Original Article

Efficacy of Three Alternative Hemostatic Agents (Chitosan, Gelatin, and Cellulose) for Soft Tissue Dissection during Lumbar Spine Surgery: A Comparative Study in an Animal Model

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Background: Intraoperative bleeding can lead to morbidity and complications. Hemostatic materials have been developed, but the relative efficacy of different agents has not been assessed.

Objective: To evaluate the hemostatic properties of four common hemostatic materials including chitosan clot pad, absorbable gelatin sponge, cellulose membrane, and gauze on para-spinal muscle bleeding using a rat lumbar spine soft tissue dissection model.

Materials and Methods: Forty 8-week-old Sprague-Dawley rats were used. Under adequate anesthesia, the para-spinal muscle of each rat was posteriorly dissected until the L5 and L6 spinous processes were found. Then the rats were randomly allocated to receive one of the four hemostatic materials. The allocated materials were placed over the raw surface of the dissected muscle and were changed every one minute until there was no further bleeding. Bleeding volume in each of the materials was recorded.

Results: The mean hemorrhage volumes (±SD) for the chitosan pad, gelatin sponge, cellulose membrane, and gauze were 0.06±0.03, 0.06±0.02, 0.07±0.03, and 0.18±0.17 ml, respectively. The overall bleeding volumes for the chitosan pad, gelatin sponge, cellulose membrane were significantly lower than the gauze; however, among those three, there were no significant differences in overall bleeding volume.

Conclusion: Chitosan pads, gelatin sponges, and cellulose membranes have better soft tissue hemostatic properties than gauze, and are all equally effective. Each of the three can be used as a hemostatic material without serious adverse effects during posterior lumbar para-spinal surgery.

Keywords: Chitosan, Cellulose, Gelatin, Rat, Para-spinal muscle

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Control of bleeding during spine surgery is important because intra-operative bleeding increases the risk of morbidity. Efficient bleeding control can also reduce complications from shock and blood transfusion. The key to stopping bleeding is to press, ligate, and cauterize the vessel, but in some danger areas, e.g., nerve root and spinal cord, this cannot be performed because the process can result in permanent tissue damage. To solve that problem, hemostatic materials have been developed, e.g., oxidized regenerated cellulose membrane, absorbable gelatin sponge, and chitosan clot pads. Absorbable gelatin sponges are used in most spinal surgeries for controlling capillary and venous bleeding(1) and are effective in reducing blood loss and have been shown to reduce adhesion of dog femoral arteries(2). Surgical cellulose pad membranes made of oxidized regenerated cellulose polymer are used extensively in maxillofacial surgery to control bleeding from the intra-alveolar artery. A cellulose membrane is used to staunch bleeding and prevent peridural fibrosis after laminectomy. Chitosan is a natural polymer with a good safety profile which has been shown in human

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studies to control bleeding, improve wound healing, and reduce adhesion formation. Chitosan pads are also used by the military to stop bleeding during field maneuvers. It is also effective for stopping bleeding from hepatic injuries in rats[3]. However, there is insufficient data to evaluate the effectiveness and safety of these materials in spinal surgery involving the para-spinal muscle and bone bleeding near neural structures.

Objective

The objectives of the present study were to compare the efficacy and safety of four commonly used hemostatic materials (cellulose membrane, gelatin sponge, chitosan pads, and gauze) in a rat model during surgical para-spinal soft tissue dissection of the lumbar area.

Materials and Methods

The present study was reviewed and approved by the Animal Ethics Committee of Khon Kaen University (AEKKU-NELAC 11/2557) before the study began.

Forty 8-week-old Sprague-Dawley rats weighing between 200 to 450 g were anesthetized by intraperitoneally injection of 60 mg/kg pentobarbital with a 1 ml syringe using a 24-gauge needle. Once the rat's toe pinch response was lost, anesthetic depth was considered sufficient for surgery. Sterile techniques were used at all times during the procedure. The rats were then intraperitoneally injected with a single dose of cefazolin 150 mg as a prophylactic antibiotic. The back skin was scrubbed with Hibitane® solution, then the skin was gently shaved. The rat was placed prone on an operating table; the back was painted with betadine solution and a surgical drape was placed on the animal. The back skin was incised and retracted bilaterally. The para-spinal muscle was dissected using a no. 11 surgical blade until exposure of the L5 to L6 spinous processes and 1 cm of bilateral laminae was achieved. At that point, the opaque sealed envelope containing the computer-generated randomization code was opened. Each rat was randomly assigned to receive one of the four sterile hemostatic materials cut to a size of 1×1 cm: (a) cellulose membrane (Surgicel Original Absorbable Hemostat; Ethicon, Somerville, New Jersey, USA); (b) absorbable gelatin sponge (Spongostan; Ferrosan Medical Device, Soeborg, Denmark); (c) chitosan pad (AnsCare ChitoClot Pad; BenQ Materials Corporation, Taoyuan, Taiwan); or (d) gauze. Each type of hemostatic material was placed over the raw surface of the muscle to stop the bleeding. The material was replaced with a new piece of the same type every minute until the bleeding stopped.

The weight of the bloody material was recorded to two decimal places using a weighing scale and then the weight was converted to volume. When there was no further bleeding, the lumbo-dorsal fascia was closed using absorbable suture (Vicryl No.3-0), then the skin was sutured using Nylon 3-0. All rats were given 300 mg/kg of paracetamol (Tylenol) syrup peroral three times a day for two days. The rats were observed for signs of pain, e.g., agitation, fatigue, difficult ambulation, and reduced food and water intake) as well as wound inflammation (redness, swelling, or pus at the surgical site). The skin sutures were removed on day 7 post-surgery.

Statistical analysis

Ten rats in each group were required to ensure 80% power. The continuous variables were analyzed using ANOVA and the independent t-test; Bonferroni correction analyzed the differences between each pair of interventions. The level of statistical significance was set at 0.05. The data were analyzed using IBM SPSS Statistics (version 20, IBM Corporation, Armonk, New York, USA).

Results

The respective average body weights of the male and female rats were 357.77±11.42 and 217.11±9.80 g; males were significantly heavier than the females ($p<0.001$). The respective average bleeding volumes of males and females were 0.08±0.04 and 0.11±0.14 ml (no significant difference, $p=0.442$) (Table 1, 2). Two-way ANOVA was conducted on the association of the two independent variables, sex and treatment group, on bleeding volume. No significant difference was found ($p=0.389$).

The average bleeding volume of the four hemostatic materials were compared and the difference was found to be statistically significant ($p=0.010$). As shown in Table 3 and Figure 1, *post hoc* comparisons between pairs of treatments using the independent t-test with Bonferroni correction showed that the groups treated with chitosan pads, absorbable gelatin sponge, and cellulose membrane had less bleeding volume than the control ($p=0.026$, 0.032, and 0.04, respectively). However, there were no statistically significant differences among the chitosan, gelatin, and cellulose groups.

Discussion

The hemostatic materials chitosan pads,

Table 1. Comparison of overall bleeding volume between male and female rats

	Male (n = 20) Mean±SD	Female (n = 20) Mean±SD	Mean difference (95% CI)	p-value
Body weight (g)	357.77±11.42	217.11±9.80	140.66 (133.85 to 147.47)	<0.001
Bleeding volume (ml)	0.08±0.04	0.11±0.14	−0.03 (−0.09 to 0.04)	0.442

SD=standard deviation; CI=confidence interval

Table 2. Body weight and bleeding volume by treatment group

	Chitosan Mean±SD	Spongostan Mean±SD	Surgicel Mean±SD	Gauze Mean±SD
Male, n	5	5	5	5
Body weight (g)	362.26±11.45	360.00±11.10	355.36±9.67	353.46±14.47
Bleeding volume (ml)	0.05±0.04	0.06±0.02	0.08±0.01	0.13±0.01
Female, n	5	5	5	5
Body weight (g)	218.26±10.17	210.70±4.07	213.08±7.58	226.40±9.86
Bleeding volume (ml)	0.07±0.02	0.07±0.02	0.05±0.03	0.23±0.25
Overall, n	10	10	10	10
Body weight (g)	290.26±76.58	258.35±79.08	284.22±75.43	289.93±67.98
Bleeding volume (ml)	0.06±0.03	0.06±0.02	0.07±0.03	0.18±0.17

SD=standard deviation

Table 3. Post hoc comparison of bleeding volume among the treatment groups

Materials (1 vs. 2)	Mean difference in bleeding volume (ml)	95% CI of mean difference (ml)	p-value
Chitosan vs. Gauze	−0.12	−0.23 to 0.01	0.026
Spongostan vs. Gauze	−0.12	−0.23 to −0.01	0.032
Surgicel vs. Gauze	−0.12	−0.23 to −0.01	0.040
Chitosan vs. Spongostan	<−0.01	−0.115 to 0.108	>0.999
Chitosan vs. Surgicel	<−0.01	−0.119 to 0.105	>0.999
Spongostan vs. Surgicel	<−0.01	−0.115 to 0.108	>0.999

CI=confidence interval

Minus sign (−) indicates material 1 had lower bleeding volume than material 2, control represents the gauze treatment group

absorbable gelatin sponge, and cellulose membrane can all stop muscle bleeding better than gauze or other conventional methods. Both cellulose membrane and gelatin sponge are absorbent. Oxidized regenerated cellulose membrane is a homo-polysaccharide composed of glucopyranose polymerized through β-glucosidic bonds[4]. In addition, it derives acidity from a carboxyl group, which provides local bactericidal and hemostyptic action. Secondary platelet activation results in formation of platelet plugs via physical scaffolding[5,6].

The absorbable gelatin sponge is a white, porous, and biodegradable product. It is prepared from sterilized dehydrated porcine skin. The hemostatic properties of the gelatin are caused by platelets damaged while entering and contacting the sponge's interstices after which thromboplastin is released from the platelets, initiating clotting[7]. A sponge can absorb as much as 40 times its weight in fluid and can expand in volume by 200%[5].

A chitosan pad is a polysaccharide polymer of glucosamine. The sponge-like material has a positive charge which allows binding with the negatively charged red blood cells and platelets, activating blood clot formation. In addition, the electropositive surface acts as an attractant for other chemotactic factors that are involved in the clotting process.

The authors compared chitosan pads, gelatin sponge, cellulose membrane, and gauze (the control material). Each of these four hemostatic agents were cut to the same size and placed over the raw muscle surface without application of force to avoid the

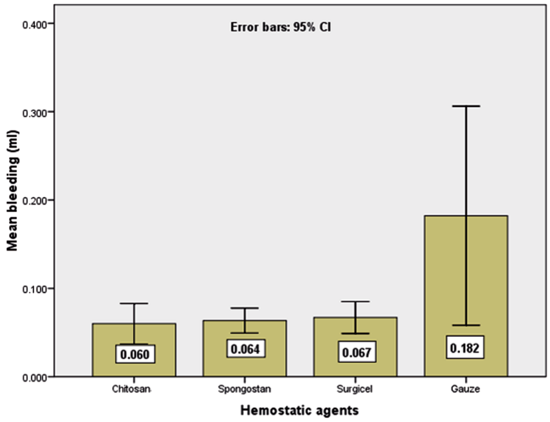

Figure 1. Mean overall bleeding volume among cellulose membrane, gelatin sponge, chitosan pad, and gauze groups.

Numbers within boxes represent mean bleeding volume (milliliter), CI=confidence interval

pressure effect, which can confound the hemorrhage volume.

Each of the three tested agents was more effective than gauze in stopping bleeding, and each of those materials can be used to staunch bleeding during para-spinal muscle surgery, although they have different mechanisms for inducing hemostasis. The absorbable materials, cellulose membrane, and gelatin sponge can remain in the operative field, while chitosan pads have to be removed when they become saturated.

Conclusion

Chitosan pads, gelatin sponge, and cellulose membrane each have better hemostatic properties than gauze when used to stop muscle bleeding after para-spinal muscle surgery in a rat model.

What is already known on this topic?

Presently, cellulose membrane and gelatin sponge are widely used to stop bleeding. However, the efficacy of these materials compared to conventional material (gauze) has not been demonstrated. Chitosan pads, a newly designed hemostatic material, has not been evaluated for safety and efficacy for stopping soft tissue bleeding in spinal surgery.

What this study adds?

This study compares the efficacy of chitosan pads, gelatin sponge, and cellulose membrane with that of gauze. The efficacy of those three materials is significantly better than gauze. However, we recommend that these materials be removed when there is no more active bleeding to avoid potential complications associated with retaining the material.

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Disclosure

This is an original work from the Department of Orthopaedics, Faculty of Medicine, Khon Kaen University, Thailand. It has not been presented elsewhere nor is it under consideration by any other journal. The authors participated in the design, data collection, analysis, and writing of the research.

Conflicts of interest

The authors declare no conflict of interest.

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