................................................................................

2.2 ทุนวิจัยที่ได้รับสำหรับโครงการนี้

☐ ได้รับทุนแล้ว ระบุแหล่งทุน................................................................. จำนวนเงิน..................

✓ กำลังยื่นขอ ระบุแหล่งทุน คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จำนวนเงิน 450,000

☐ ทุนส่วนตัว จำนวนเงิน.................................................................

2.2.1 หากเป็นโครงการที่ทำต่อเนื่องโครงการเดิมที่ได้ผ่านการพิจารณาจรรยาบรรณการใช้สัตว์ทดลองแล้วไปประมวลผลโครงการที่ผ่านความเห็นชอบ และยังมีความเหมือนและความแตกต่างของโครงการนี้กับโครงการเดิมด้วย

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

..........................................................................................................................................................

...................................................................................................................................................
2.3 ลักษณะของโครงการ (Discipline)

☐ 2.3.1 งานวิจัย (Research)
☐ งานวิจัยทั่วไป
☐ 2.2.1.1 งานวิจัยพื้นฐานทางด้านสาขา (Basic research)
☐ พฤติกรรมศาสตร์ (Behavioral science)
☐ จุลชีววิทยา (Microbiology)
☐ พยาธิชีววิทยา (Pathobiology)
☐ สรีรวิทยา (Physiology)
☐ พิษวิทยา (Toxicology)
☐ ชีวเคมี (Biochemistry)
☐ ชีววิทยา (Biology)
☐ ปรากฏการณ์ที่อื่นๆ (ระบุ)

☑ 2.2.1.2 งานวิจัยประยุกต์ (Applied research) (ระบุด้าน/สาขา) Spine Surgery

☐ 2.3.2 งานทดสอบ (Testing and / or monitoring)
☐ อาหาร (Food)
☐ ยา (Drugs)
☐ น้ำ (Water)
☐ เครื่องสัมผัส (Cosmetic)
☐ อื่นๆ (ระบุ)

☐ 2.3.3 งานผลิตชีววัตถุ (Biological Products)
☐ วัคซีน (Vaccine)
☐ เอนไซม์ (Enzymes)
☐ แอนติบอดี (Antibodies)
☐ คอมพลีเมนท์ (Complement)
☐ อื่นๆ โปรดระบุ

☐ 2.3.4 งานการเรียนการสอน ระบุวิชา (รหัสวิชา) / Subject (Code #)

☐ 2.3.5 งานบริการวิชาการ

2.4 สารหรือชีววัตถุที่นำมาใช้กับสัตว์และผลกระทบที่มีต่อสัตว์

<table>
<thead>
<tr>
<th>ประเภท</th>
<th>ชื่อสารหรือชีววัตถุ/บริการที่นำมาใช้</th>
<th>แสดงผลเป็นการดื้อย่อยว่ายะที่อาจได้รับอันตราย</th>
</tr>
</thead>
</table>

55
<table>
<thead>
<tr>
<th>สารเคมี</th>
<th>สารก่อมะเร็ง</th>
<th>ยา NSAIDs</th>
<th>สมุนไพร</th>
<th>สารพิษ</th>
<th>อาหาร</th>
<th>น้ำ</th>
<th>เชื้อรา</th>
<th>ไวรัส</th>
<th>แบคทีเรีย</th>
<th>ปรสิต</th>
<th>วัคซีน</th>
<th>Freund’s adjuvant</th>
<th>อื่นๆ โปรดระบุ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parecoxib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.5 การให้ยา/สารเคมี/เชื้อโรค/สารติดเชื้อ/สารรังสีหรือสิ่งแปลกปลอม ในกรณีที่เป็นพิษหรือวัตถุอันตรายแก่สัตว์ทดลอง มีโอกาสที่จะสามารถแพร่กระจายจากร่างกายสัตว์ทดลอง ผ่านเยื่อหุ้มดูราและรากประสาท ไปยังผู้ดูแลสัตว์ ผู้ที่ทำงานกับสัตว์ ผู้ที่อยู่ในบริเวณรอบๆ หรือผู้ที่มีการสัมพันธ์กับสัตว์ ผู้ที่มีการสัมผัสกับสิ่งแวดล้อมโดยตรง หรือผู้ที่มีการสัมผัสกับสิ่งแวดล้อมโดยอ้อม อาศัยวิธีการป้องกันการแพร่กระจายของสารพิษหรือเชื้อที่ทำให้เกิดการศึกษาที่อาจมีต่อสัตว์ทดลอง ต่อผู้ดูแลสัตว์ และต่อสัตว์แวดล้อมภายในและภายนอก

- ☐ ได้ (ตอบคำถามข้างล่าง)
- ☑ ไม่ได้ (ข้ามไปข้อ 3)

2.5.1 การมีผลพลิกผันทางการแพทย์ ให้ระบุรายละเอียดข้อควรระวังและวิธีการป้องกันการแพร่กระจายของสารพิษหรือเชื้อที่ทำให้เกิดการศึกษาที่อาจมีต่อสัตว์ทดลอง ต่อผู้ดูแลสัตว์ และต่อสัตว์แวดล้อมภายในและภายนอก

Cellulose membrane | เยื่อหุ้มดูรา และรากประสาท

เยื่อหุ้มดูรา และรากประสาท
ภายนอกสถานที่เลี้ยงสัตว์ รวมถึงต้องเลี้ยงดูสัตว์เป็นกรณีพิเศษอย่างไรจึงจะไม่เกิดการแพร่กระจาย พร้อมทั้งอธิบายวิธีการป้องกันความปลอดภัยของบุคคลใน การใช้สารดังกล่าว (กรุณาแนบเอกสารอ้างอิงถึงระดับความอันตราย รวมทั้ง Standard Operating Procedure: SOP)

-----------------------------------------------------------------------------------------

i. ระบุวิธีการกำจัดสารพิษ/วัตถุอันตราย/เชื้อโรค และการปฏิบัติกับสัตว์หรือวัสดุพื้นฐานในการเลี้ยงสัตว์อย่างไรเมื่อสิ้นสุดการทดลอง

3. เหตุผลที่ต้องใช้สัตว์

3.1 มีวิธีการซึ่งอาจนำมาใช้แทนสัตว์ได้หรือไม่ ให้เหตุผลด้วยว่าทำไมไม่ใช้วิธีการอื่นดังกล่าวมาใช้แทนสัตว์ (Replacement)

ไม่มีวิธีการซึ่งน่าจะนำมาใช้แทนสัตว์ทดลอง เนื่องจากเป็นการวิจัยถึงความปลอดภัย และประสิทธิภาพของยาการอักเสบชนิดที่ไม่ใช้สเตียรอยด์ และเยื่อชุ้นตุ้มเส้น ซึ่งยังไม่มีการทดสอบมาก่อน และไม่สามารถทำการศึกษาในสัตว์อื่นได้

3.2 หากมีเหตุผลความจำเป็นที่จะต้องใช้สัตว์ทดลองและไม่สามารถใช้วิธีการอื่นทดแทน ทำมีวิธีการหรือแนวทางปฏิบัติในการลดจำนวนสัตว์ทดลองให้น้อยที่สุดอย่างไร (Reduction)

มีการคำนวณจำนวนตัวอย่าง (sample size) โดยใช้หลักทางสถิติ ในการที่ใช้จำนวนสัตว์ทดลองที่พอตัว (ไม่ใช่มากเกินไป) เพื่อให้ตอบคำถามวิจัยได้ (α =0.05, power of study=80%)

3.3 ทำมีวิธีในการปฏิบัติกับสัตว์ทดลองอย่างไร เพื่อให้สัตว์มีความเจ็บปวดน้อยที่สุด (Refinement)

มีการให้ยาแก่ผู้พักผ่อนได้ตามปกติ ในสถานภาพเดิมที่ดี
3.4 กรณีที่จำเป็นต้องใช้สัตว์ป่าให้เห็นผลด้วยว่าทำไปก็ใช้สัตว์ทดลองหรือสัตว์อื่นแทนไม่ได้  

3.5 ประโยชน์ต่อมนุษย์หรือสัตว์และผลประโยชน์ทางวิชาการที่คาดว่าจะได้รับ  

3.5.1 สรุปผลประโยชน์ต่อมนุษย์หรือสัตว์  
หากการศึกษาได้ผลว่า ยาต้านการอักเสบชนิดที่ไม่ใช้สเตียรอยด์ และเยื่อเซลลูโลส มีความปลอดภัย และสามารถป้องกันการเกิดพังผืดรอบซูรูา หรือจากประสานหลังได้ หากนำมาใช้ในมนุษย์แล้วได้ผลเช่นเดียวกันจะสามารถป้องกันการเกิดพังผืดรอบซูรูา หรือจากประสานหลัง ซึ่งเป็นภาวะแทรกซ้อนที่สาคัญ และเกิดขึ้นได้ในผู้ป่วยทุกรายที่มีการผ่าตัดเปิดแต่ละเกิดการขึ้นของซูรูา

3.5.2 สรุปผลประโยชน์ต่อความก้าวหน้าทางวิชาการ  
เนื่องจากปัจจุบันนี้องค์การอาหารและยาสรุปว่า ยังไม่มีวิธีการป้องกันการเกิดพังผืดรอบซูรูาที่ได้ผล หากการวิจัยนี้ให้ผลว่า ยาต้านการอักเสบชนิดที่ไม่ใช้สเตียรอยด์ และเยื่อเซลลูโลส มีความปลอดภัย และสามารถป้องกันการเกิดพังผืดรอบซูรูา หรือจากประสานหลังได้ สามารถนำมาพัฒนาใช้ในมนุษย์อันนำมาสู่การรักษาใหม่ในมนุษย์ได้ 

4. สัตว์ทดลองที่ใช้ในงานวิจัย  

4.1 ชนิดของสัตว์ทดลองที่ใช้  

☐ หนูเม้าส์ / Mouse  ✔ หนูแรท / Rat  ☐ หนูแฮมสเตอร์ / Hamster  
☐ หนูตะเภา / Guinea Pig  ☐ กระต่าย / Rabbit  ☐ อื่นๆ (ระบุ).........................

4.2 สายพันธุ์  

☐ Outbred stock ซึ่งสายพันธุ์ ................... ☐ Inbred strain ซึ่งสายพันธุ์ ..................
4.3 เพศ □ ผู้ □ เมีย
4.4 อายุ 8 สัปดาห์เดือน..............วัน
4.5 น้ำหนัก 200-250 กรัม
4.6 ระยะเวลาระหว่างสัตว์ทดลองตั้งแต่วันที่ (วัน/เดือน/ปี) 1 มีนาคม 2555 ถึง 31 กรกฎาคม 2555 รวมระยะเวลา 5 เดือน
4.7 จำนวนที่ใช้ทดลองโครงการ 51 ตัว
4.8 ทำไมจึงเลือกใช้สัตว์ทดลองชนิดนี้ในการทดลอง มีการศึกษามากมายแล้วหรือไม่ ทดลองนี้ หลักเหตุผลในการกำหนดจำนวนสัตว์ทดลองที่ใช้อย่างไร เลือกใช้ Sprague-Dawley Rat เนื่องจากมีการศึกษาในหนูชนิดนี้ในการผ่าตัด laminectomy มาก่อน การกำหนดจำนวนสัตว์ทดลองได้มาจากค่าขนาด sample size โดยหลักการทางสถิติ

5. ระเบียบวิธีวิจัยที่เกี่ยวข้องกับสัตว์ทดลอง
5.1 การแบ่งกลุ่มการทดลอง แบ่งเป็น 3 กลุ่มการทดลอง กลุ่มละ 17 ตัว (ระบุรายละเอียด) เนื่องจาก นำหนูทดลองทั้งหมด 51 ตัว มาทำการผ่าตัด L4-L5 laminectomy จากนั้นทำการสุ่มแยกหนูทดลองออกเป็น 3 กลุ่ม กลุ่มละ 17 ตัว นำกลุ่มแรก ใช้เยื่อเซลลูลูสคลุมยาต้านการอักเสบชนิดไม่ใช่เดียร์รยีด (parecoxib) วางคลุมบริเวณที่ผ่าตัด กลุ่มที่สอง ใช้เยื่อเซลลูลูสวางคลุมบริเวณที่ผ่าตัด และกลุ่มที่สาม หยดน้ำเกลือรอบบริเวณที่ผ่าตัด จากนั้นหนูทุกตัวได้รับการเย็บปิดแผลตามวิธีปกติ ภายหลังจากนั้น 4 สัปดาห์ทำการช้าและ หยดเจลกีซใช้เพื่อปรับสภาพของบริเวณที่ทำการผ่าตัด โดยวัดปริมาณ fibroblast density, inflammatory cell density และ fibrous adherence grading เปรียบเทียบกันในหนูทั้งสามกลุ่ม
**Study flow chart**

Spraque – Dawley rats

L4-5 laminectomy

Randomization & Allocation

**Study group**
(peridural NSAIDs soaked cellulose)

All rats are sacrificed at 4 weeks

**Study group**
(epidural cellulose membrane)

All rats are sacrificed at 4 weeks

**Control group**
(peridural saline dripping)

All rats are sacrificed at 4 weeks

**Histopathology**
(Outcomes)

Blinded 2 outcome assessors

**Statistical analysis**
ระบุขั้นตอนและวิธีการทดลองโดยละเอียด เช่นการสลบสัตว์, การผ่าตัด, การดูแลก่อนและหลังการผ่าตัด, การเลี้ยงสัตว์และการดูแลสัตว์ทดลองตลอดการทดลอง เป็นต้น หากขับขันโปรดเขียน Flow chart ประกอบด้วย  เช่นการสลบสัตว์, การผ่าตัด, การดูแลก่อนและหลังการผ่าตัด, การเลี้ยงสัตว์และการดูแลสัตว์ทดลองตลอดการทดลอง เป็นต้น หากขับขันโปรดเขียน Flow chart ประกอบด้วย

นำสลบหนูทดลองด้วย intraperitoneal ketamine ขนาด 8 mg/100 g จากนั้น ทำความสะอาดหนูด้วยน้ำยา hibiscrub ขับให้แห้ง แล้วเตรียมบริเวณหลังหูด้วยน้ำยา betadine solution จากนั้นใช้ด้าสีเหลืองจากกลางครูมหลังหนูเปิดให้เห็นเฉพาะบริเวณ L4-L5 หนู ใช้มีดผ่าตัดกรีดเป็นแนวยาวบริเวณ L4-L5 เลาะเนื้อเยื่อและกล้ามเนื้อรอบๆกระดูกสันหลังเปิดให้เห็นกล้ามเนื้อกระดูก ใช้คีมผ่าตัดกระดูกสันหลังม้วน lamina ของ L4-L5 ออก จากนั้นทำการทำทดลองด้วยการหยดยา, ใช้เซลลูโลสหรือน้ำเกลือตามแต่กลุ่มที่ได้รับการสุ่มทำการทำเป็นแม่ตักด้วย nylon 3-0 ภายหลังการทำผ่าตัดให้ยาแกปิด paracetamol ขณะนำหนูให้แก่สัตวแพทย์ทุก 6 ชั่วโมงใน 3 วัน

ภายหลังจากนั้น 4 สัปดาห์ ใช้ยา sodium pentobarbital ขนาด 100 mg/kg เพื่อทำจนยาขาด แล้วขับแห้งส่วนกระดูกสันหลัง L4-L5 พร้อมกับเนื้อเยื่อและกล้ามเนื้อแช่ใน 10% formalin solution 1 สัปดาห์ แล้วแช่ใน decalcifying solution ต่อ 1 สัปดาห์ จากนั้นนำไป embed ด้วย paraffin แล้วตัดด้วย microtome ให้ตัดชิ้นเนื้อขนาด 5 ในเครื่อง ทำการทำย้อมเส้น hemotoxylin & eosin เพื่อนำไปวัดผล fibroblast density, inflammatory cell density และ fibrous adherence grading

โปรดระบุข้อกำหนดในการตัดสินใจที่จะหยุดการทำทดลองกับสัตว์ก่อนสิ้นสุดการทำทดลอง ตัวอย่างเช่นสัตว์อยู่ในสภาพทรุดโทรม น้ำหนักลด ส่งเสียงร้องด้วยความเจ็บปวด และไม่สามารถเคลื่อนไหวได้ หนูมีอาการติดเชื้อเป็นหนองที่ผ่าตัด หรือมีอาการบาดเจ็บต่อมระบบที่มีผล (เคลื่อนไหวไม่ได้)

6. แหล่งผลิตสัตว์ (Animal Resources)

6.1 แหล่งที่มาของสัตว์

เพาะขยายพันธุ์สัตว์ในหน่วยงาน คณะหรือสถาบัน (ระบุที่มา ศูนย์สัตว์ทดลองภาคตะวันออกเฉียงเหนือ.)
ดังข้อจากแหล่งเพราะขยายพันธุ์ต่างประเทศ (ระบุที่มา…………………………………………………)

ดังข้อจากแหล่งเพราะขยายพันธุ์อื่นๆภายในประเทศ (ระบุที่มา…………………………………………………)

อื่นๆ (ระบุ)……………………………………………………………………………………………………….

6.2 คุณภาพของสัตว์จากแหล่งผลิต
✓ มีหลักฐานแสดงสืบสายพันธุ์ และความคงที่ทางพันธุกรรมของสายพันธุ์ที่ต้องการตรวจสอบ

☐ มีหลักฐานตรวจสอบได้ว่าเป็นสัตว์เลี้ยงด้วยระบบอนามัยเข้ม (Strict hygienic conventional system)

☐ มีหลักฐานตรวจสอบได้ว่าเป็นสัตว์เลี้ยงด้วยระบบปลอดเชื้อจุ้ปน (SPF System)

(ระบุชนิดเชื้อ…………………………………………………………………………………………..)

☐ มีหลักฐานตรวจสอบได้ว่าเป็นสัตว์เลี้ยงด้วยระบบปลอดเชื้อสมบูรณ์ (Germ Free system)

☐ ไม่มีหลักฐาน หรือเอกสารรับรอง

☐ อื่น ๆ

(ระบุ)…………………………………………………………………………………………………………

6.3 ศักยภาพของแหล่งผลิต
✓ มีวิธีเพาะขยายพันธุ์ที่แสดงว่าสามารถเพราะขยายพันธุ์สายพันธุ์สัตว์ที่ต้องการได้ทุกรูปแบบของพันธุ์ อายุ น้ำหนัก และจำนวนตามที่ต้องการ

✓ สามารถจัดบริการส่งโดยใช้มาตรฐานการขนส่งสัตว์สากล

☐ อื่น ๆ

(ระบุ)…………………………………………………………………………………………………………

7. การขนส่งและจัดเก็บปุกิฏิกาและการเตรียมสัตว์ทดลอง

7.1 การขนส่งสัตว์ (ตอบเฉพาะกรณีมีการขนส่งสัตว์ทดลองมาจากภายนอกมหาวิทยาลัย)

☐ มีการควบคุมอุณหภูมิ

☐ มีการระบายอากาศเพียงพอ
มีการป้องกันการติดเชื้อ

มีภาชนะบรรจุสัตว์มั่นคงแข็งแรง (ระบุชนิดของภาชนะ)

มีพื้นที่เพียงพอ (ระบุขนาดของพื้นที่)

ถึงจุดหมายปลายทางภายใน 1 วัน

อื่น ๆ (ระบุ)

7.2 การเตรียมสัตว์ก่อนการทดลอง

ถ่ายพยาธิ

ฉีดวัคซีน

พักสัตว์เป็นระยะเวลา 14 วัน

อื่น ๆ (ระบุ)

8. สภาพแวดล้อมของการเลี้ยงสัตว์

8.1 มาตรฐานการเลี้ยง

☑ อานามัยเข้ม

☑ ปลอดเชื้อจุลพืช

☑ ปลอดเชื้อสมบูรณ์

☐ โรงเรือนปิด

☐ โรงเรือนเปิด / ฟาร์ม

☑ อื่น ๆ (ระบุ)

8.2 สภาพแวดล้อมของห้อง / สถานที่ปฏิบัติงาน

☐ อุณหภูมิ …25…°C

☐ ความชื้นสัมพัทธ์ …70….. %

☐ แสงสว่าง ………700……..Lux หรือ …………… Footcandle

☑ ไม่มีเสียงดังรบกวน

☑ ไม่มีกลิ่นเหม็นรบกวน

☑ การถ่ายเทอากาศดี

☑ มีระบบไฟฟ้าและน้ำสำรอง

8.3 กรง / คอกสัตว์ / พื้นที่เลี้ยงสัตว์

☑ คอกสัตว์ ขนาดพื้นที่ ……2x2 ตารางเมตร

☑ กรงสี่เหลี่ยมผืนผ้า / Shoebox

ขนาด (กว้างXยาวXสูง) ………..เซนติเมตร

☑ แผ่นสแตนเลส

☑ แผ่นเหล็ก

☑ อื่น ๆ (ระบุ)
8.4 อาหาร

8.4.1 ชนิดของอาหาร

✔ อาหารเม็ดจากโรงงาน (Commercial pellets)  ☐ อาหารปลอดเชื้อ (Sterile diet)

☐ อาหารปั่นผลิตเองสูตรพิเศษ (Ground diet special formula)

สารที่เพิ่มในอาหาร ☐ มี (ระบุ).......................... ☐ ไม่มี

☐ อื่น ๆ (ระบุ)..........................................................

8.4.2 การให้อาหาร

✔ ให้อาหารในอัตราปกติมีกินตลอดเวลา  ☐ มีกำหนดเวลาและปริมาณอาหาร

☐ อื่น ๆ (ระบุ)..........................................................

8.5 น้ำดื่ม

8.5.1 ชนิดของน้ำ

✔ น้ำประปา  ☐ น้ำกรองตะกอน

☐ น้ำเติมคลอรีนความเข้มข้น...............ppm  ☐ อื่น ๆ (ระบุ)..........................

8.5.2 การให้น้ำ

✔ ให้น้ำในอัตราปกติมีกินตลอดเวลา  ☐ มีกำหนดเวลาและปริมาณน้ำ
8.5.3 วิธีการให้น้ำ

☐ โดยบรรจุน้ำในขวดมีจุกและหลอด  ☑ โดยวิธีการให้น้ำอัตโนมัติ

☐ อื่นๆ (ระบุ) .................................................................

8.6 วัสดุรองนอน

8.6.1 ชนิดของวัสดุรองนอน

☐ ขี้กบ (Wood shaving)  ☑ ขี้เลื่อย (Sawdust)  ☑ กระดาษ (Paper)

☐ วัสดุรองนอนปลอดเชื้อ (Sterile bedding)  ☐ วัสดุอื่นๆ (ระบุ).................................

☐ ไม่ใช้วัสดุรองนอน

8.6.2 การเปลี่ยนวัสดุรองนอน

☐ วันเว้นวัน  ☐ ทุก 2 หรือ 3 วัน ☐ ทุกสัปดาห์  ☐ อื่นๆ (ระบุ)...................

9. ความพร้อมของสถานที่และการจัดการในหน่วยงานเลี้ยงสัตว์ (Nature of facility needed)

☑ โปรดระบุหน่วยงานสถานที่เลี้ยงและดูแลสัตว์ทดลองด้วยโครงการให้ชัดเจน (ระบุ ศูนย์สัตว์ทดลองภาคตะวันออกเฉียงเหนือ)

☑ ท่านต้องการให้ศูนย์สัตว์ทดลองภาคตะวันออกเฉียงเหนือรับผิดชอบดูแลสัตว์ตามที่ระบุไว้ในโครงการ และยินดีจะปฏิบัติตามเงื่อนไขและระเบียบที่ทางศูนย์สัตว์ทดลองฯได้กำหนดทุกประการ

10. เทคนิคการปฏิบัติการกับสัตว์

10.1 อะไรบ้างต่อไปนี้ที่ท่านต้องนำมาใช้ปฏิบัติกับสัตว์ในโครงการ
การจับและควบคุมสัตว์  การเก็บตัวอย่างจากสัตว์  การแยกเพศ  การสลบสัตว์  การทําเครื่องหมายบนตัวสัตว์  การทําการผ่าตัด  การทําการผ่าซาก  อื่น ๆ (ระบุ) ..............................

มีประสบการณ์การผ่าตัด laminectomy และ sterile technique อย่างดี

10.2 การให้สาร บริเวณ และปริมาณที่ใช้ในโครงการ  □ ไม่มี  □ มี

สารที่ให้…………………… paracetamol…… □ ให้ทางปาก (Oral) ...

สารที่ให้………………………………… □ ชั้นผิวหนัง (Intradermal) ปริมาณที่ใช้ ………………… □ ปริมาณ……

สารที่ให้………………………………… □ เข้าใต้ผิวหนัง (Subcutaneous) ปริมาณที่ใช้ ………………… □ ปริมาณ……

สารที่ให้………………………………… □ เข้ากล้ามเนื้อ (Intramuscular) ปริมาณที่ใช้ ………………… □ ปริมาณ……

สารที่ให้ sodium pentobarbital  □ เข้าช่องท้อง (Intraperitoneal) ปริมาณที่ใช้ หน้าท้อง ปริมาณ 100 mg/kg

สารที่ให้ ketamine  □ เข้าช่องท้อง (Intraperitoneal) ปริมาณที่ใช้ หน้าท้อง ปริมาณ 8 mg/100g

สารที่ให้………………………………… □ เข้าหลอดเลือดต่ํา (Intravenous) หลอดเลือดที่ใช้ ………………… □ ปริมาณ……

สารที่ให้………………………………… □ อื่น ๆ โปรดระบุ…………………………………………………… □ ปริมาณ……
10.3 การเก็บตัวอย่างเลือดที่ใช้ในโครงการภารกิจ (กรณีใช้ยาสลบให้ระบุใช้ยาสลบอะไร)  

<table>
<thead>
<tr>
<th>ประเภทของการเก็บเลือด</th>
<th>ชนิดของหลอดเลือด และตำแหน่งที่เก็บ</th>
<th>ยาสลบที่ใช้/ชนิดยา</th>
<th>ปริมาณที่เก็บ</th>
<th>ความถี่ที่เก็บ</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ จากหลอดเลือดดำ</td>
<td>(Venous blood)</td>
<td>☐ไม่มี</td>
<td>☑มี</td>
<td></td>
</tr>
<tr>
<td>☐ จากหลอดเลือดแดง</td>
<td>(Arterial blood)</td>
<td>☐ไม่มี</td>
<td>☑มี</td>
<td></td>
</tr>
<tr>
<td>☐ เจาะจากหัวใจ</td>
<td>(Cardiac puncture)</td>
<td>☐ไม่มี</td>
<td>☑มี</td>
<td></td>
</tr>
<tr>
<td>☐ อื่นๆ</td>
<td>(ระบุ).........................</td>
<td>☐ไม่มี</td>
<td>☑มี</td>
<td></td>
</tr>
</tbody>
</table>

10.4 การเก็บตัวอย่างอื่น ๆ ที่ใช้ในโครงการ  

10.4.1 ชิ้นส่วนเนื้อเยื่อ/อวัยวะ/อื่น ๆ  

<table>
<thead>
<tr>
<th>☐ เก็บขณะสัตว์มีชีวิตอยู่</th>
<th>☑เก็บหลังจากสิ้นสุดการทดลอง</th>
</tr>
</thead>
<tbody>
<tr>
<td>ชิ้นส่วนเนื้อเยื่อ/อวัยวะที่เก็บ/อื่น ๆ</td>
<td>ชิ้นส่วนเนื้อเยื่อ/อวัยวะที่เก็บ/อื่น ๆ</td>
</tr>
</tbody>
</table>

| 1. ......................... บรรยาย ......................... | 1. กระดูกสันหลัง L4-L5 บรรยายทั้งหมด |
| 2. ......................... บรรยาย ......................... | 2. ......................... บรรยาย ......................... |
| 3. ......................... บรรยาย ......................... | 3. ......................... บรรยาย ......................... |
| 4. ......................... บรรยาย ......................... | 4. ......................... บรรยาย ......................... |
10.5 การเก็บตัวอย่างอุจจาระ          □ มี □ ไม่มี

10.5.1 ใช้ วิธีการ……………………………………………………………………………………………………

10.5.2 ชนิดของกรงหรือวัสดุอุปกรณ์ที่ช่วยในการเก็บตัวอย่าง………………………………………………

10.6 การเก็บตัวอย่างปัสสาวะ          □ มี □ ไม่มี

10.6.1 ใช้ วิธีการ……………………………………………………………………………………………………

10.6.2 ชนิดของกรงหรือวัสดุอุปกรณ์ที่ช่วยในการเก็บตัวอย่าง………………………………………………

11. การปฏิบัติต่อสัตว์หลังจากเสร็จสิ้นโครงการ

11.1 วิธีปฏิบัติต่อสัตว์หลังเสร็จสิ้นโครงการ          □ สัตว์ตายหลังจากการผ่าตัดหรือเก็บตัวอย่าง □ สัตว์มีชีวิตอยู่หลังเสร็จสิ้นโครงการ

โดยจะมีการทำการให้สัตว์ตายอย่างสงบนั้น (Euthanasia) ด้วยวิธี:

☐ ฉีดยาสลบเกินขนาดที่กำหนด (ระบุชนิด) sodium pentobarbital

☐ สูดดมยาสลบเกินขนาด (ระบุชนิด) …………………………………………………………………………

☐ การเคลื่อนข้อต่อกระดูกสันหลังบริเวณคอ (cervical dislocation)

☐ การทำให้หายโดยการสูดดมแก๊ส carbon dioxide (CO2)

☐ การทำให้หายโดยการใช้ไบโอตินตัดศีรษะ

☐ การทำให้หายโดยการยิงกระแทกตัวอย่างเป็น

☐ อื่น ๆ (ระบุ)……………………………………………………………………………………………………

☐ ถ้าไม่มีการทำการให้สัตว์ตายอย่างสงบ โปรดระบุวิธีการดำเนินการทั้งสัตว์หลังเสร็จสิ้นโครงการ

ต่อไป………………………………………………………………………………………………………………

68
11.2 การปฏิบัติต่ออาสาสัตว์สัตว์เลี้ยงโครงการ

☑ เก็บไว้ในห้องเย็นและส่งมาต่อภายตาม

☐ มีกลบดิน

☐ อื่น ๆ(ระบุ).................................................................

ค่ารับรอง

ข้าพเจ้าขอรับรองว่าจะปฏิบัติต่ออาสาสัตว์สัตว์เลี้ยงโครงการที่ได้เสนอไว้ในโครงการ ไม่ปล่อยปละละเลย ดูแลให้น้ำและอาหารในปริมาณที่เพียงพอแก่ความต้องการ เจ้าหน้าที่สัตวแพทย์ในการป้องกันการติดเชื้อและ การแพร่กระจายของเชื้อตามมาตรฐานการปฏิบัติงานที่ได้กำหนดไว้ และขอรับรองว่าจะใช้สัตว์ที่มีประสิทธิภาพสูงสุด โดยระลอกข้อมูลล่าสุดของชีวิตและศีลธรรมตามหลักศาสนา ตลอดจนยินดีให้คณะกรรมการจรรยาบรรณและมาตรฐานการใช้สัตว์ในงานวิทยาศาสตร์ ติดตามและตรวจสอบได้

ลงชื่อ…………………………………………หัวหน้าโครงการวิจัย

(นพ. สุรชัย แซจึง)

วันที่........... เดือน.............................. พ.ศ. ..........

ลงชื่อ……………………………………..ประธานกรรมการที่ปรึกษาวิทยานิพนธ์

(รศ.ดร.นพ. กิตติ จิระรัตนโพธิ์ชัย)

วันที่........... เดือน.............................. พ.ศ. ..........

ลงชื่อ…………………………………………หัวหน้าภาควิชา/หน่วยงาน

(........................................)

วันที่........... เดือน.............................. พ.ศ. ...........

(ผู้ขอรับการพิจารณาส่งแบบฟอร์ม จย สท. จำนวน 4 ชุด , โครงการวิจัยฉบับสมบูรณ์ จำนวน 4 ชุด และ แผ่นบรรจุข้อมูลโครงการวิจัยทั้งหมด (diskette หรือ cd-record) จำนวน 1 ชุด)

“ข้อมูลจะถูกเก็บเป็นเอกสารลับ”
Appendix 5

The randomization procedure

This picture showed the technique of randomization using the Microsoft Excel 2007 software.

Column A is the treatment sets. That is NSAIDs, cellulose or control groups.

Column B is the excel command. .......... “=rand()”
Column C is the command. ............ “=rank(B1,$B$1:$B$51)”

Column D is the command. ............ “=index($A$1:$A$51, match(rows($D$1:D1),$C$1:$C$51,0))”

The column D will show the randomization codes that can keep in the opaque sealed envelopes.

Table 25: The example of randomization codes for 51 rats

<table>
<thead>
<tr>
<th>1. Cellulose</th>
<th>18. control</th>
<th>35. Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. control</td>
<td>19. Cellulose</td>
<td>36. NSAIDs</td>
</tr>
<tr>
<td>3. NSAIDs</td>
<td>20. NSAIDs</td>
<td>37. NSAIDs</td>
</tr>
<tr>
<td>4. NSAIDs</td>
<td>21. control</td>
<td>38. control</td>
</tr>
<tr>
<td>5. NSAIDs</td>
<td>22. Cellulose</td>
<td>39. Cellulose</td>
</tr>
<tr>
<td>6. control</td>
<td>23. Cellulose</td>
<td>40. control</td>
</tr>
<tr>
<td>7. NSAIDs</td>
<td>24. Cellulose</td>
<td>41. NSAIDs</td>
</tr>
<tr>
<td>8. NSAIDs</td>
<td>25. control</td>
<td>42. control</td>
</tr>
<tr>
<td>10. control</td>
<td>27. Cellulose</td>
<td>44. NSAIDs</td>
</tr>
<tr>
<td>11. control</td>
<td>28. Cellulose</td>
<td>45. Cellulose</td>
</tr>
<tr>
<td>12. Cellulose</td>
<td>29. control</td>
<td>46. control</td>
</tr>
<tr>
<td>13. Cellulose</td>
<td>30. control</td>
<td>47. NSAIDs</td>
</tr>
<tr>
<td>14. Cellulose</td>
<td>31. control</td>
<td>48. NSAIDs</td>
</tr>
<tr>
<td>15. NSAIDs</td>
<td>32. NSAIDs</td>
<td>49. control</td>
</tr>
<tr>
<td>16. Cellulose</td>
<td>33. control</td>
<td>50. NSAIDs</td>
</tr>
<tr>
<td>17. Cellulose</td>
<td>34. NSAIDs</td>
<td>51. control</td>
</tr>
</tbody>
</table>
Appendix 6

The researcher

ประวัติผู้รับผิดชอบแผนงานวิจัย

1. ชื่อ-สกุล
   (ภาษาไทย)  นาย สุรชัย แซ่จึง
   (ภาษาอังกฤษ)  Surachai Sae-Jung

2. รหัสประจำตัวนักวิจัยแห่งชาติ

3. ตำแหน่งปัจจุบัน
   รองศาสตราจารย์ ภาควิชาวิทย์อิเล็กทรอนิกส์

4. หน่วยงานที่สังกัด
   ภาควิชาวิทย์อิเล็กทรอนิกส์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
   123 ถนนมิตรภาพ อ.เมือง จ.ขอนแก่น 40002
   โทรศัพท์ 043-348398
   E-mail : sursea@kku.ac.th

5. ประวัติการศึกษา

<table>
<thead>
<tr>
<th>Degree / Status</th>
<th>Institution(Year Conferred)</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>• M.D.</td>
<td>Khon Kaen University(1994)</td>
<td>Medicine</td>
</tr>
<tr>
<td>• Member</td>
<td>Medical Council(1994)</td>
<td>Medicine</td>
</tr>
<tr>
<td>• Certificate, Clinical science</td>
<td>Khon Kaen University(1995)</td>
<td>Orthopaedics</td>
</tr>
<tr>
<td>• Diploma, Certified Board</td>
<td>Khon Kaen University (Medical Council)(1998)</td>
<td>Orthopaedics</td>
</tr>
<tr>
<td>• Fellow(FRCOST)</td>
<td>Royal College of Orthopaedic Surgeons of Thailand</td>
<td>Orthopaedics</td>
</tr>
<tr>
<td>• Fellow (AO/ASIF)</td>
<td>AO/ASIF Foundation(2002)</td>
<td>Spinal Surgery</td>
</tr>
<tr>
<td>• Diploma, Certified Board</td>
<td>Khon Kaen University (Medical Council)(2003)</td>
<td>Family Medicine</td>
</tr>
<tr>
<td>Role</td>
<td>Institution</td>
<td>Specialty</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>--------------------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>AOSpine Member (code: 14204)</td>
<td>AOSpine Asia Pacific of AOSpine International (2005)</td>
<td>Spinal Surgery</td>
</tr>
<tr>
<td>Fellow (FRCFPT)</td>
<td>The Royal College of Family Physicians of Thailand (2007)</td>
<td>Family Medicine</td>
</tr>
<tr>
<td>Certificate of clinical fellow</td>
<td>Charité – University Medicine Berlin, Humboldt-University, Germany (2007)</td>
<td>Musculoskeletal surgery</td>
</tr>
</tbody>
</table>

6. สาขาวิชาการที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา) ระบุสาขาวิชาการที่เป็นสาขาวิชาการหลัก

7. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ โดยระบุสถานภาพในการทำวิจัยว่าเป็นผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในแต่ละข้อเสนอการวิจัยเป็นต้น

7.1 ผู้อำนวยการแผนงานวิจัย: ชื่อแผนงานวิจัย

7.2 หัวหน้าโครงการวิจัย: ชื่อโครงการวิจัย

งานวิจัยที่ทำเสร็จแล้ว: ชื่อชื่อเสนอการวิจัย ปีที่พิมพ์ การเผยแพร่ และสถานภาพในการทำวิจัย


7.3 ผู้ร่วมโครงการวิจัย : ชื่อโครงการวิจัย


7.4 งานวิจัยที่กำลังทำ: ชื่อข้อเสนอการวิจัยและสถานภาพในการทำวิจัย
1. Functional Outcomes for Lumbar facet Syndrome Treated with Oral Diclofenac or Methylprednisolone Facet Injection: A Prospective Randomized Trial (หัวหน้าโครงการวิจัย,กำลังเขียนต้นฉบับเพื่อส่งให้พิจารณาตีพิมพ์ในวารสาร)