EFFICIENCY AND SAFETY OF MICROPOROUS POLYSACCHARIDE HEMISPHERES FROM POTATO STARCH IN BRAIN SURGERY

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Dedicated to Acad. Bogdan C. Simionescu on the occasion of his 70th anniversary

Efficient hemostasis is important in neurosurgery and in other surgical areas, in which bleeding is a challenge. Thus, topical hemostatic agents have become a highly important armamentarium. After the 2000s, surgery has come to use microporous polysaccharide hemispheres, natural macromolecular biopolymers obtained from potato starch, and over time, these hemostatic agents proved their efficiency and safety in performing topical hemostasis, both in clinical studies and in experimental ones. This article undertakes to highlight the advantages, the adverse reactions and the applicability of microporous polysaccharide hemispheres in the neurosurgical field and present new directions in chemical recombination of microporous polysaccharide hemispheres.

Keywords: microporous polysaccharide hemispheres, potato starch, hemostasis, neurosurgery, brain surgery

INTRODUCTION

In neurosurgery, focal and diffuse brain injury might prove a very serious complication. Thus, when managing hemostasis in brain surgery, topical hemostatic agents have become a highly important armamentarium for neurosurgeons, since their use has led not only to cutting down on costs and products, but also to the improvement of the neurosurgical technique,[1,2] as cancer treatment and research have become a priority nowadays because of the high burden the disease represents for the health system worldwide.[3,4] Although nowadays, most neurosurgeons use bipolar electrocoagulation and various hemostatic agents and techniques, these bring about a series of advantages and disadvantages.

After the 2000s, surgery has come to use microporous polysaccharide hemispheres (MPHs) (Arista™ AH, TraumaDex™, Bleed-X™, HemaDerm™), which are spherical particles of controlled porosity, obtained from vegetable polysaccharides. These make hemostasis smoother by creating the conditions of rapid fluid assimilation in the blood and by speeding up the clotting and the aggregation of the platelets[5,6,7] (Fig. 1). Over time, these hemostatic agents proved their efficiency and safety in performing topical hemostasis, both in clinical studies[5,8-13] (Tables 1-2) and in experimental ones[6,14-20] (Table 3). Due to a huge surface area, high porosity and great water absorption capacity, MPHs have been considered a remarkable and attractive candidate for hemostasis.[21]

Polysaccharides represent a type of natural macromolecular biopolymers defined by the International Union of Pure and Applied Chemistry (IUPAC) as carbohydrates with more than 10 monomeric units.[22] A polysaccharide is usually composed of 10 monosaccharides joined through glycosidic linkages in branched or linear chains, with a molecular weight that varies from

tens of thousands to millions.[23] Similarly to proteins and polynucleotides, the polysaccharide is a major macromolecule in the biological life cycle and it also has a significant role in immune molecular recognition, cellular communication and cell adhesion.[24]

The naturally occurring food polysaccharides are classified into 3 groups: structural components of the plant cell walls (*e.g.* pectins, cellulose, hemicelluloses), storage polysaccharides (*e.g.* starch, galactomannans, fructans) and isolated polysaccharides (*e.g.* pectin, gums, mucilages).[25]

Owing to their specific functional properties, such as stabilizing, thickening and gel formation, MPHs are employed not only in petroleum oil drilling and cosmetic or food industries,[25] but also in medicine, pharmacy and biochemistry, due to their high efficiency, safety and non-toxic properties.[26-31] In 2002, MPHs received the approval for intraoperative applications and began to be used clinically as a topical hemostatic agent.[32]

DEFINITION. MECHANISMS OF ACTION

The first starch-derived hemostatic agent was described by Murat *et al.*,[6] when evaluating the hemostasis in a partially open porcine nephrectomy model, and was called MPH. It had a porous surface, which facilitated the absorption of water, but also of low molecular weight compounds (<40,000 Da) in the blood.[6] Two years later, in 2006, the U.S. Food and Drug Administration made it available for legal use on the medical market.[33]

MPHs are particles made from biologically inert plant polysaccharides derived from potato starch, so they are a 100% plant-based polysaccharide. MPHs are generated by cross-linking starch with epichlorohydrin to form glycerol-ether links (1-3 dioxypropanol).[2,21] MPH particles measure 30-100 μm[9] and do not contain human or animal components. Moreover, they are biocompatible, non-pyrogenic and can be assimilated in 24-48 hours.[34]

Starch is derived from plants as a branched glucose polymer (α4-glucose chains with α6 branches). The polymer consists of amylose and amylopectin, is very similar to glycogen, the animal equivalent to starch, only differing in a shorter branch length for the glycogen molecule, and these similarities make starch an ideal biomaterial for medical purposes.[32]

MPHs represent a fluid powder engineered to dehydrate blood and enhance clotting on contact, used as hemostatic agent in controlling capillary, venous and arteriolar bleeding.[35] After application, MPHs act as a "molecular sieve", absorbing the fluid components of blood and concentrating clotting factors, platelets, red blood cells and blood proteins on the surface of the particles (Fig. 1), resulting in an elastic, natural clot, within a few minutes, like a gel matrix, regardless of the patient's coagulation,[34] although some studies place it in an interdependent relationship with the patient's clotting status.[35] Actually, this powerful osmotic action causes the particles to swell and condense on their surface the platelets, serum proteins and other formed elements.[5,15,16,35]

In neurosurgery, it has been proved that MPHs act best in diffuse moderate bleeding in the resection cavity walls. The white powder also facilitates the identification of recurrent bleeding. In most of the cases, the diffuse bleeding from the resection cavity has immediately ceased.[34] MPHs produce a durable hemostasis on brain tissue, Tschan *et al.*[34] recording an average period length of 57 seconds (8-202 seconds range), while Galaraza *et al.*[35] recorded an average period length greater than 120 seconds until the cessation of the bleeding (Table 2).

In spite of this, MPHs manifested an insufficient hemostatic capacity to stop severe bleeds,[7,10,36,37] and this aspect finds an explanation in the fact that MPHs assimilate a number of proteins that reaches 40,000 Da. In this case, α-thrombin, β-thrombin and γ-thrombin are retained in the hemispheres during bleeding in amounts of 39,000 Da, 28,000 Da and 28,000 Da, respectively.[38,39]

MPH is completely destroyed by alpha amylase as quickly as 6 h after application,[17] and therefore, the short-lived clot created by MPHs could lead to postoperative bleeding, which might inflict the need of another surgical intervention. According to Hamdi and Ponchel (1999), MPHs are enzymatically destroyed in water soluble fragments in as little as 12 hours with a stable intact clot remaining.[40] Further studies are needed to throw light on this aspect. Moreover, MPHs permit the body's own enzymes to break them into oligosaccharides, maltose and eventually glucose, which are assimilated in 24-48 hours.[41,42] The degrading rate depends on the activity of endogenous amylase and the degree of cross-linking of the spheres.[40]

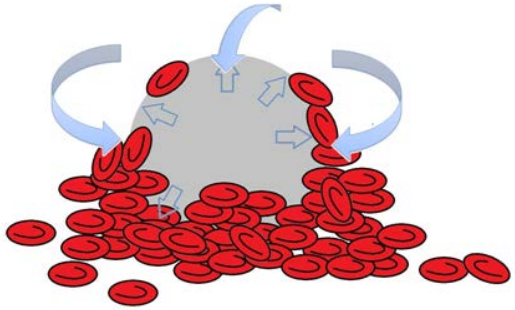

Figure 1: Scheme of MPHs and mechanism of hemostasis action. MPH particles (grey ball) act as a molecular sieve that expands (transparent arrows) by absorbing the fluid (blue arrows) and concentrating erythrocyte, platelets and blood proteins on the surface of the particles (from the personal collection of the authors)

Table 1
Clinical studies of MPHs

Author	Medical field	Procedure	Patients (MPHs)	Hemostasis obtained after MPH application	Adverse reactions
Reynbakh[46]	Interventional	Electrophysiology device implantation	77	Significant reduction in the rate of overall post-procedural complications, reduction of the infection and implantation site hematoma rate	no
Bruckner[47]	Thoracic surgery	Cardiothoracic surgical procedures	103	Significant reduction in hemostasis	no
Nunez-Nateras[42]	Urology	Radical prostatectomy	10	Postoperative decrease in hemoglobin was less	-
Antisdel[8]	ENT	Endoscopic sinus surgery	40	40% reduction in bleeding	no
Sindwani[45]	ENT	Endoscopic sinus surgery	65	30-45 seconds	no
Tan[5]	Dermatology	Mohs micrographic surgery	22	Did not have an increased incidence of active bleeding upon dressing removal	no

Table 2
Clinical studies in neurosurgery of MPHs

Author	Neurosurgical procedures	Patients	Hemostasis	Adverse reactions
Tschan[34]	Glioma, meningioma, brain metastasis, microsurgical brain tumor resection	33	8-202 seconds (mean 57 seconds)	no
Galarza[35]	5 cerebral convexity meningiomas, 5 corticosubcortical gliomas	10	<2 minutes	no

Table 3
Experimental studies on MPHs

Author	Medical field	Model		Hemostasis	Observations
Ereth[16]	Brain surgery	Rat	228	60 seconds	Equally effective hemostatic properties with other hemostatics, no foreign body reaction
Antisdel[8]	Intact sinonasal mucosa	Rabbit	10	-	No foreign material or foreign body reaction
Humphreys[13]	Laparoscopic trocar injury to the spleen	Porcine	3	165.3-200.7 seconds	-
Humphrey[19]	Laparoscopic renal injuries	Porcine	4	100.2-196.2 seconds	No foreign body reaction
Ersoy[44]	Severe femoral artery bleeding	Rat	6	30, 60, 90 seconds	MPHs and compression significantly decreased the time of hemostasis
Biondo-Simoes[7]	Heaptic injuries	Rat	10	6 minutes	-
Murat[20]	Laparoscopic partial nephrectomy	Porcine	6	2 minutes (range of 1-3)	Provides effective parenchymal hemostasis
Murat[6]	Open partial nephrectomy	Porcine	12	2.67-4.67 minutes	No complications, no evidence of residual foreign material

ADVANTAGES OF USING MPHs

MPHs have been used for the first time in experimental studies in renal surgery,[6,43] cardiovascular surgery,[10,44] spleen and liver surgery,[13,19] but also in brain surgery[16] (Table 3). Authors notice that MPHs do not inhibit bone healing[15] and degrade faster than a gelatin matrix,[17] Surgicel, Avitene and a gelatin-thrombin matrix hemostatic sealant (FloSeal),[16] that unlike the gelatin matrix, they have a lower infection rate,[17] as well as a lower inflammation rate compared with other topical hemostatic agents.[18] Last but not least, no foreign body reactions have been recorded.[16]

In clinical studies, MPHs had excellent results in proving rapid and effective hemostasis in dermatologic surgery,[5,12] laparoscopic surgery[13] and endoscopic nasal sinus surgery[8,45] (Tables 1 and 2).

One of the major strengths of MPHs is that they are not derived from animals or humans, and therefore, the risk of a hypersensitivity reaction or infectious disease transmission is avoided.[34] Due to their natural composition, MPHs have the advantage of being hypoallergenic,[5] non-mutagenic, non-toxic, non-irritating, non-immunogenic and non-hemolytic.[46] Moreover, MPHs do not require any prior heating or mixing when used.[9]

In addition to this, the recent studies of Bruckner *et al.*[47] emphasize intraoperative specimens from mediastinum blood clots in order to understand better the mechanism of action of MPHs, while the microscopy of the samples showed that the MPHs interact in order to concentrate blood at the site of application, including clotting factors, in accordance with the initial findings on the mechanisms of action of MPHs. Bruckner also highlighted the efficiency of MPH hemostatic powder in reducing hemostatic time during surgeries.

Recently, in 2018, Reynbakh *et al.*[46] used MPH hemostatic powder for electrophysiology device implantation in a study led on 283 subjects. According to their conclusions, MPHs not only decreased the bleeding rate and hematoma events, but also reduced post-procedural complications of device implantation, the site hematoma rate and the infection rate. Similar findings have been identified in other intraoperative uses of MPHs, performed in different surgical specialties, including neurosurgery.[34,35,42] Also, the authors observed that by applying MPH hemostatic powder, the bleeding areas can be much more easily localized, while the time of hemostasis generation is reduced, especially for patients on dual and triple anticoagulation[46] (Fig. 2).

Furthermore, no expansion or significant swelling of MPHs has been detected, as it seems that there is rapid enzymatic absorption in the cerebral parenchyma.[34] The use of MPHs in neurosurgery has the advantage of reducing the thermal side effects of bipolar coagulation to healthy brain parenchyma, a very important aspect in tumoral resections from eloquent areas.

In what the contraindications of MPHs are concerned, the only known possible clinical contraindication is the history of allergic reactions to potato starch,[6,44] although this occurrence has not been identified in any patient.[48,49]

Figure 2: Intraoperative application of MPH hemostatic powder in “Prof. Dr. N. Oblu” Emergency Clinical Hospital, Iasi

APPLICABILITY OF MPHs IN BRAIN SURGERY

Bleeding after brain tumor resection occurs in 0.8%-1.5% cases: out of all these, 60% are intracerebral, 30% extracerebral and 10% subdural.[50] Besides, the risk of bleeding in malignant brain tumors reaches 4%.[51,52]

In brain tumor surgery, conventional hemostasis is mostly generated by electric tissue coagulation (bipolar coagulation). Even so, potential diffuse bleeding can prove difficult to manage. Although bipolar coagulation offers control over bleeding, it is time-consuming and can lead to a wide enlargement of the working channel, along with the involved disadvantages.[53,54] Also, hemostatic agents can be difficult to apply on the wall of the operative cave, while the MPH powder presents the advantage of being easily applied deep into hemorrhagic wounds by using a plastic device applicator.[35,55]

Although MPHs are used on a large scale, together with several topical hemostatic agents, they can simulate tumor relapse or infection in postoperative MRI and can produce allergic reactions, determine the formation of granuloma and increase the infection rate.[34]

Using MPHs in neurosurgery helps in obtaining efficient and rapid control over superficial brain bleeding, reduces the use of bipolar coagulation and the surgical time.[34,35] At the same time, diffuse capillary bleeding may be problematic at the end of the tumoral resection, but Galarza *et al.*,[35] in their studies on five cerebral convexity meningiomas and five cerebral gliomas, found that this complication can be easily solved by using MPHs. They also highlighted the efficiency of MPHs in cases of arteriolar bleeding at the cortex (their use facilitates control over the hemostasis), without implying the use of bipolar coagulation.

As for the inflammatory reaction to MPHs in the brain, Ereth *et al.*[16] identified the same reaction as that to the other commonly used neurosurgical hemostatic agents, but with a shorter median time to degradation, compared to other hemostatic materials. Despite of this, an experimental study led on 12 rabbit brains emphasized a slightly higher inflammation in the MPH group than the one involving oxidized regenerated cellulose, even though there was no significant difference between the two hemostatic agents in what the pericellular edema, bleeding or neuronal degeneration are concerned.[56]

On the one hand, the MPH enhanced clot is enzymatically broken into small water-soluble fragments,[40] and it does not permit radiographic evidence of deployment within 12 hours from application.[6,13,34] On the other hand, common hemostatic agents, such as microfibrillar collagen, oxidized cellulose, gelatin matrix thrombin sealants and gelatin sponge, are characterized by a longer degrading time and their presence has been demonstrated on computed tomography up to 7 months after the surgery, mimicking tumor recurrence.[15,16,57]

One of the alleged risks of using MPHs in brain surgery is the aggravation of perilesional brain edema preexistent in brain tumors, subsequent to MPH power concentration, but Galarza *et al.*[35] did not identify any evidence of this on control head computed tomography scan performed after 10 brain tumor surgeries.

Another advantage of using MPHs is the absence of any interaction with arachnoidian villi. Tschan *et al.*,[34] who carried out the first study of MPH application to human brain tissue, pointed out, in a study led on 33 subjects, that 8 of them (25%) had their ventricles opened during the tumoral resection, which caused MPHs to come into contact with the cerebrospinal fluid. The authors did not take notice of the development of postoperative hydrocephalus and, as such, suggested that this hemostatic agent could be further used at skull base or within the cerebrospinal fluid.

NEW DIRECTIONS IN RECOMBINATION OF MPHs

Hemostatic materials may be divided into active and passive hemostatic agents, or agents that are a combination of the two types.[58]

Passive agents (bovine, porcine or equine collagen, gelatin, oxidized cellulose) act by absorption of the excess fluid from the blood, and therefore they concentrate endogenous coagulation factors at the bleeding site. Practically, this material offers a matrix for formation of a clot. Some passive hemostatic agents have platelet-activating properties that improve hemostasis.

Active agents are exogenous coagulation factors (thrombin, bovine or equine fibrinogen) that interact with the patient's coagulation system and accelerate fibrin formation,

creating a strong hemostatic clot. These agents do not provide a matrix that protects the newly created clot from fragmentation.[32]

Hemostatic agents combining active and passive agents improve the hemostatic capability, but increase the risk of secondary effects.[59]

MPHs are passive agents that do not increase platelet activation nor coagulation,[32] therefore the combination of MPHs with active agents, such as thrombin or fibrinogen, would be an efficient way to increase clotting formation and durability.[21] Starting from this premise, Björses *et al.*[32] were the first to propose the chemical modification of MPHs, achieving this by diethylaminoethyl chloride, chloroacetic acid, N-octenylsuccinic anhydride, ellagic acid and acetic anhydride, with increased capacity of activating platelets. Unfortunately, the results were obtained *in vitro* and chemically modified MPHs could not be used in clinical applications due to toxicity and poor degradability (Table 4).

The increase of the hemostatic effect was also reported by Alam *et al.*,[37] when MPHs were combined with a recombinant factor VIIa, fibrinogen or thrombin,[10] or more recently combined with mesoporous zinc-calcium silicate.[60]

Chen *et al.*[21] experimented on rabbits the use of a hemostatic agent, calcium-modified microporous starch prepared by oxidization and self-assembly with Ca^{2+}. It proved its efficiency in bleeding control due to the acceleration of Ca^{2+} for blood clotting. The authors showed that this hemostatic agent activates intrinsically the coagulation cascade pathway, induces platelet adherence and promotes water absorption due to the large surface and the porous structure of starch (Table 4).

Table 4
Experimental studies of chemical MPHs changes

Author	Chemical modifications of MPHs	Evaluated	Conclusion	Disadvantage
Chen[61]	Cationic modified starch microspheres (CS)	Hemostatic performance	Induced the adhesion of red blood cell and platelet (activated the blood chemical coagulation system due to positive charge, improved the degradation of CS	-
Chen[21]	Calcium-modified microporous starch	Hemostasis efficiency, degradation behavior	Improved hemostatic performance and degradability	-
Björses[32]	N-Octenylsuccinic anhydride, chloroacetic acid, acetic anhydride diethylaminoethyl chloride and ellagic acid	Thrombin generation, platelet adhesion	Superior in haemostatic capacity	Toxic modifications, poor degradability

A few years later, in 2017, Chen *et al.*[61] showed that cationic modified starch microspheres (CSs) have an excellent porous structure and due to their electro-positivity, they can aggregate red blood cells and platelets. CS was initially developed *via* enzymatic hydrolysis and assembled with quaternary ammonium groups by etherification reaction with microporous starch. By its synergetic effects with the mechanism of hemostasis, it proved its efficiency in both *in vitro* and *in vivo* studies on rabbits. *In vitro*, CS induced the adhesion of red blood cells and platelets, activating in this way the blood coagulation system due to its positive charge, and *in vivo*, in rabbit liver injuries, improved the hemostatic capacity a lot (Table 4), while other authors optimized the drug release from chitosan-starch crosslinked beads using response surface methodology.[62]

In future research seeking to find a better hemostatic agent, hemostasis disorders in patients with anticoagulant therapy, hematological patients with primary

hemostasis impairment, as well as patients with chronic alcohol consumption with associated diseases should be also taken into account.[63-65]

Even if in neurosurgery the ideal hemostatic agent has not been found yet, a good hemostasis must be obtained and this can be a challenge even to expert neurosurgeons. Thus, medicine faces various legal and ethical issues,[66] in which the decision to treat the patient is established by mutual agreement between the patient and the neurosurgeon, undoubtedly for the benefit of the patient, respecting his personal values.[67]

CONCLUSION

In neurosurgery, hemostasis is critical and the ideal topical hemostatic agent is not yet available. The hemostatic agents currently found on the market have the disadvantages of deficient hemostasis, non-degradability, high costs and potential safety issues. Based on literature data, the primary benefits of using MPHs include reduction of the time to achieve hemostasis, reduction of blood loss, improvement of the operatory technique with advantages for the surgeon and for the patient. MPHs may be considered an important element in the management of topical arterial bleeding, having significant advantages compared to other hemostatic agents.

REFERENCES

[1] M. Gabay and B. A. Boucher, *Pharmacotherapy*, **33**, 935 (2013).
[2] K. M. Lewis, H. Atlee, A. Mannone, L. Lin and A. Goppelt, *J. Surg. Res.*, **193**, 825 (2015).
[3] P. de Robles, K. M. Fiest, A. D. Frolkid, T. Pringsheim, C. Atta *et al.*, *Neuro. Oncol.*, **17**, 776 (2015).
[4] G. M. Nitulescu, G. Iancu, G. Nitulescu, R. C. Iancu, C. Bogdanici *et al.*, *Rev. Chim.*, **68**, 754 (2017).
[5] S. R. Tan and W. D. Tope, *Dermatol. Surg.*, **30**, 908 (2004).
[6] F. J. Murat, M. H. Ereth, Y. Dong, M. P. Piedra and M. T. Gettman, *J. Urol.*, **172**, 1119 (2004).
[7] M. L. P. Biondo-Simões, R. Petrauskas, A. G. Dobrowolski, G. Godoy, F. Kaiber *et al.*, *Acta Cir. Bras.*, **22**, 29 (2007).
[8] J. L. Antisdel, C. G. Janney, J. P. Long and R. Sindwani, *Laryngoscope*, **118**, 1265 (2008).
[9] J. L. Antisdel, J. L. West-Denning and R. Sindwani, *Otolaryngol. Head. Neck Surg.*, **141**, 353 (2009).
[10] K. Björses and J. Holst, *Eur. J. Vasc. Endovasc. Surg.*, **33**, 363 (2007).
[11] J. M. Buchowski, K. H. Bridwell, L. G. Lenke and C. R. Good, *Spine* (*Phila. Pa. 1976*), **34**, 473 (2009).
[12] J. Ho and G. Hruza, *Dermatol. Surg.*, **33**, 1430 (2007).
[13] M. R. Humphreys, E. P. Castle, P. E. Andrews, M. T. Gettman and M. H. Ereth. *Am. J. Surg.*, **195**, 99 (2008).
[14] E. Benlier, H. Top, A. C. Aygit, U. Usta and Y. Unal, *J. Reconstr. Microsurg.*, **23**, 311 (2007).
[15] M. Ereth, J. Sibonga, W. Oliver, G. Nuttall, J. Henderson *et al.*, *Orthopedics*, **31**, 222 (2008).
[16] M. H. Ereth, M. Schaff, E. F. Ericson, N. M. Wetjen, G. A. Nuttall *et al.*, *Neurosurgery*, **63**, 369 (2008).
[17] M. H. Ereth, Y. Dong, L. M. Schrader, N. A. Henderson, S. Agarwall *et al.*, *Surg. Infect.* (*Larchmt*), **10**, 273 (2009).
[18] N. E. Hoffmann, S. A. Siddiqui, S. Agarwal, S. H. McKellar, H. J. Kurtz *et al.*, *J. Surg. Res.*, **155**, 77 (2009).
[19] M. R. Humphreys, J. E. Lingeman, C. Terry, E. P. Castle, P. E. Andrews *et al.*, *J. Endourol.*, **22**, 1375 (2008).
[20] F. J. Murat, C. Q. Le, M. H. Ereth, M. P. Piedra, Y. Dong *et al.*, *J.S.L.S.*, **10**, 302 (2006).
[21] F. Chen, X. Cao, X. Chen, J. Wei and C. Liu, *J. Mater. Chem. B.*, **3**, 4017 (2015).
[22] S. P. Plaami, *Food Rev. Int.*, **13**, 29, (1997).
[23] J. H. Xie, M. L. Jin, G. A. Morris, X. Q. Zha, H. Q. Chen *et al.*, *Crit. Rev. Food. Sci. Nutr.*, **56**, S60 (2016).
[24] R. A. Dwek, *Chem. Rev.*, **96**, 683 (1996).
[25] H. M. M. Hassan, *J. Appl. Sci. Res.*, **6**, 89 (2010).
[26] C. W. Cho, C. J. Han, Y. K. Rhee, Y. C. Lee, K. S. Shin *et al.*, *Int. J. Biol. Macromol.*, **72**, 519 (2015).
[27] L. J. Li, M. Y. Li, Y. T. Li, J. J. Feng, F. Q. Hao *et al.*, *Marine Drugs*, **10**, 2648 (2012).
[28] N. Li, L. Li, J. C. Fang, J. H. Wong, T. B. Ng *et al.*, *Biosci. Rep.*, **32**, 221 (2012).
[29] J. H. Xie, M. Y. Xie, S. P. Nie, M. Y. Shen, Y. X. Wang *et al.*, *Food Chem.*, **119**, 1626 (2010).
[30] M. Huang, F. Q. Wang, X. H. Zhou, H. X, Yang and Y. Z. Wang, *Carbohyd. Polym.*, **117**, 91 (2015).
[31] L. Yang and L. M. Zhang, *Carbohyd. Polym.*, **76**, 349 (2009).
[32] K. Björses, L. Faxälv, C. Montan, K. Wildt-Persson, P. Fyhr *et al.*, *Acta Biomater.*, **7**, 255 (2011).
[33] https://www.accessdata.fda.gov/cdrh_docs/pdf5/P050038c.pdf (2006).
[34] C. A. Tschan, M. Nie, E. Schwandt and J. Oertel, *Neurosurgery*, **69**, 49 (2011).
[35] M. Galarza, O. P. Porcar, R. Gazzeri and J. F. Martínez-Lage, *World Neurosurg.*, **75**, 491 (2011).
[36] M. C. Neuffer, J. McDivitt, D. Rose, K. King, C. C. Cloonan *et al.*, *Mil. Med.*, **169**, 716 (2004).

[37] H. B. Alam, G. B. Uy, D. Miller, E. Koustova, T. Hancock *et al*., *J. Trauma*, **54**, 1077 (2003).
[38] K. G. Mann, C. M. Heldebrant and D. N. Fass, *J. Biol. Chem*., **246**, 6106 (1971).
[39] J. J. Gorman, P. A. Castaldi and D. C. Shaw, *Biochim. Biophys. Acta*, **439**, 1 (1976).
[40] G. Hamdi and G. Ponchel, *Pharm. Res*., **16**, 867 (1999).
[41] P. Rajagopal and N. Hakim, *Transplant. Proc*., **43**, 424 (2011).
[42] R. Nunez-Nateras, K. J. Hurd, E. N. Ferrigni, E. P. Castle, P. E. Andrews *et al*., *World J. Urol*., **31**, 523 (2013).
[43] K. Björses and J. Holst, *J. Trauma*., **66**, 602 (2009).
[44] G. Ersoy, M. F. Kaynak, O. Yilmaz, U. Rodoplu, F. Maltepe *et al*., *Adv. Ther*., **24**, 485 (2007).
[45] R. Sindwani, *Otolaryngol. Head. Neck. Surg*., **140**, 262 (2009).
[46] P. Reynbakh, P. Akhrass, N. Souvaliotis, C. K. Pamidimukala, H. Nahar *et al*., *Curr. Med. Res. Opin*., **19**, 1 (2018).
[47] B. A. Bruckner, L. N. Blau, L. Rodriguez, E. E. Suarez, U. Q. Ngo *et al*., *J. Cardiothorac. Surg*., **9**, 134 (2014).
[48] I. Wedmore, J. G. McManus, A. E. Pusateri and J. B. Holcomb, *J. Trauma*, **60**, 655 (2006).
[49] A. E. Pusateri, J. B. Holcomb, B. S. Kheirabadi, H. B. Alam, C. E. Wade *et al*., *J. Trauma*, **60**, 674 (2006).
[50] D. Moskopp and H. D. Wassmann (eds.), "Neurochirurgie: Handbuch Für Die Weiterbildung und interdisziplinäres Nachschlagewerk", Stuttgart, Germany, Schattauer- Verlag, 2004.
[51] S. M. Chang, I. F. Parney, M. McDermott, F. G. Barker, M. H. Schmidt *et al*., *J. Neurosurg*., **98**, 1175 (2003).
[52] G. Henke, T. Kucinski, M. Simon and U. Herrlinger, *Onkologe*, **12**, 546 (2006).
[53] I. H. Kalfas and J. R. Little, *Neurosurgery*, **23**, 343 (1988).
[54] J. D. Palmer, O. C. Sparrow and F. Iannotti, *Neurosurgery*, **35**, 1061 (1994).
[55] R. Gazzeri, M. G. Galaraza, C. Fiore, G. Callovini and A. Alfieri, *Neurosurgery*, **11**, 32 (2015).
[56] H. Emmez, M. Tonge, N. Tokoz, E. Durdag, I. Gonul *et al*., *Turk. Neursurg*., **20**, 485 (2010).
[57] T. Ribalta, I. E. McCutcheon, A. G. Neto, D. Gupta, A. J. Kumar *et al*., *Arch. Pathol. Lab. Med*., **128**, 749 (2004).
[58] S. Samudrala, *A.O.R.N. J*., **88**, S2 (2008).
[59] C. Lomax and O. Traub, *Pharmacotherapy*, **29**, 8S (2009).
[60] Y. Hou, Y. Xia, Y. Pan, S. Tang, X. Sun *et al*., *Mater. Sci. Eng. C*, **76**, 340 (2017).
[61] F. Chen, X. Cao, J. Yu, H. Su, S. Wei *et al.*, *Colloids. Surf. B*, **159**, 937 (2017).
[62] K. Kumari, B. Singh and V. Singh, *Cellulose Chem. Technol*., **50**, 925 (2016).
[63] S. B. Dimmitt, V. Rakic, I. B. Puddey, R. Baker, R. Oostryck *et al*., *Blood. Coagul. Fibrinol*., **9**, 39 (1998).
[64] A. B. Ciubara, R. C. Tudor, L. Nechita, O. Tita, A. Ciubara *et al*., *Rev. Chim*. (*Bucharest*), **69**, 1247 (2018).
[65] J. Koscielny, G. F. von Tempelhoff, S. Ziemer, H. Radtke, M. Schmutzler *et al*., *Clin. Appl. Thromb. Hemost*., **10**, 155 (2004).
[66] P. Craciun, V. Astarastoae, S. Turliuc and M. C. Vicol, *Revista de Cercetare si Interventie Sociala*, **42**, 310 (2013).
[67] P. Craciun, M. C. Vicol, S. Turliuc and V. Astarastoae, *Romanian Journal of Bioethics*, **10**, 92 (2012).