

The Influence of Haemostatic Dressing Prototypes for the Emergency Services on the Histopathological Parameters of Porcine Muscle

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Abstract. *Background/Aim: Haemostatic dressings for the uniformed and rescue services are an integral part of life-saving equipment for controlling post-traumatic haemorrhage. The aim of this study was to assess the influence of active constituent substances and materials of haemostatic dressings on muscle tissue and muscle regeneration after traumatic injury. Materials and Methods: Three hemostatic dressing prototypes were analysed: OBR/G/S sponge: dressing material sponge made of Na-Ca chitosan/alginate composite microfibers and nanofibers; OBR/MBT/S: tactic gauze modified with a polymer mixture of Na-Ca chitosan/alginate composite microfibers and nanofibers, impregnated with a moderate amount of procoagulants (22.9 g/m²); and OBR/MS/S: seton gauze modified with a polymer mixture of Na-Ca chitosan/alginate composite microfibers and nanofibers, impregnated with a moderate amount of procoagulants (18.0 g/m²), with chitosan (ChitoClearhqg 95) and sodium alginate (Protanal LF10/60 FT) as the coagulants. The experiment was conducted on 20 pigs which were euthanised 24 h, 7 or 14*

days after wound dressing. Samples of porcine muscle tissue were subjected to qualitative histopathological analysis. Results: Histopathological analysis of muscle tissues from the experimental pigs revealed that the application of modified seton (OBR/MS/S) produced the most satisfactory results. The observed changes were similar on all dates that samples were collected and in all experimental groups, and minor differences in their extent were observed between groups. Regenerative processes were most advanced, and retrograde changes were least apparent in animals treated with OBR/MS/S. Conclusion: Modified seton (OBR/MS/S) induced the least tissue reaction and was most effective in promoting tissue regeneration after injury.

Individuals who take part in military, police or rescue operations often suffer damage to large arterial and venous trunks as a consequence of gunshot wounds, stab wounds or extensive lacerated wounds (1, 2). Outside the hospital setting, profusely bleeding wounds are treated with haemostatic dressings according to Trauma Life Support and Tactical Combat Casualty Care guidelines (3). The achievement of complete haemostasis with the use of haemostatic dressings is the main priority during combat or rescue operations (4, 5). Most combat wounds are deep and extensive, and dressings have to be applied deeply in between tissues, mostly thick layers of muscle (6, 7). This study evaluated three haemostatic dressing prototypes composed of various materials and substances that stimulate clotting at the wound site (8). The application of various materials and substances to promote clotting affects not only blood vessels,

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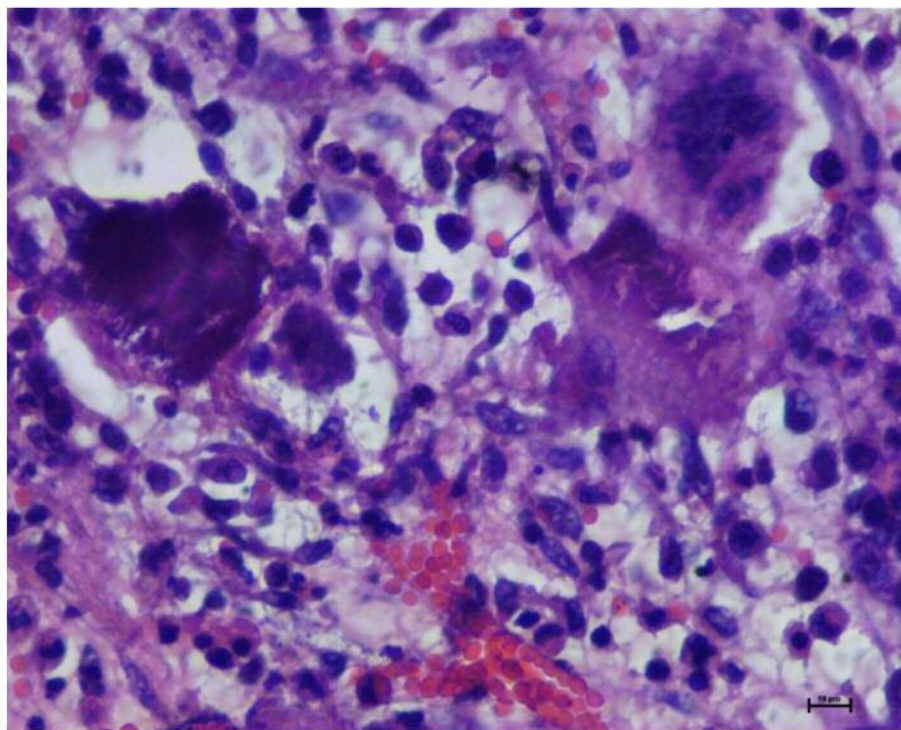


Figure 1. Group II, day 7 – giant muscle cell and focus of calcification. Haematoxylin and eosin staining. Bar: 10 μm .

but also the surrounding muscle tissue. Some haemostatic agents can cause serious endothelial damage and substantial transmural injury that rendered the vessels non-viable for primary surgical reparation. Necrosis of fat and muscle, as well as full- and partial-thickness cutaneous burn, were also noted as a result of topical administration of a granular mineral haemostatic agent. Studies showed the chitosan sponge degraded much more slowly than collagen sponge after subcutaneous implantation in rabbits. Additionally, tissue reactions for chitosan sponges were much greater than for collagen sponges. Haemostatic agents may affect changes in blood electrolyte concentration and cause acceleration of the intrinsic pathway of blood coagulation *in vitro* (9-12). The aim of this study was to perform a histopathological evaluation of the influence of three haemostatic dressing prototypes on muscle tissue.

Materials and Methods

The study was approved by the Institute for Animal Welfare and the Bioethics Committee. All animals were handled humanely in compliance with the Policy on the Humane Care and Use of Laboratory Animals and the standards of the Polish Council on Animal Care. The study was also approved by the Local Committee for Animal Care in Olsztyn (Decision No.44/2014/N).

The experiment was performed on 20 Polish Large White pigs with an average body weight of 45 kg, divided into four groups.

OBR/G/S sponge made of Na-Ca chitosan/algal composite microfibers and nanofibers (MASKPOL S.A., Panki, Poland) was used in six animals (group I); OBR/MBT/S tactic gauze modified with a polymer mixture of Na-Ca chitosan/algal composite microfibers and nanofibers (MASKPOL S.A.) and impregnated with a moderate amount of procoagulants (22.9 g/m²) was applied in another six animals (group II); and OBR/MS/S seton gauze modified with a polymer mixture of Na-Ca chitosan/algal composite microfibers and nanofibers (MASKPOL S.A.) and impregnated with a moderate amount of procoagulants (18.0 g/m²) was used in another six animals (group III). Group IV was composed of two animals in which haemostatic dressings were not applied.

The coagulants in all dressings were chitosan (ChitoClearhq 95) and sodium alginate (Protanal LF10/60 FT) (IBWCH, Lodz, Poland). Haemostatic dressings were sterilised by electron beam irradiation. The animals were premedicated with atropine (Atropinum Sulfuricum; Polfa S.A., Warsaw, Poland) at 0.05 mg/kg body weight (BW) *intramuscularis* (*i.m.*) and azaperone (Stresnil; Janssen Pharmaceutica NV, Beerse, Belgium) at 2.5 mg/kg BW *i.m.* ketamine (Bioketan; Vetoquinol Biowet Sp. z o.o., Gorzów Wielkopolski, Poland) at 8 mg/kg BW *i.m.* was used to induce anaesthesia and maintained with propofol (Scanofol; ScanVet, Gniezno, Poland) administered intravenously to clinical response. The pain was managed with buprenorphine (Bupaq Multidose; Orion Pharma, Warsaw, Poland) at 20 $\mu\text{g}/\text{kg}$ BW *intravenosa* (*i.v.*). All animals were intubated with the maintenance of spontaneous ventilation. Ringer's lactate solution was administered intravenously during the procedure.

A sterile surgical site was prepared in the region of the left inguinal fossa. The skin, muscles and the femoral artery were

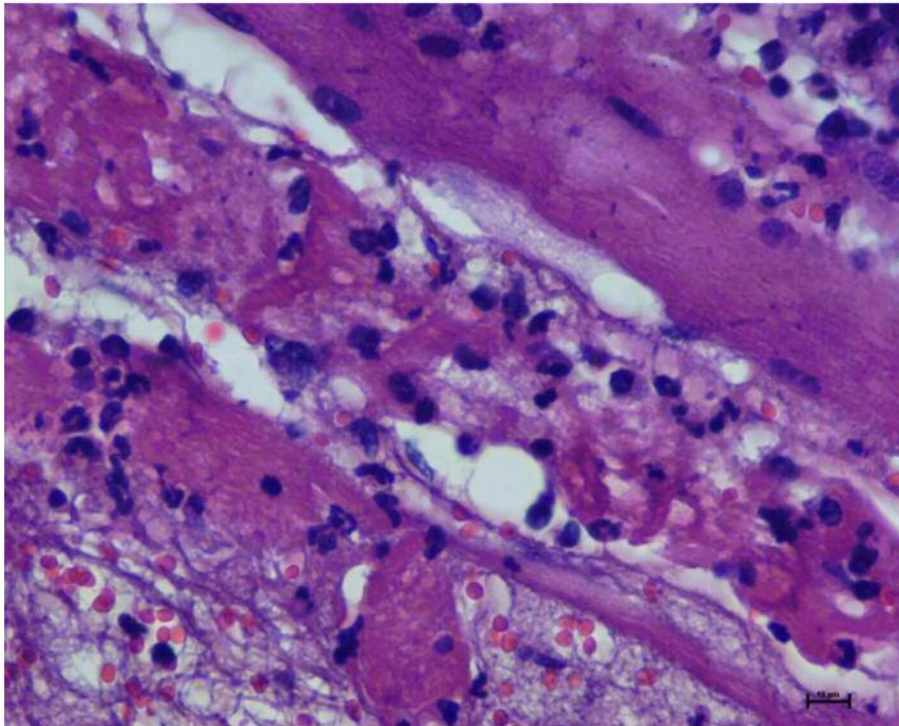


Figure 2. Group I, day 14 – sarcoplasmic degeneration with infiltrating phagocytes. Haematoxylin and eosin staining. Bar: 10 μ m.

incised in all patients. The size of the incision made with a sterile scalpel was about 8 cm. Haemostatic dressings were left inside the wound, which was sutured with the dressing in animals all experimental groups. The dressings were removed after 12 hours. The analgesic drug metamizole (Biovetalgin; BIOWET DRWALEW S.A., Drwalew, Poland) at 40 mg/kg BW *i.m.* was administered for 5 days. The surgical procedure was not performed in control animals. Two animals from each group were euthanised 24 hours, 7 days and 14 days after the procedure. The two control group pigs were euthanised on experimental days 7 and 14. Muscle tissue was sampled from all animals. Tissue samples were collected in sterile conditions using Metzenbaum scissors from the muscle tissue surrounding the incised femoral artery. The samples were fixed in 10% buffered formalin, dehydrated and embedded in paraffin blocks. Microtome sections were stained with haematoxylin and eosin (HE) and Masson's trichrome. Qualitative assessment of muscle tissue included determination of the type and severity of morphological changes, *e.g.* circulatory disorders, reversible and irreversible changes, regeneration.

Results

This study showed that in all experimental groups, the morphological changes in the muscles were similar 24 h after the surgical procedure and the application of haemostatic dressings. Circulatory disorders were observed in all groups, mostly extensive haemorrhaging and blood extravasation in the endomysium, perimysium and epimysium. Hyperaemia

was also noted, in particular in the capillaries, and oedema was observed in all areas of muscle tissue and connective tissue. Oedema was frequently accompanied by the presence of exudate and infiltration of granulocytes and macrophages. Visible regressive lesions involved focal degeneration of muscle fibres. In all experimental groups, differences in the diameter of muscle fibres in bundles, hyaline degeneration of the sarcoplasm in large fibres and the presence of atrophic, polygonal fibres with a reduced diameter were observed. Early necrotic changes in myofibres were considerably more prevalent in the experimental groups than in the control group. Sarcoplasmic vacuolation in muscle cells and, in individual cases, the local absence of muscle striation (group I) and changes in sarcoplasmic architecture (group II), were observed in all evaluated muscle tissue samples. Infiltrating granulocytes and macrophages were also detected in the vicinity of necrotic muscle fibres.

Extensive organising haematomas, in some cases surrounded by a thin layer of connective tissue and infiltrating granulocytes, were observed in muscle tissue sampled from pigs 7 days after wound dressing in all groups. Oedema was observed in all groups, mainly in the perimysium and epimysium. Extensive oedema was noted in groups I and II. Visible retrograde changes involved extensive necrosis of muscle tissue with disseminated giant cells and calcification of fibres (Figure 1). The prevalence of

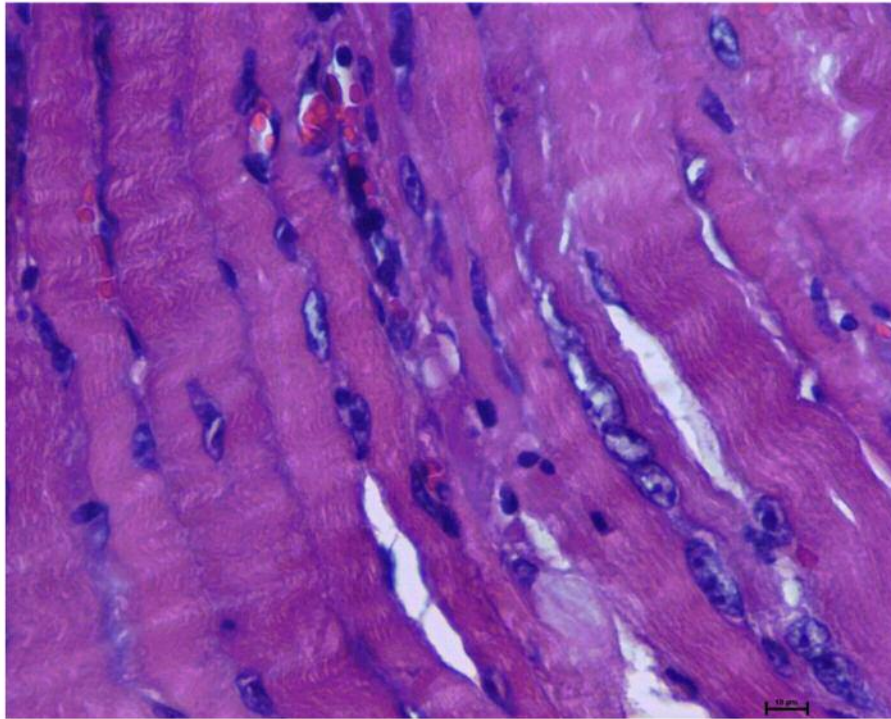


Figure 3. Group III, day 14 – myotube formation. Haematoxylin and eosin staining. Bar: 10 μ m.

regressive changes was similar in the experimental groups, but it was the lowest in group III, where modified seton was used. Infiltrating cells, mainly mononuclear cells and, in some cases, granulocytes, were identified in necrotic foci. Most infiltrating cells formed small disseminated foci. Extensive infiltration was observed in one pig from group II, and eosinophils were noted in group I. Hemosiderin-laden macrophages were observed in two pigs from groups I and III. Perivascular infiltration was noted in group I. Infiltrating adipocytes in necrotic foci were identified in all animals. Muscle fibres in bundles differed in diameter in all experimental animals. Polygonal fibres were present in one. Hyaline degeneration and vacuolation of the sarcoplasm were observed sporadically; hyaline degeneration was noted only in group I, and vacuolation only in groups II and III. The disappearance of muscle striation was observed in all experimental animals, mostly in muscle fibres localised in the proximity of necrotic foci. Small sections of supercontracting fibres were sporadically noted in every experimental group. Regenerative changes, mostly myotube formation and stimulation of satellite cells, were also observed in muscle tissues sampled 7 days after surgery. The number of myotubes was highest in group III, and lowest in group I. Extensive granulation tissue with numerous thin-walled and irregular vessels was observed in most animals, excluding group III pigs. Stimulation of connective tissue

cells and their accumulation in muscle fascicles were noted in group II.

Minor focal oedema was observed in muscle samples collected 14 days after the surgery, mainly in the perimysium, epimysium, around blood vessels and in the surrounding connective tissue. No significant differences were found between experimental groups, and the presence of exudate and infiltration of mononuclear cells were observed in individual pigs from groups I and III. Circulatory disorders, mostly an expansion of the vascular lumen and venous insufficiency, were noted in individual animals. Haemorrhaging and extravasation were observed in most cases. Extensive hematomas were accompanied by granulocyte infiltration in groups I and II. Small disseminated extravasations were most frequently observed in group III. Regressive changes involving sarcoplasmic degeneration in muscle fibres were observed sporadically and in individual cells only. In one pig from group I, sarcoplasmic degeneration was extensive and accompanied by infiltration of mononuclear cells (Figure 2). Sarcoplasmic vacuolation and the presence of fibres with centrally positioned nuclei were noted sporadically in individual animals in every experimental group and in individual muscle cells. Muscle tissue necrosis was observed in all animals. Necrotic changes were extensive in most groups, and the presence of necrotic foci was noted only in group III, where wounds were dressed with modified seton.

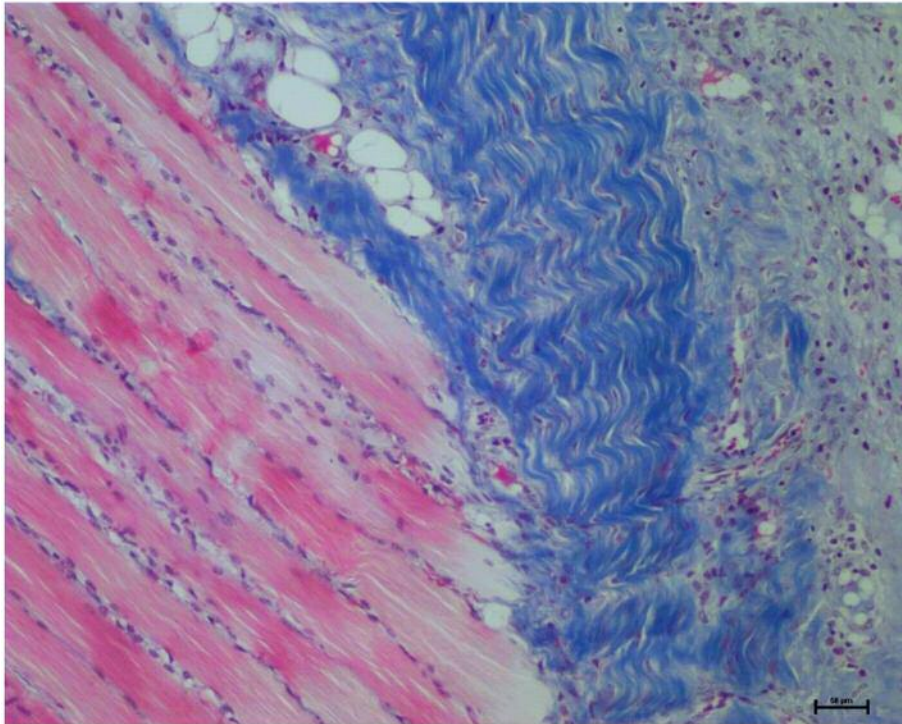


Figure 4. Group III, day 14 – fibrocyte band surrounding the wound site. Masson's trichrome stain. Bar: 50 μ m.

Necrotic changes were accompanied by giant muscle cells which were most numerous in group II. Small foci of calcification were observed sporadically, mainly in groups II and III. Foci of muscle fibres in early stages of necrosis with granulocyte infiltration were also noted in group II. The diameter of muscle fibres in bundles differed in all animals. The highest number of atrophic, thin and polygonal fibres was observed in group I, whereas large fibres with signs of hyaline degeneration were noted mainly in group II. The disappearance of striation and the presence of supercontracting fibres were observed in individual pigs in every experimental group. The extent of regenerative changes differed considerably between groups. Myotube formation (Figure 3), new muscle fibres and stimulation of satellite cells were observed. Regenerative changes were least pronounced in group I and most highly apparent in group III. Infiltration differed considerably in the analysed fragments of muscle tissue. Extensive infiltration, mainly with granulocytes, was noted in group I animals. Granulocytes were also the main infiltrating cells in group II and III pigs, but the observed changes were mostly focal and disseminated, and perivascular infiltration was also noted in individual animals. Inflammatory cells were localised mainly in the perimysium and epimysium. Disseminated eosinophils were also observed in individual animals in the experimental groups. Infiltration of mononuclear cells was noted, and it was least pronounced in

group III; however, abscesses were found in one pig from group III. Granulation tissue filling wound sites was characterised by different degrees of maturation. The largest foci of granulation tissue were observed in group I animals, whereas mature connective tissue was more frequently noted in the remaining groups. Significant stimulation of connective tissue and its accumulation in muscle fascicles, in particular in the perimysium and epimysium, were observed in all animals. Bands of fibrocytes were formed around the wounds in muscle tissue (Figure 4).

Minor morphological changes, mainly capillary hyperaemia, were observed in control group animals which did not undergo haemostatic dressing. Early necrotic changes were visible in individual myofibres. Only minor differences in the diameter of muscle fibres were observed, and thin, polygonal fibres were sporadically noted. The disappearance of muscle striation was noted sporadically and only locally.

Discussion

The experiment was a pilot study because there has been no previous research on the haemostatic dressings used in this study. The changes in control group animals differed significantly in comparison to the lesions observed in the experimental animals, and most of them were indicative of normal physiological processes. The type and extent of the

observed changes were examined to determine the presence of pathological processes, such as systemic infections, in pigs.

The muscles of the experimental animals were characterised by similar histopathological changes whose extent did not differ significantly between the groups. Symptoms of acute tissue damage, such as extensive haemorrhaging, blood extravasation and infiltration of inflammatory cells, mostly granulocytes and macrophages (13), were observed in tissue samples collected 24 hours after wound dressing. Regressive changes involved early necrosis of muscle fibres and sarcoplasmic degeneration. The extent of the observed changes was similar in all experimental groups. In muscles sampled 7 days after the surgery, the changes were more diversified, ranging from circulatory disorders to regressive and regenerative changes. Regenerative processes (myotube formation and stimulation of satellite cells) (14) were most pronounced in the muscles of group III pigs, where wounds were dressed with modified seton (OBR/MS/S) whereas regressive lesions were least extensive in this group. Similarly, various changes indicative of coexisting regressive and progressive changes were observed in muscle tissue sampled 14 days after wound dressing. However, the severity of the regenerative changes was higher compared to the muscle tissue collected 24 hours and 7 days after surgery, especially in group III where the highest number of myotubes, stimulated satellite cells and new fibres was observed. Furthermore, in this group granulation tissue in wound sites was replaced with mature connective tissue faster than in groups I and II, where immature granulation tissue was more extensive than mature connective tissue.

Conclusion

The results of this study demonstrate that modified seton (OBR/MS/S) was most effective in protecting muscle tissue from pathological changes induced by mechanical damage and in stimulating regenerative processes.

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Conflicts of Interest

The Authors declare that there are no conflicts of interest in regard to this study.

Authors' Contributions

PH conducted the experiments, wrote and drafted the manuscript. ZA designed the study and conducted the experiments. IB

performed all histopathological analyses, wrote and drafted the manuscript. MJ and PJ conducted the experiments and helped with sample collection. LG, MB and JB critically revised the manuscript. AT and JG conducted the experiments, wrote and drafted the manuscript. All authors read and approved the final manuscript.

References

- 1 Eastridge BJ, Hardin M, Cantrell J, Oetjen-Gerdes L, Zubko T, Mallak C, Wade CE, Simmons J, Mace J, Mabry R, Bolenbaucher R and Blackburne LH: Died of wounds on the battlefield: causation and implications for improving combat casualty care. *J Trauma Acute Care Surg* 71: 4-8, 2011. PMID: 21795876. DOI: 10.1097/TA.0b013e318221147b
- 2 Zhang YJ, Gao B and Liu XW: Topical and effective hemostatic medicines in the battlefield. *Int J Clin Exp Med* 8: 10-19, 2015. PMID: 25784969.
- 3 Gegel BT, Austin PN and Johnson AD: An evidence-based review of the use of a combat gauze (QuikClot) for hemorrhage control. *AANA J* 81: 453-458, 2013. PMID: 24597007. DOI: 10.7205/MILMED.170.1.63
- 4 Alam HB, Burris D, DaCorta JA and Rhee P: Hemorrhage control in the battlefield: role of new hemostatic agents. *Mil Med* 170: 63-69, 2005. PMID: 15724857. DOI: 10.7205/MILMED.170.1.63
- 5 Arnaud F, Parreno-Sadalan D, Tomori T, Delima MG, Teranishi K, Carr W, McNamee G, McKeague A, Govindaraj K, Beadling C, Lutz C, Sharp T, Mog S, Burris D and McCarron R: Comparison of 10 hemostatic dressings in a groin transection model in swine. *J Trauma Inj Infect Crit Care* 67: 848-855, 2009. DOI: 10.1097/TA.0b013e3181b2897f
- 6 Adamiak Z, Jastrzebski P, Pomianowski A, Otrocka-Domagala I, Holak P, Zhalniarovich Y, Przyborowska P and Glodek J: Effect of 24-hour application of three hemostatic dressings to porcine thigh muscles. *Pol J Vet Sci* 17: 519-521, 2014. PMID: 25286664. DOI: 10.2478/pjvs-2014-0076
- 7 Adamiak Z, Bukowiecka D, Jastrzebski P, Jalynski M, Holak P, Glodek J and Gudzbeler G: The effectiveness of modified seton and modified combat gauze in controlling severe hemorrhaging during operations of uniformed services. *Pol J Vet Sci* 19: 503-507, 2016. PMID: 27760028. DOI: 10.1515/pjvs-2016-0063
- 8 Adamiak Z, Krystkiewicz W, Pomianowski A, Bukowiecka D, Zubrzycki W, Jalynski M, Holak P, Glodek J and Jastrzebski P: The effect of hemostatic dressing prototypes for the uniformed services on selected blood coagulation parameters in pigs. *Acta Vet Scand* 59: 29, 2017. PMID: 28499437. DOI: 10.1186/s13028-017-0297-9
- 9 Wright JK, Kalns J, Wolf EA, Traweek F, Schwarz S, Loeffler CK, Snyder W, Yantis LD and Eggers J: Thermal injury resulting from application of a granular mineral hemostatic agent. *J Trauma Inj Infect Crit Care* 57: 224-230, 2004. PMID: 15345965. DOI: 10.1097/01.TA.0000105916.30158.06
- 10 Wang X, Yan Y and Zhang R: A comparison of chitosan and collagen sponges as hemostatic dressings. *J Bioact Compat Polym* 21: 39-54, 2006. DOI: 10.1177/0883911506060201
- 11 Kheirabadi BS, Mace JE, Terrazas IB, Fedyk CG, Estep JS, Dubick MA and Blackburne LH: Safety evaluation of new hemostatic agents, smectite granules, and kaolin-coated gauze in a vascular injury wound model in swine. *J Trauma Acute Care Surg* 68: 269-278, 2010. PMID: 20154537. DOI: 10.1097/TA.0b013e3181c97ef1

- 12 Li J, Cao W, Lv XX, Jiang L, Li YJ, Li WZ, Chen SZ and Li XY: Zeolite-based hemostat QuikClot releases calcium into blood and promotes blood coagulation *in vitro*. *Acta Pharmacol Sin* 34: 367-372, 2013. PMID: 23334236. DOI: 10.1038/aps.2012.159
- 13 Nikolaou PK, Macdonald BL, Glisson RR, Seaber AV, Garrett WE Jr: Biomechanical and histological evaluation of muscle after controlled strain injury. *Am J Sports Med* 15: 9-14, 1987. PMID: 3812867. DOI: 10.1177/036354658701500102
- 14 Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, Partridge T and Buckingham M: Direct isolation of satellite cells for skeletal muscle regeneration. *Science* 309: 2064-2067, 2005. PMID: 16141372. DOI: 10.1126/science.1114758

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