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Structural and biological evaluation of new chitosan membrane for dural closure

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Objective: to study the structural and biological evaluation of chitin-chitosan based membrane for dura mater replacement.

Materials and methods. Chitosan-based films were made out of 3% solution of chitosan for the research. We used 200, 500 and 700 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 dried out during 3 days at room temperature.

To obtain information about the structure of membrane surface and cross-section scanning, electron microscopy was performed.

Hydrolytic degradation was studied by pouring into SBF solution. To determine the rate of enzymatic degradation, trypsin solution was used. To determine the mass loss percentage, we measured the sample weight after 7, 14, 21, 30 and 60 days after being in the appropriate solutions.

Relative elongation and strength were measured by digital dynamometer to study membranes mechanical properties such as the strength and elasticity. MLO-A5 cells were used to assess biocompatibility of new materials.

Results. Macroscopic view of obtained samples has shown their relative transparency with impregnation of chitin particle that elevated over the membrane surface without any diversity between different chitosan molecular weight samples.

Due to scanning electron microscopy, principal diversity between the samples of different molecular weight has being seen: rough pore surface at 200 and 500 kDa and flat with minimal roughness surface of 700 kDa membranes. Cross-section of 500 and 700 kDa membranes are dense with no pores, but 200 kDa membrane are sponge like and it can be prediction for fluid sorption and cell migration during healing process.

Chitin-chitosan membranes are biocompatible and degrade in aqueous and enzymatic solutions. Due to polysaccharide nature of chitosan and chitin, enzymatic degradation has shown higher trend compare to the hydrolytic ones. 200 kDa membrane degrades faster with final mass loss 83.2 % and completely due to porous structure that allows fluid sorption.

Membrane mechanical parameters strongly depend on their structure. 200 kDa membrane has shown 2-fold higher elongation compared to 500 kDa and 3-fold — compared to 700 kDa ones. The compensation of mechanical forces ensured by porous structure is better than in dense ones. Tensile strength was in 2-fold better in 200 kDa membranes than in 500 and 700 kDa ones.

Cell culture experiment has shown the better adhesion at the 3rd day for 200 kDa membrane and minimal cell adhesion for 700 kDa membrane, probably due to smooth surface. The reduction rate between all samples and PCT control differ a lot, except for 200 kDa membrane that has the same proliferation rate as TCP.

Conclusion. Chitin-chitosan membranes, made from different molecular weight chitosan, are transparent and has appropriate structure for being used as a dura mater substitute. They are biocompatible and degrade in aqueous and enzymatic solutions. Due to porous structure, excellent mechanical properties as well as better cell adhesion and proliferation, 200 kDa chitosan membrane is more applicable for neurosurgical issues.

Key words: *dura mater substitute; chitin; chitosan; degradation; mechanical properties; cell culture*

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Вивчення структурних та біологічних властивостей нової хітозанової плівки для закриття твердої мозкової оболонки

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Мета: вивчити структурні та біологічні властивості плівки на основі хітину і хітозану для заміщення твердої мозкової оболонки.

Матеріали і методи. Для дослідження були виготовлені методом розчинення та випаровування хітозанові плівки з 3% розчину хітозану. Використано хітозан з молекулярною масою 200, 500 та 700 кДа (рівень деацетилювання – 80–90%). Для посилення механічних властивостей та зменшення деградації в хітозановий розчин додавали частинки хітину. Співвідношення хітозану та хітину – 80:20. Висихання хітин-хітозанового розчину в чашці Петрі відбувалося за кімнатної температури впродовж 3 діб. Для отримання даних щодо структури поверхні мембрани та поперечного перерізу використовували растрову електронну мікроскопію. Гідролітичну деградацію вивчали додаванням розчину SBF. Для дослідження рівня ензиматичної деградації використовували розчин трипсину. Для визначення відсотка втрати маси вимірювали вагу зразків на 7, 14, 21, 30 та 60-ту добу після перебування в відповідному розчині. Відносне подовження і міцність вимірювали цифровим динамометром для вивчення механічних властивостей, таких як міцність і еластичність. Для оцінювання біосумісності нових матеріалів використовували клітинну лінію MLO-A5.

Результати. Макроскопічний вигляд отриманих зразків свідчив про їх відносну прозорість з просоченням частинками хітину, які здіймалися над поверхнею мембрани без будь-яких відмінностей між зразками з різною молекулярною масою хітозану.

Завдяки растровій електронній мікроскопії встановлено принципову різницю між зразками з різною молекулярною масою: груба пориста поверхня при використанні мембрани з хітозану з молекулярною масою 200 та 500 кДа і гладенька мінімально шорстка при застосуванні мембрани з хітозану з молекулярною масою 700 кДа.

На поперечному перерізі плівка з хітозану з молекулярною масою 500 і 700 кДа щільна без пор, тоді як мембрана з хітозану з молекулярною масою 200 кДа – губчаста, що може бути передумовою для сорбції рідини і міграції клітин під час процесу загоєння. Хітин-хітозанові плівки є біосумісними та деградують у водних і ферментативних розчинах. Завдяки полісахаридній природі хітозану та хітину спостерігали тенденцію до швидшої ферментативної деградації порівняно з гідролітичною. Мембрана з хітозану з молекулярною масою 200 кДа швидше деградує з кінцевою втратою маси 83,2%, що зумовлено пористою структурою, яка сприяє сорбції рідини.

Механічні параметри мембран значною мірою залежать від їх структури. Плівка з хітозану з молекулярною масою 200 кДа продемонструвала вдвічі більше подовження порівняно з мембраною з хітозану з молекулярною масою 500 кДа і тричі більше порівняно з плівкою з хітозану з молекулярною масою 700 кДа. Пориста структура краще, ніж щільна, забезпечує посилення механічних властивостей. Міцність на розрив була вдвічі кращою в мембрани з хітозану з молекулярною масою 200 кДа, ніж у плівок з хітозану з молекулярною масою 500 і 700 кДа.

Експеримент на культурі клітин показав кращу адгезію на 3-тю добу у плівки з хітозану з молекулярною масою 200 кДа та мінімальну адгезію клітин у мембрани з хітозану з молекулярною масою 700 кДа, ймовірно, через гладеньку поверхню. Швидкість рівня проліферації в усіх зразків порівняно з контролем ТСР дуже відрізнялася, за винятком мембрани з хітозану з молекулярною масою 200 кДа, яка мала таку саму швидкість проліферації, як і ТСР.

Висновки. Хітин-хітозанові мембрани, виготовлені з хітозану з різною молекулярною масою, є прозорими і мають відповідну структуру для використання як замінник твердої мозкової оболонки. Вони є біосумісними і деградують у водних та ферментативних розчинах. Завдяки пористій структурі, відмінним механічним властивостям, а також кращій клітинній адгезії та проліферації плівка з хітозану з молекулярною масою 200 кДа більш придатна для нейрохірургічних втручань.

Ключові слова: *імплантат твердої мозкової оболонки; хітин; хітозан; деградація; механічні властивост;, культура клітин.*

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Изучение структурных и биологических свойств новой хитозановой пленки для закрытия твердой мозговой оболочки

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Цель: изучить структурные и биологические свойства пленки на основе хитина и хитозана для замещения твердой мозговой оболочки.

Материалы и методы. Для исследования были изготовлены методом растворения и выпаривания хитозановые пленки из 3 % раствора хитозана. Использовали хитозан с молекулярной массой 200, 500 и 700 кДа (степень деацетилирования – 80–90%). Для усиления механических свойств и уменьшения деградации в хитозановый раствор добавляли частички хитина. Соотношение хитозана и хитина – 80:20. Высыхание хитин-хитозанового раствора в чашке Петри происходило при комнатной температуре в течение 3 суток. Для получения информации о структуре поверхности мембраны и поперечного сечения использовали растровую электронную микроскопию. Гидролитическую деградацию изучали путем добавления раствора SBF. Для исследования уровня энзиматической деградации использовали раствор трипсина. Для определения процента потери массы измеряли вес образцов на 7, 14, 21, 30 и 60-е сутки после нахождения в соответствующем растворе. Относительное удлинение и прочность измеряли цифровым динамометром для изучения механических свойств, таких как прочность и эластичность. Для оценки биосовместимости новых материалов использовали клеточную линию MLO-A5.

Результаты. Макроскопический вид полученных образцов свидетельствовал об их относительной прозрачности с пропиткой частицами хитина, которые возвышались над поверхностью мембраны без каких-либо отличий между образцами с разной молекулярной массой хитозана.

Благодаря растровой электронной микроскопии установлена принципиальная разница между образцами с разной молекулярной массой: грубая пористая поверхность при использовании мембраны из хитозана с молекулярной массой 200 и 500 кДа и гладкая минимально шершавая при применении мембраны из хитозана с молекулярной массой 700 кДа. В поперечном сечении пленка из хитозана с молекулярной массой 500 и 700 кДа плотная без пор, а мембрана из хитозана с молекулярной массой 200 кДа – губчатой, что может быть предпосылкой для сорбции жидкости и миграции клеток в процессе заживления. Хитин-хитозановые пленки биосовместимы и деградируют в водных и ферментативных растворах. Благодаря полисахаридной природе хитозана и хитина наблюдали тенденцию к более быстрой ферментативной деградации по сравнению с гидролитической. Мембрана из хитозана с молекулярной массой 200 кДа быстрее деградирует с конечной потерей массы 83,2%, что обусловлено пористой структурой, которая способствует сорбции воды.

Механические параметры мембранв значительной степени зависят от их структуры. Пленка из хитозана с молекулярной массой 200 кДа продемонстрировала в 2 раза большее удлинение по сравнению с мембраной из хитозана с молекулярной массой 500 кДа и в 3 раза большее по сравнению с пленкой из хитозана с молекулярной массой 700 кДа. Пористая структура лучше, чем плотная, обеспечивает усиление механических свойств. Прочность на разрыв была в два раза лучше у мембраны из хитозана с молекулярной массой 200 кДа, чем у пленок из хитозана с молекулярной массой 500 и 700 кДа.

Эксперимент на культуре клеток показал лучшую адгезию на 3-и сутки у пленки из хитозана с молекулярной массой 200 кДа и минимальную адгезию клеток у мембраны из хитозана с молекулярной массой 700 кДа, вероятно, из-за гладкой поверхности. Скорость уровня пролиферации у всех образцов по сравнению с контролем ТСР очень отличалась, за исключением мембраны из хитозана с молекулярной массой 200 кДа, которая имеет такую же скорость пролиферации, как и ТСР.

Выводы. Хитин-хитозановые мембраны, изготовленные из хитозана с разной молекулярной массой, являются прозрачными и имеют соответствующую структуру для использования в качестве заменителя твердой мозговой оболочки. Они биосовместимы и деградируют в водных и ферментативных растворах. Благодаря пористой структуре, отличным механическим свойствам, а также лучшей клеточной адгезии и пролиферации пленка из хитозана с молекулярной массой 200 кДа более пригодна для нейрохирургических вмешательств.

Ключевые слова: *имплантат твердой мозговой оболочки; хитин; хитозан; деградация; механические свойства; культура клеток*

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Introduction

Cranial surgery in many cases, for example the removal of meningiomas, require the following reconstruction of the dura mater [1]. The dura has to be meticulously closed following craniotomy [2] almost always.

But primary dural closure with sutures alone can be difficult in a loss of native dural tissue, when dura mater edges have shrunken and cannot be sutured directly and sufficiently watertight.

The usage of dural grafts is a common neurosurgical practice when primary closure is not possible. An ideal graft wouldn't show inflammation in the host body, neurotoxicity and adhesion to the underlying brain. At the same time, it should be affordable and inexpensive, as well as durable, flexible, and easily prepared and shaped. Ideally, at the same time it should be rapidly resorbed, allowing the endogenous connective tissue to build up. Additionally, while providing adequate protection for the underlying brain, it should ensure watertight closure [3].

A common classification of dural substitutes includes: autografts (fascia lata, temporalis fascia), allografts (amniotic and placental membranes, pericardium, fascia, lyophilized dura), xenografts (bovine or porcine pericardium, peritoneum, dermis) and synthetic materials (polytetrafluoroethylene, polyester urethane). However, each material poses drawbacks that limit their usage and require suturing to the endogenous dura to obtain a watertight closure [4].

Autograft is a tissue that is taken from one part of a person's body and transplanted to a different part of the same person. There are several autografts, the most important of which are temporalis fascia, pericranium, autologous fat and fascia lata. Autografts do not transmit diseases and do not give immuno-mediated responses in the host, but dimensions and qualities of tissues used for transplant are insufficient, especially when there is a considerable loss or retraction of dura mater [5]. Furthermore, autograft requires an additional incision, resulting in an increase of surgical time with a consequent increase of anesthesic time, and a graft hypoxia potentially causes an inflammatory response of underlying cortex [6].

The most severe complications associated with autologous fat transplant are early fat necrosis and liquefaction [7], fat dissemination in the subarachnoid space [8] and subsequent lipoid meningitis [9].

Allograft is a transplant from one person to another, but not an identical twin. In past years, neurosurgeons have used several allogenic tissues for duraplasty (amniotic and placental membranes, pericardium, cadaveric lyophilized dura mater), many of which are now rejected [10]. Cadaveric lyophilized dura mater is a fragile tissue and creates adherences with surrounding tissues and underlying brain; it can give immunomediated inflammatory reactions and transmit Creutzfeldt-Jakob disease. In fact, it was hypothesized that prions could survive at any type of sterilization; however, it is important to remember that prions can be found in nervous system and have never been shown in dura mater. Moreover, reoperation has demonstrated atrophy of the allograft, even in well-performed surgery, and the transmission of Creutzfeldt-Jakob disease raised doubts about a possible viral transmission [11].

Bovine or porcine pericardium and other xenografts have been used as dural substitutes for many years [12]. Equine Achilles tendon, bovine or porcine pericardium are the surgical graft or tissue from one species to an unlike species. Bovine or porcine pericardium is an example of processed whole tissues. It is strong, pliable and easy to handle, economically advantageous, but requires watertight suturing. Bovine pericardium is well tolerated, with a low incidence of postoperative complications (2 %) [13]; however, among the most common complication with this type of graft, there are the onset of foreign body reactions, aseptic meningitis and the transmission of Creutzfeldt-Jakob disease.

Synthetic grafts are also widely used in neurosurgery [14]. In recent years, several materials have been introduced in surgical practice (polytetrafluoroethylene, polyester urethane). However, these materials present a lot of drawbacks that place them in the background. Despite their theoretical uniform thickness and no risk of infection transmission, polytetrafluoroethylene and other synthetic grafts have often a rigid structure, resulting difficult to handle, are not often able to be replaced by dura mater and are burdened by numerous inflammatory and foreign body reactions. These reactions can create an inflammation of surrounding tissues and underlying brain, an excessive fibrin production with graft encapsulation, cerebrospinal fluid bleeding, meningitis, graft rejection, scarring, infections, delayed bleeding, for which a reoperation is often required [15, 16]. Moreover, in watertight closure, holes created by suturing graft to dura mater could cause a cerebrospinal fluid leakage. The strength of synthetic absorbable grafts is only guaranteed for the first 2 weeks: these materials are often brittle and they tend to give cerebrospinal fluid leaks, without preserving the guest from serious inflammatory reactions [17]. Other drawbacks of polytetrafluoroethylene are represented by its strong surface tension and its lack of adaptability, frequent appearance of friction injury with underlying brain and meninges, which may cause bleeding and inflammation [6].

Resorbable natural-based polymeric implants seem to be best able to replicate the physiological composition of dura mater and to guarantee a natural scaffold for integration and replacement of the entire graft with normal dura mater. Collagen is most promising polymer for dura mater substitute [18].

Collagen has many properties; because it is a resorbable protein, its degradation can be controlled by crosslinking. Collagen is hemostatic and able to induce cellular growth with a final tissue reconstruction. The major problem of these types of substitutes is the risk of an immunological or severe inflammatory reaction: the collagen should be highly purified, not pyrogenic, and without telopeptides. Hence, various chemical or physical procedures have been used to inactivate viruses, bacteria, and prions. To date, many collagen-based dural substitutes have been synthesized and tested in experimental *in vitro* and *in vivo* studies, and data on the integration with normal tissue are available [19, 20].

There is a large number of these dural substitutes and the growing interest in finding "ideal" dural substitutes that can mimic the physiological structure of human dura mater and avoid complications. Chitosan is one of the promising polymer for tissue replacement, including dura mater.

Chitosan, an amino polysaccharide (poly-1, 4-D-glucoamine), is known as a biological material in promoting the healing process of soft and hard connective tissues. It is biocompatible, biodegradable, bioactive, non-toxic, non-expensive and non-immunogenic, with antibacterial capability [21]. Some researchers have shown possible

This article contains some figures that are displayed in color online but in black and white in the print edition

application of chitosan for duraplasty, but currently they focus on bilayer structures that are made of not-porous low degradable layer and high porous chitosan substrate [25]. The physical characteristics of this membrane allows dural closure without cerebrospinal fluid leak. But difference in degradation affects histological structure of newly formed tissues.

Objective

The aim of this research was the structural and biological evaluation of chitin-chitosan based membrane for dura mater replacement.

Materials and methods

Chitin-chitosan membrane preparation

Chitosan-based films were made out of a 3% solution of chitosan (molar mass 200, 500 and 700 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 over 48–72 hours. The obtained film was treated with 5% NaOH for 2 hours, washed frequently with distilled water and then incubated in 10% aqueous solution of glycerine for 30 min in order to enhance elasticity and softness. Chitin particles (1–2 mm) were added to chitosan solution to enhance mechanical properties and reduce the degradation rate of the film. The ratio of chitosan and chitin 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.

Scanning electron microscopy

Scanning electron microscopy was performed using the electron microscope REMMA102 (SELMI, Ukraine) to obtain information about structure of membrane surface and cross-section. To avoid surface charge accumulation in the electron-probe, experimental membranes were covered with the thin (30–50 nm) layer of silver in the vacuum set-up VUP-5M (SELMI, Ukraine).

Degradation study

To study the rate of degradation, the samples were dried to a constant weight in a thermostat at a temperature of 37° C. Samples weighing 100 mg were placed in a Petri dish and poured into SBF (simulated body fluid) solution for the study of hydrolytic degradation and 1% trypsin solution to determine the rate of enzymatic degradation. To determine the mass loss percentage, we measured the sample weight after 7, 14, 21, 30 and 60 days after being in the appropriate solutions. Solution was changed every 3 days to remove degradation products. The percentage of weight loss was calculated by the formula:

$$C = 100\% - \left(\frac{M1 - M2}{M1}\right) * 100\%,$$

where C is the percentage of weight loss, M1 is the initial mass of the sample, M2 is the mass of the sample after being in the solution.

Mechanical properties

For mechanical study, one side of membrane was fixed to laboratory table and other to digital dynamometer WH-A Series Portable Electronic Scale. The sample fixed on the laboratory table stretched with constant effort until the break along with fixed force of tension. The results were presented as: *Li* — initial length, *Lf* — finite length, F — force at break and *Si* — membrane area at the beginning of the experiment. To estimate the strength and elasticity of the samples, the calculation of relative elongation, %, (1) and strength, g/mm^2, (2) were measured:

$$\Delta L = (LfLi * 100) - 100 \quad (1)$$

$$St = \frac{F}{Si} \quad (2)$$

Cell culture

All reagents were obtained from Sigma-Aldrich (UK) unless otherwise stated. Prior to cell culture, dura matter substitutes (200, 500 and 700 kDa) were cut using a cork borer into circular disks with a diameter of 1.2 cm and sterilized in 100% ethanol for 1 hour at room temperature. All grafts were washed in phosphate-buffered saline (PBS) and placed in 12-well plates. Grafts were placed with either the smooth or rough surface facing upwards. Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of fetal calf serum, 1% L-glutamine, 1% penicillin and streptomycin and 0.25% fungizone, was added to each graft and incubated at 37° C in a humidified environment with 5% CO_2. After 24 hours, stainless steel rings were placed on the scaffolds to hold them in place and MLO-A5 cells (passage 34) were seeded at 1.0 × 10^5 cells per scaffold. MLO-A5 cells were selected due to their frequent usage in cell toxicity experiments. After 24 hours, the rings were removed and 1 ml of supplemented DMEM was added to each scaffold. Scaffolds were incubated at 37° C with 5% CO_2, and media was changed every 3 days during a 14-day culture period. Resazurin sodium salt assay was used to access cell viability and proliferation rate on day 3, 7 and 14 after cell seeding. Media was removed from each well and washed with PBS twice. Resazurin sodium salt solution of 1 ml was added to each scaffold and incubated for 4 hours. Three aliquots of 200 µl of resazurin sodium salt solution was collected (***Fig. 1***) from each scaffold and read at a wavelength of 620 nm in a colorimetric plate reader (Multiskan FC Microplate Photometer, Thermo Fisher Scientific) to obtain baseline values of colorimetric absorbance.

Statistic methods

Data were expressed as means ± standard deviation. Student's t-test on unpaired data was used to assess the statistical significance of the difference between the results obtained from the tested specimens. Statistical significance was assumed at a confidence level of 95 % ($p<0.05$).

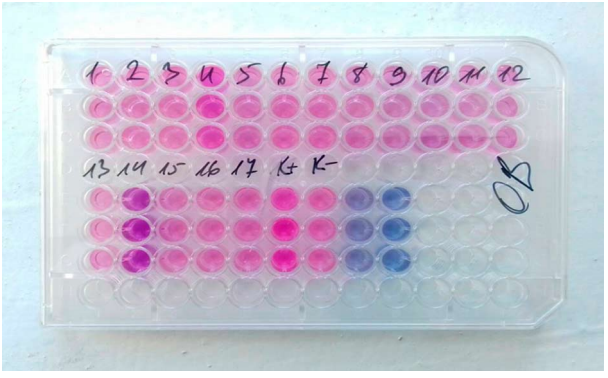

Fig. 1. Image of 96-well plate with aliquots of 200 µl of resazurin sodium salt solution collected from each scaffold: 1–17 — samples; K+ — a well with cells cultured on tissue culture plastic (TCP); K- — a well with media without cells; A — resazurin sodium salt solution

Results and discussion

Macroscopic view of obtained samples has shown their relative transparency with impregnation of chitin particle that elevated over the membrane surface (***Fig. 2***). We did not observe any visual difference between samples made from chitosan with different molecular weight. Chitin particle uniform distributed over the membrane surface and did not make any congestions. Transparent membranes are capable for surgical procedure due to clean observation of injured tissues under the materials.

Scanning electron microscopy showed principal difference between membranes made from chitosan with different molecular weights. 200 and 500 kDa chitin-chitosan membranes have rough surface with pore in different size (***Fig. 3***). Surface of 700 kDa membrane

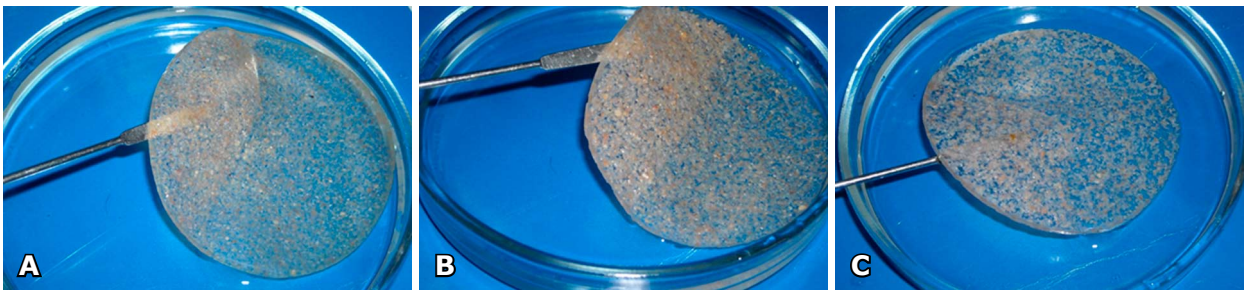

Fig. 2. General view of chitin-chitosan membrane after synthesis in Petri dish: A — made from chitosan 200 kDa, B — 500 kDa and C — 700 kDa

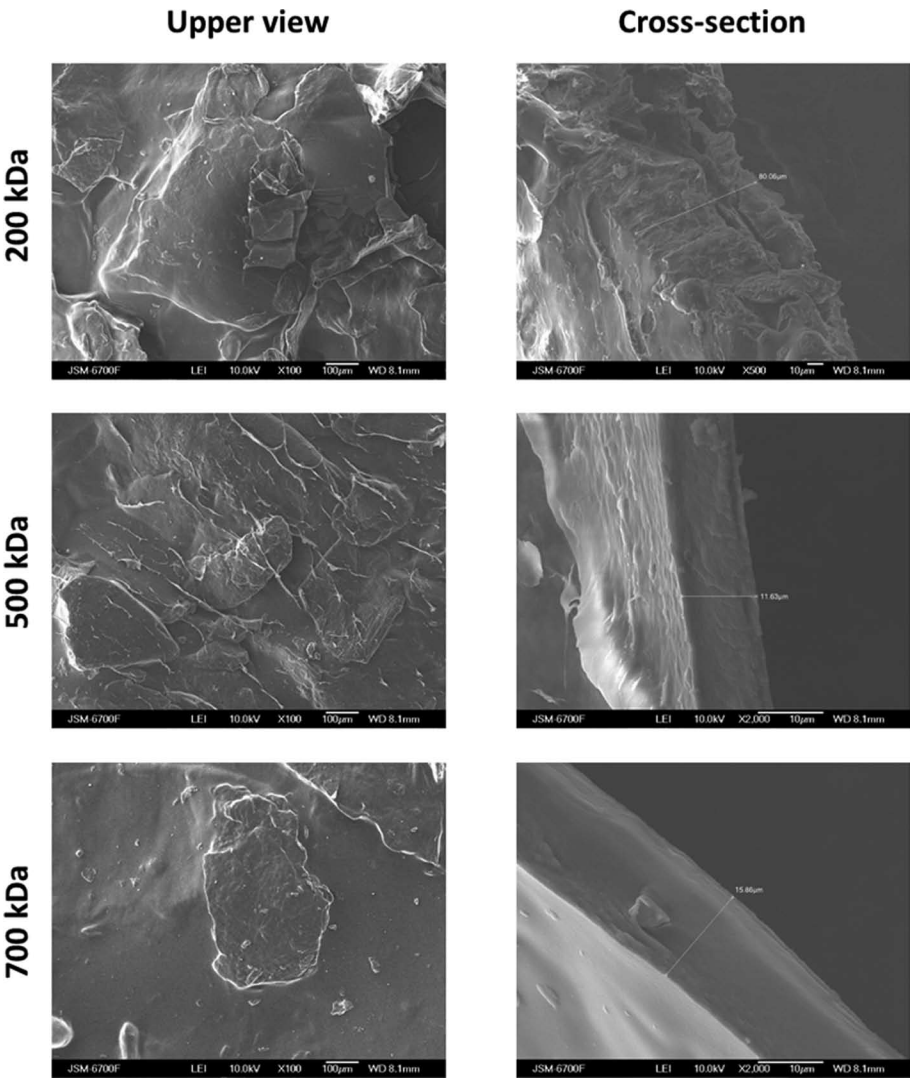

Fig. 3. Scanning electron microscopy of chitin-chitosan membranes made from chitosan with different molecular weights. Left column — upper view, right column — cross section

is flat with minimal roughness. Some researchers have shown that rough surface has greater potential for cell adhesion that is principal for biocompatibility [22]. Just after material interacts with body fluids, protein absorbs onto the surface and plays a role of vesical substrate for cell adhesion. Flat surface is less attractive for proteins. At the same case, rough surface is more attractive for bacteria adhesion compared to the smooth ones [23], but chitosan has antibacterial properties and can protect from bacterial colonization [24].

Cross-section of 500 and 700 kDa membranes are dense and do not have any pores. At the same case, 200 kDa membranes are sponge-like in cross section and it can be prediction for fluid sorption and cell migration during healing process.

Degradation in SBF solution has shown low weight loss during 7 and 14 days and no significant difference between membranes. In day 21, 200 kDa lost 18.9 % from initial weight compared to 500 and 700 kDa materials that had 11.3% and 8.4% mass loss. Next 2 months, membranes degraded faster and in last time point the loss was 45.7 %, 35.7 % and 32.1 % from initial weight (***Fig. 4A***). Due to polysaccharide nature of chitosan and chitin, enzymatic degradation (***Fig. 4B***) has shown higher trend compared to the hydrolytic ones. But 200 kDa membrane degrades faster with final mass loss of 83.2 % and completely due to porous structure that allows fluid sorption.

Mechanical parameters of membranes strongly depended on their structure. Elongation of 200 kDa membrane was 2-fold higher compared to 500 kDa and 3-fold compared to 700 kDa ones (***Fig.5***). Porous structure allows compensate mechanical forces much better compared to the dense ones. This feature can allow surface modeling during the operation in dura mater defect. We also did not reveal significant difference in tensile strength between 500 and 700 kDa membranes, but this indicator was 2-fold better in 200 kDa ones.

Cell culture experiment has shown the better adhesion to 200 kDa membrane on the 3rd day (***Fig. 6***). Resazurin reduction rate is similar to positive TCP control. 700 kDa membrane has shown minimal cell adhesion, probably due to smooth surface. Cells proliferate in all surfaces on the 7th and 14th day after seeding, but 500 and 700 kDa membranes show less cell viability at all-time points. We can see significant difference in reduction rate between all samples and PCT control, except for 200 kDa membrane that has the same proliferation rate as TCP.

Conclusion

Chitin-chitosan membranes made from chitosan of different molecular weight are transparent and have appropriate structure for being used as a dura mater substitute. They are biocompatible and degrade in aqueous and enzymatic solutions. 200 kDa chitin-chitosan membranes are more applicable

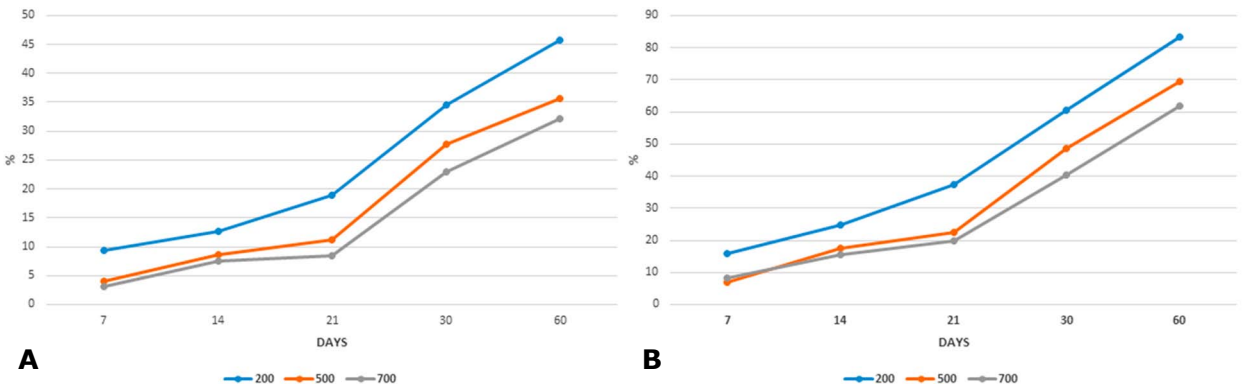

Fig. 4. Degradation rate of chitin-chitosan membranes in SFB (A) and trypsin (B) solutions

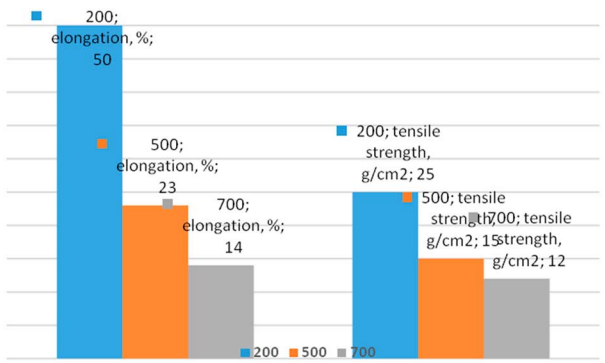

Fig. 5. Mechanical parameters of chitin-chitosan membranes made from chitosan with different molecular weights

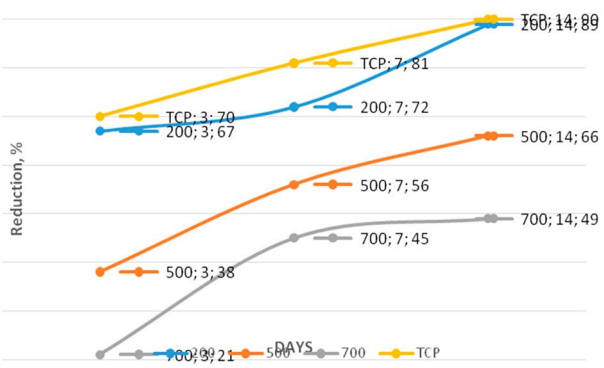

Fig. 6. Cell viability of MLO-A5 cells on chitin-chitosan materials made of chitosan of molecular weights (200, 500 or 700 kDa)

for neurosurgical issues due to porous structure and excellent mechanical properties as well as better cell adhesion and proliferation.

Disclosure

Conflict of interest

The authors declare no conflict of interest.

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