Cerebrospinal fluid composition after duraplasty with different substitutes in early and late postoperative periods (an experimental study)

Volodymyr O. Pyatikop¹, Anna V. Kravtsova¹, Yuliya G. Sergiyenko¹, Liliya P. Abramova²

¹Neurosurgery Department, Kharkiv National Medical University, Kharkiv, Ukraine
²Central Research Laboratory, Kharkiv National Medical University, Kharkiv, Ukraine

Received: 04 October 2019
Accepted: 12 November 2019

Address for correspondence:
Anna V. Kravtsova, Neurosurgery Department, Kharkiv National Medical University, 4, Nauki av., Kharkiv, 61022, Ukraine, e-mail: anna.kravtsova.20@gmail.com

Objective. To study cerebrospinal fluid (CSF) changes after the duraplasty with autologous fascia, collagen-based material and chitin-chitosan membrane in early and late postoperative periods.

Materials and methods. Chitosan-based films were made out of 3% solution of chitosan for the research. We used 200 kDa chitosan (deacetylation rate 80–90%) to produce chitin-chitosan membrane by using solvent evaporation method. For enhancing mechanical properties and reducing the degradation, chitin particles were added to the chitosan solution. Chitosan and chitin ratio was 80/20. The chitin/chitosan solution in Petri dishes was being dried out during 3 days at room temperature.

Cerebrospinal fluid composition has been studied after the duraplasty with autofascia, collagen-based material and innovative chitosan-based graft in early and late postoperative periods. The duraplasty was performed by applying these materials to 90 Chinchilla rabbits breed. Animals were divided into three groups: I group — duraplasty using the fascia lata autograft, II group — duraplasty with the collagen-based material, III group — duraplasty using the chitin-chitosan membrane. The animals in the II and III groups were divided into 2 subgroups: A — plasty without fixing the material, B — plasty with fixing the material using atraumatic suture. CSF composition was studied before and after the operation had been performed in 2 weeks, 2 and 6 months.

Results. The results of our study demonstrated the increase in density and protein level, the decrease in pH and glucose level and the extreme increase of cells, mostly neutrophils after the use of fascia lata for dural closure. At the same time, there were no substantial changes after dural closure with artificial collagen- and chitosan-based materials, the CSF composition normalized in 2 months after operation.

Conclusion. The use of autologous fascia for duraplasty leads to an acute response of the cerebrospinal fluid in the early postoperative period and to residual pleocytosis. The chitosan-based graft application was followed by applying these materials to 90 Chinchilla rabbits breed. Animals were divided into three groups: I group — duraplasty using the fascia lata autograft, II group — duraplasty with the collagen-based material, III group — duraplasty using the chitin-chitosan membrane. The animals in the II and III groups were divided into 2 subgroups: A — plasty without fixing the material, B — plasty with fixing the material using atraumatic suture. CSF composition was studied before and after the operation had been performed in 2 weeks, 2 and 6 months.

Keywords: cerebrospinal fluid; dura mater; duraplasty; implant; substitute; chitosan
Состав спинномозговой жидкости после пластики твердой мозговой оболочки разными заменителями в ранний и поздний послеоперационный период (экспериментальное исследование)

Пятикоп В.А.1, Кравцова А.В.1, Сергienenко Ю.Г.1, Абрамова Л.П.2

1 Кафедра нейрохирургии, Харьковский национальный медицинский университет, Харьков, Украина
2 Центральная научно-исследовательская лаборатория, Харьковский национальный медицинский университет, Харьков, Украина

Поступила в редакцию 04.10.2019
Принята к публикации 12.11.2019

Адрес для переписки: Кравцова Анна Витальевна, кафедра нейрохирургии, Харківський національний медичний університет, пр. Науки, 4, Харків, 61022, Україна, e-mail: anna.kravtsova.20@gmail.com

Цель: изучить изменения спинномозговой жидкости после пластики твердой мозговой оболочки (ТМО) аутологичной фасцией, материалом на основе коллагена и хитин-хитозановой мембраны в ранний и поздний послеоперационный период.

Материалы и методы. Хитозановые пленки для исследования были изготовлены из 3% раствора хитозана. Использовали хитозан 200 кДа (степень деацетилирования – 80-90%) для получения мембран методом испарения растворителя. Для усиления механических свойств и уменьшения времени деградации к раствору хитозана добавляли хитиновые частицы. Соотношение хитозана и хитина составило 80:20. Хитозан-хитозановый раствор в чашках Петри высушивали в течение 3 сут при комнатной температуре.

Изучали состав спинномозговой жидкости после пластики аутофасцией, материалом на основе коллагена и хитин-хитозановой мембраны в ранний и поздний послеоперационный период. В исследовании использованы 90 кроликов породы шиншила, которым проводили пластику ТМО указанными материалами. Животных разделили на три группы: группа I – пластика с использованием fascia lata, группа II – пластика материалом на основе коллагена, группа III – пластика хитин-хитозановой пленкой. Группы II и III поделили на две подгруппы: A – пластика без фиксации материала, B – пластика с фиксацией материала. Состав спинномозговой жидкости изучали до операции и через 2 нед, 2 и 6 мес.

Результаты. После использования аутофасции для пластики дефекта ТМО отмечено увеличение удельного веса спинномозговой жидкости и содержания белка, уменьшение рН и уровня глюкозы, выраженный плейоцитоз с преобладанием лейкоцитов. Применение коллагенсодержащей мембраны и хитозановой пленки не вызывает существенных изменений спинномозговой жидкости, которая возвращается к норме через 2 мес после пластики.
Introduction
The need for substitution of lost native dural tissue arises in the presence of its traumatic or neoplastic destruction, after operative procedures, when the dura mater is involved [1–3]. The applying of patient’s fascia lata is the classic method. This technique has its advantages due to the absence of an immune response to the native material, ease of execution and lack of the financial burden on the patient or clinic. But both the probability of postoperative complications and an additional trauma limit the use of this technique [4, 5]. To date, numerous medical products have been developed for the dura mater replacement based on natural (collagen) and synthetic materials.

An ideal dural substitute should not induce neurotoxicity, should be biocompatible, should have no allergenic and antigenic properties and have a relatively low cost [6, 7]. Most of the available materials do not fully meet all requirements, thus this encourages the development of new implants for duraplasty. Biocompatible implants can be developed on the base of natural polysaccharide chitosan and its derivatives [8].

Chitosan is biodegradable, non-toxic and biocompatible material with antibacterial properties and the ability to stimulate regeneration, does not induce an immunological response [9, 10]. A reasonable cost of the material is also one of the advantages. Recent studies have shown the benefits of chitosan-based materials for skin plastic and the treatment of burns and trophic ulcers, for the hemostatic purpose [11]. Unfortunately, there is a few of information concerning the use of chitosan-based materials as a dural substitute.

Objective: To study cerebrospinal fluid (CSF) changes after the duraplasty with autologous fascia, collagen-based material and chitin-chitosan membrane in early and late postoperative periods.

Materials and methods
Graft preparation
Chitosan-based films were made out of a 3% solution of chitosan (molar masses 200 kDa, deacetylation rate 80–90%). Firstly, 10 ml of 3% solution of chitosan in 1% acetic acid was poured onto a round teflon support (with a diameter of 8 cm) until the height of the solution layer reached 5 mm. Then the solvent was evaporated at room temperature for 48–72 h. The obtained film was treated with 5% NaOH for 2 h, washed frequently with distilled water and then incubated in a 10% aqueous solution of glycerine for 30 min in order to enhance elasticity and softness. Chitin particles (1–2 mm) were added to the chitosan solution to enhance mechanical properties and reduce the degradation rate of the film. Chitosan and chitin ratio was 80/20. The chitin particles were dispersed by stirring within the volume of a viscose solution to form a homogeneous solution. The solution of chitin/chitosan was placed into Petri dishes and dried for 3 days at room temperature [12, 13]. After the drying, materials had a visually smooth lower surface and a rough upper surface (Fig. 1A).

For the duraplasty, we used a membrane made from 200 kDa molecular weight chitosan because of the results of the previous experiments. They have shown antibacterial properties and significant better cell viability [14].

Design of the experiment and surgery
We used 90 Chinchilla breed rabbits for the experiments, which were divided into three groups:

I group (18 animals) — duraplasty using the fascia lata autograft.
II group (36 animals) — duraplasty using the collagen-based material.
III group (36 animals) — duraplasty using the chitin-chitosan membrane.

The animals in the II and III groups were divided into 2 subgroups:
A — plasty without fixing the material,
B — plasty with fixing the material using atraumatic suture.

The duraplasty procedure was as follows: after general anaesthesia (ketamine 10 mg/kg) and peripheral vein catheterization, the animals’ heads were shaved from the superciliary arch to the ear butts. The surgical site was treated with C-4 solution and then a T-shaped incision was made: the transverse incision was made in the frontal plane between the ear butts; the vertical incision was made from midline directing almost to the bridge of the nose and it was perpendicular to the transverse incision. The triangular skin flaps were retracted. The transverse incision was made at a distance...
0.5 cm from the point of attachment to the bone in order to expose the temporal muscle. At the place to be trepanned, the periosteum was separated in a lateral direction with a bone rasp. The hole was drilled using a trepan with a sharp point of 0.5 cm diameter. Using the Luer and Liston forceps, a trepanation opening was created and the sharp edges were scraped. If it was necessary, bleeding was stopped in the diploe. A cross-shaped incision was created in the dura mater. The fascia lata was placed in the dura mater and was sown up with atraumatic suture material [12].

Atraumatic suture material was also used for the fixing in the subgroup B of the II and III groups to compare the effectiveness of fixing of the membranes. Suture material was not used for the subgroup A of the II and III groups because of good adhesion properties of the collagen-based and chitosan-based implants. The implant larger than the defect was placed between the undamaged dura mater and internal bone surface (Fig. 1B).

Than myorrhaphy was conducted without closure of the bone defect; the wound was closed with simple interrupted sutures and we applied an aseptic dressing.

The follow-up of the experiment was 2 weeks, 2 and 6 months after the operation.

Cerebrospinal fluid research
Cerebrospinal fluid was collected at all experimental time-points. General anaesthesia (ketamine 10 mg/kg) and peripheral vein catheterization were provided before manipulation. Using standard 10ml syringe, 2 ml of cerebrospinal fluid was taken through the dura mater puncture 5–7 mm back from the edge of the implant avoiding brain tissue trauma. We studied the following indicators: clarity, density (using the method of Gauzner S.I. et al.), pH by pH-meter (Δ=±0.05) “CyberScan pH/Ion 510” (Germany), protein level by biuret test and glucose level by glucose oxidase method, using reagent kits “Lachema” (the Czech Republic), the cellular components.

P-value test was used to assess the difference between groups using GraphPad Prism 8.0 software (free-access). Before statistical analysis, all data were assessed for normal distribution using GraphPad Prism 8.0 software. Statistical significance was assumed at a confidence level of 95% (p<0.05).

The study was carried out in accordance with the principles of bioethics, regulated by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986), Directive 2010/63/EU “On the Protection of Animals Used for Scientific Purposes” (2010), the Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelties” (2006) and approved by the Ethics and Bioethics Commission of Kharkiv National Medical University (Minutes No. 1 of 01/02/2012).

Results and discussion
Cerebrospinal fluid of the animals before the operation was crystal clear. The density was 1.004–1.007, pH — 7.3±0.3. The protein and glucose level was 0.27±0.06 g/l and 4.2 ± 0.18 mmol/l, respectively. Cells were rare, near 2 x 10^5/l. Small lymphocytes, which are the body immunologically competent cells, prevailed. Acute inflammation cells (neutrophils) and erythrocytes were not found in CSF of the animals.

CSF became less clear after 2 weeks after the duraplasty with fascia lata, density increased up to 1.014 (p =0.034), which could indicate both bacterial inflammation and reaction to the foreign body. Significant decrease in the cerebrospinal fluid pH to 6.6 ± 0.4 (p=0.021) was the confirmation of the inflammation hypothesis. The presence of a pathological inflammatory process in the subarachnoid space was also confirmed by an increase in protein level to 0.41 ± 0.04 g/l (p=0.04) and a decrease in glucose concentration to 3.47 ± 0.13 mmol/l (p=0.02).

Sediment microscopy revealed an increase in the absolute number of cells up to 280 x 10^6/l (p=0.001), which is characterized as a pleocytosis and is observed in inflammatory processes. Microscopy did not reveal bacterial cells, but the percentage of neutrophils was 65% of the total number of cells, which indirectly indicated the presence of bacterial inflammation. Lymphocytes (23%), monocytes (7%) and erythrocytes (5%) were found in the cerebrospinal fluid in addition to neutrophils in the animals’ CSF. The presence of the erythrocytes may be associated with vessels injury during plastic and implant suturing. Inflammatory response of the cerebrospinal fluid decreased in 2
months after the duraplasty, which is characterized by the normalization of the density up to 1.009 (p = 0.064). However, this indicator still did not reach the level of healthy animals. Instead, the cerebrospinal fluid pH was 7.2 ± 0.5 (p = 0.12), reaching preoperative levels. Protein level decreased unreliably from the previous term — up to 0.38 g/l (p = 0.046) while the glucose level normalized up to 4.12 mmol/l (p = 0.12). The cerebrospinal fluid cell composition was almost unchanged from the previous observation period: the percentage of neutrophils was 57%, lymphocytes — 31%, monocytes — 12%. Instead, microscopic examination revealed no erythrocytes in the samples. The number of cell elements was significantly reduced compared to the previous term — up to 100 x 10^6/l (p = 0.001), despite the constancy of cell composition, that corresponded to medium pleocytosis. There was complete normalization of the animals’ cerebrospinal fluid in 6 months after the duraplasty with the use of autologous fascia (Table 1). Density, protein and glucose decreased to the level of healthy animals. The number of cell elements remained a little bit increased — 14 x 10^6/l (p = 0.01), the most part of them were small lymphocytes — 95% and only 5% were neutrophils.

The dural closure with collagen-based graft resulted in less evident changes in CSF after 2 weeks compared with animals that used a fascia lata. However, we did not find a significant difference between the suture and non-suture subgroups. CSF density increased with no significant difference with the control group, fluid pH became significantly reduced up to 6.8 ± 0.3 (p = 0.038) in subgroup IIB. The use of collagen may lead to the tissue immune response stimulation, increasing protein level might indicate the presence of an inflammatory process. However, the glucose level did not change and did not exceed 4.1 ± 0.09 mmol/l (p = 0.17). Moderate pleocytosis with a significant increase in the number of cells up to 150 x 10^6/l (p = 0.001) in subgroup IIB with lymphocytes and single erythrocytes prevailed in that group of the animals. The number of the cells was higher in the group of the animals with the substitute fixation by suture. The absence of neutrophils indicated the absence of an inflammatory process of infectious genesis. The presence of a large number of lymphocytes along with increasing protein level might indicate the presence of an immune response to the foreign protein, but the degree of its severity was moderate.

After 2 and 6 months, there was complete normalization of all main indicators of CSF (Table 2), except for the number of cells in the sediment. The pleocytosis became 45 x 10^6/l (p = 0.018) in the subgroup without implant suture and 60 x 10^6/l (p = 0.001) in the subgroup with suture in the next research term. Cell composition normalized to the 2nd month of follow-up. Small and medium lymphocytes were the prevailing elements in the sediment as in the previous observation period.

The use of a chitin-chitosan composite as an implant did not lead to significant changes in the density and pH of the cerebrospinal fluid, indicating the relative inertness of the material. The protein level in cerebrospinal fluid increased unreliably in the animal subgroup without the use of suture material and accounted for 0.3 ± 0.05 g/l (p = 0.033). There was a slight but significant protein level increase up to 0.37 ± 0.07 g/l (p = 0.01) while using the implant suture fixation, which might be a consequence of the reaction to the suture material or additional dura injury. Glucose level was somewhat reduced over the preoperative period, which might be a manifestation of the general animal body response to surgery. Pleocytosis corresponded to the previous animal groups level and

| Parameters | Before operation | 2 weeks | 2 months | 6 months |
|------------|------------------|---------|----------|---------|
| Density    | 1.005            | 1.014*  | 1.009*   | 1.006   |
| pH         | 7.3 ± 0.3        | 6.6 ± 0.4* | 7.2 ± 0.5 | 7.4 ± 0.3 |
| Protein (g/l) | 0.27 ± 0.06 | 0.41 ± 0.04* | 0.38 ± 0.03* | 0.29 ± 0.06 |
| Glucose (mmol/l) | 4.20 ± 0.18 | 3.47 ± 0.13* | 4.12 ± 0.18 | 4.25 ± 0.21 |
| Cell number/l | 2 x 10^5 | 280 x 10^5** | 100 x 10^5** | 14 x 10^5** |

* P ≤ 0.05

**Table 2.** Cerebrospinal fluid parameters before and after duraplasty with collagen-based material in different periods

| Parameters | Before operation | Subgroup IIA | Subgroup IIB | Subgroup IIA | Subgroup IIB | Subgroup IIA | Subgroup IIB |
|------------|------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Density    | 1.005            | 1.07         | 1.006        | 1.003        | 1.004        | 1.005        | 1.007        |
| pH         | 7.3 ± 0.3        | 6.9 ± 0.3*   | 6.8 ± 0.5*   | 7.1 ± 0.2    | 7.2 ± 0.1    | 7.2 ± 0.5    | 7.3 ± 0.3    |
| Protein (g/l) | 7.3 ± 0.3 | 6.9 ± 0.3*   | 6.8 ± 0.5*   | 7.1 ± 0.2    | 7.2 ± 0.1    | 7.2 ± 0.5    | 7.3 ± 0.3    |
| Glucose (mmol/l) | 4.20 ± 0.18 | 4.10 ± 0.09  | 4.00 ± 0.12  | 4.30 ± 0.15  | 4.40 ± 0.08  | 4.10 ± 0.19  | 4.00 ± 0.23  |
| Cell number/l | 2 x 10^6 | 110 x 10^6** | 150 x 10^6** | 45 x 10^6** | 60 x 10^6**  | 3 x 10^6    | 3 x 10^6    |

* P ≤ 0.05

http://theunj.org
was 95 x 10^6/l (p =0.001) for the subgroup without suture fixation and 135 x 10^6/l (p =0.001) with suture, but the main difference was the absence of erythrocytes in CSF in subgroup IIIA. There was no any significant difference with the previous group in pH, protein and glucose level as well as in cell number. Small lymphocytes are the main cellular elements of the sediment, which indicates a weak immune response to the implant. The absence of erythrocytes in the subgroup without suture may be associated with its atraumatic adhesion fixation and the absence of injury of the dura vessels. All the CSF parameters came to the preoperative level after 2 months, including the cell number of the sediment, which was 4 x 10^6/l (p =0.09). This parameter is an important indicator of the safety of chitin-chitosan implant, unlike collagen-based graft, when after its using moderate pleocytosis persists.

The CSF parameters were not significantly different from the control after 6 months after duraplasty (Table 3).

### Discussion

An ideal dural graft should be biocompatible, should not be allergenic and neurotoxic, have to stimulate regeneration in addition to the basic function of good watertight closure. Besides, control of the inflammatory process and the presence of antibacterial properties are desirable [15]. In clinical practice auto- and xenografts and materials based on synthetic and natural derivatives are used today. The use of auto- and xenografts is limited due to a number of complications that occur both at the material receiving stage and after operations. Artificial implants, especially of biological origin, are being used more actively now. Numerous studies report about a number of complications even after using of these materials [1, 4, 5].

Researches studying the effectiveness of chitosan-based materials for duraplasty are scarce. Furthermore, chitosan has proven efficiency in the skin and bone plastic defects that give prerequisites of its using for duraplasty grafts [16, 17]. Unfortunately, pure chitosan-based materials are poorly resistant to biodegradation and are destroyed during the physical intervention [18, 19]. Therefore, it is advisable to use its complex compounds, particularly with chitin. Our study has shown that the chitin-chitosan membrane is completely biocompatible, does not cause allergy and implant rejection. The same results have been confirmed by numerous in vitro and in vivo researches [11, 20]. Chitosan has anti-inflammatory and antibacterial properties that significantly reduces inflammatory manifestations in the cerebrospinal fluid analysis and reduces the number of bacterial cells. Normalization of cerebrospinal fluid tests has been seen within 2 months after surgery, as opposed to the groups using fascia lata and collagen-based material. There is no information about the clinical or experimental application of chitosan-based graft but the results of our experiment coincide with others with an application of collagen-based experimental grafts [21].

### Conclusions

1. The classical technique of using autofascia for duraplasty leads to an acute response of the cerebrospinal fluid in the early postoperative period and to residual pleocytosis.

2. The chitosan-based material application was followed by only slight CSF response in the early postoperative period and no complications at 6 months after surgery in our study. The study found no significant difference between the innovative chitosan-based material and the commercial collagen-based one.

3. The study didn’t show any differences between suture fixation and adhesion of the duraplasty substitute except for a slight increase in erythrocyte number in CSF in the early postoperative period. Thus, the choice of material fixation method is entirely dependent on the clinical situation and does not affect the cerebrospinal fluid state.

### Disclosure

**Conflict of interest**

The authors declare no conflict of interest.

**Ethical approval**

All procedures performed on experimental animals comply with the regulations and ethics and approved by the ethics committee of the scientific institution on which the study was conducted.

**Funding**

The research had no sponsor support.

### References

1. Barrientos S, Leif M, Hon HH, Aizenberg M, Wong S. Duraplasty Using Autologous Fascia Lata and Latissimus Dorsi Free Flap for Chronic Cerebrospinal Fluid Leak. J Craniofac Surg. 2019 Oct;30(7):e671-e674. doi: 10.1097/SCS.0000000000005747. PMID: 31574789.
2. McCabe C, Warren R. Trauma: An annotated bibliography of the literature - 2001. Am J Emerg Med. 2002 Jul;20(4):352-66. doi: 10.1016/j.ajem.2002.03.019. PMID: 12098186.
3. Pyatikop VA, Moroz IS, Tarasenko VI, Babalyan YuA, Bibichenko SI, Gunko BV, Genkin AV, Teslenko DS, Masalitin IN. [Cranioplasty of bone defects with differentiated usage of implants]. Ukrainian Neurosurgical Journal. 2011;3:22-25. Russian. doi: 10.25305/unj.57791.
4. Lin J, German M, Wong B. Use of copolymer polyactic and polyglycolic acid resorbable plates in repair of orbital floor fractures. Facial Plast Surg. 2014 Oct;30(5):581-6. doi: 10.1055/s-0034-1393640. PMID: 25397715.
5. Zang G, Yang W, Jiang Y, Zeng T. Extensive duraplasty with autologous graft in decompressive craniectomy and subsequent early cranioplasty for severe head trauma. Chin J Traumatol. 2010 Oct;13(5):259-64. PMID: 20804049.
6. Guo S, He L, Yang R, Chen B, Xie X, Banat F, Sahabudeen S, Ramachandran S, Devanand Venkatasubbu G. Fabrication of Chitosan/PVA/GO/CuO patch for potential wound healing application. Int J Biol Macromol. 2019 Oct 14. pii: S0141-8130(19)35397-8. doi: 10.1016/j.ijbiomac.2019.10.029. PMID: 31622704.
7. Gazzeri R, Neroni M, Alfieri A, Galarza M, Faiola A, Esposito S, Giordano M. Transparent equine collagen biomatrix as dural repair. A prospective clinical study. Acta Neurochir (Wien). 2009 May;151(5):537-43. doi: 10.1007/s00701-009-0290-9. PMID: 19337680.