J Mater Sci: Mater Med (2017)28:34
DOI 10.1007/s10856-017-5845-3

CLINICAL APPLICATIONS OF BIOMATERIALS

Original Research

Experimental evaluation of new chitin–chitosan graft for duraplasty

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Received: 11 August 2016 / Accepted: 4 January 2017

Abstract Natural materials such as collagen and alginate have promising applications as dural graft substitutes. These materials are able to restore the dural defect and create optimal conditions for the development of connective tissue at the site of injury. A promising material for biomedical applications is chitosan—a linear polysaccharide obtained by the deacetylation of chitin. It has been found to be nontoxic, biodegradable, biofunctional and biocompatible in addition to having antimicrobial characteristics. In this study we designed new chitin–chitosan substitutes for dura mater closure and evaluated their effectiveness and safety. Chitosan films were produced from 3 % of chitosan (molar mass—200, 500 or 700 kDa, deacetylation rate 80–90%) with addition of 20% of chitin. Antimicrobial effictively and cell viability were analysed for the different molar masses of chitosan. The film containing chitosan of molar mass 200 kDa, had the best antimicrobial and biological activity and was successfully used for experimental duraplasty in an in vivo model. In conclusion the chitin–chitosan membrane designed here met the requirements for a dura matter graft exhibiting the ability to support cell growth, inhibit microbial growth and biodegradade at an appropriate rate. Therefore this is a promising material for clinical duroplasty.

1 Introduction

After cerebral or spinal operative procedures, it is imperative to provide a complete and watertight dural closure to minimize the risks of cerebrospinal fluid fistulas, infections, brain herniation, cortical scarring, and adhesions [1, 2]. Dural closure is necessary in cases of substitution of lost native dural tissue (i.e., in neoplastic or traumatic destruction); repair of dural fistulas; enlargement of the dural compartment (i.e., in Arnold-Chiari malformation or inoperable intramedullary tumors); and in dura graft surgery (i.e., myelomeningocele) [3, 4].

The history of duraplasty began in 1890 when Beach suggested the use of gold foil to prevent meningocerebral adhesions [5]. Currently, several materials are used experimentally and clinically as dural substitutes, [6, 7] but these all have limitations [2, 3]. Autografts, such as pericranium or temporal fascia are easy to handle, nontoxic, inexpensive, and have a favorable biological behavior [8]. However, autograft use is not possible when defects are of a large size [9]. The use of autologous fascia needs an additional operation, which increases operation time and possible complications at the donor site [2]. Homologous dura mater is also widely used but current sterilization methods do not guarantee it free from risk of latent virus and prion infections [10].

Some synthetic materials (for example: silastic and expanded polytetrafluoroethylene) can be used for dural closure but they can lead to unfavourable tissue reactions,

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Published online: 21 January 2017

excessive scar formation, meningitic symptoms or hemorrhage risk [6].

Natural materials such as collagen or alginate have promising results as dural graft substitutes. These materials are able to restore the dural defect and create optimal conditions for the development of connective tissue at the site of injury [11, 12]. Alternatively, synthetic substrates such as polyethylene glycol, and poly(lactic-co-glycolic acid) are easily modifiable, can serve as a scaffold for matrix molecules and cellular implants and are immunologically inert and resorbable [13, 14].

The design requirements for a material used for dural closure are that it should not induce an immunological or inflammatory response, not induce neurotoxicity, should be resorbable for endogenous neodura to grow, should provide a watertight closure, should keep its shape after application, and should not rupture. An ideal dural substitute will not create a risk of transmission of viral and prion infections and be of reasonable cost for adoption by national healthcare systems.

A new promising material for biomedical applications is chitosan—a linear polysaccharide obtained by the deacetylation of chitin, which is a structural biopolymer present in the exoskeletons of crustaceans and mollusks, as well as the cell wall of fungi [15]. Chitin is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be nontoxic, biodegradable, biofunctional and biocompatible, in addition to having antimicrobial characteristics [16, 17]. Previous studies have shown that chitin and chitosan-based dressings can accelerate the repair of different tissues, facilitate contraction of wounds, and regulate secretion of inflammatory mediators such as interleukin 8, prostaglandin E_2, interleukin 1β, and others [18]. Many researchers have shown that the biodegradable chitin or chitosan itself provided bacteriostatic and fungistatic activities [19, 20]. 200 kDa molecular weight chitosan has been shown to have advantageous antimicrobial and biological activity [21].

Previous studies have described chitosan-based materials for wound treatments and bone replacement applications [16, 22, 23], but there is limited information concerning their use for dural closure [24, 25]. Chitosan was shown to have degraded be complete dura mater regeneration [10], but chitin undergoes slower degradion in biological media [20]. Therefore, we hypothesied that a chitin/chitosan composite could be an effective dura matter substitute. In the present study we evaluate the effectiveness and safety of a set of novel chitin–chitosan films for dural closure.

2 Materials and methods

2.1 Graft preparation

Chitosan-based films were made out of a 3% solution of chitosan (molar masses—200, 500 and 700 kDa, deacetylation rate 80–90%). Firstly, a 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 were in the ratio 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. After the drying, materials had a visually smooth lower surface and a rough upper surface (Fig. 1).

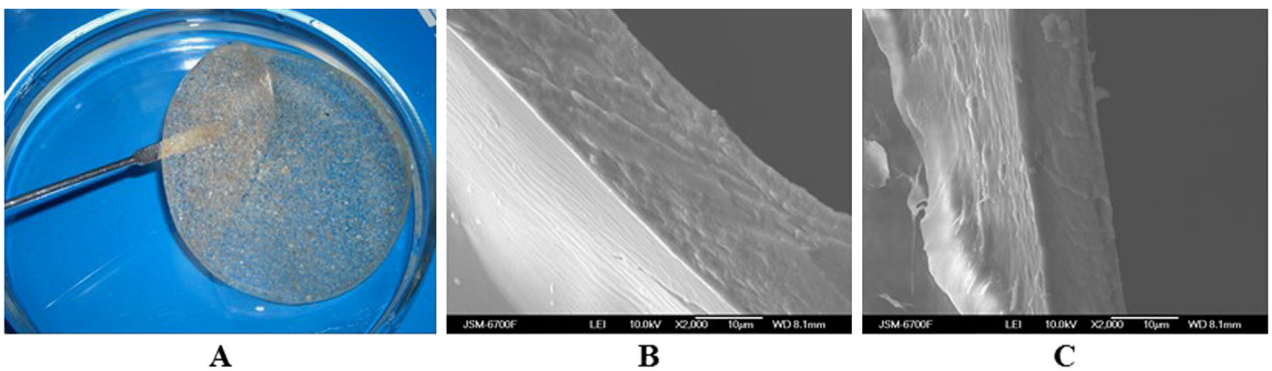

Fig. 1 Photograph of the chitin/chitosan graft after the drying procedure (**a**). Scanning electron microscopy of the *lower* (*smooth*) **b** and *upper* (*rough*) surfaces of the chitin/chitosan graft. *Scale bar* 10 μm. Representative images of 15 grafts

2.2 Antibacterial activity of chitin–chitosan grafts (in vitro test)

Chitin–chitosan grafts (200, 500 and 700 kDa) were cut using a knife to create disks of 1.2 cm and sterilized in 100% ethanol for 1 h at room temperature. The antimicrobial activity of chitin–chitosan films was tested against *Pseudomonas aeruginosa, Klebsiella pneumonia (*Gram-negative) and *Staphylococcus aureus*, *Streptococcus pyogenes* (Gram-positive). The final concentration of bacteria was 1×10^7 colony formed unit per 1 ml. Samples were applied to sterile McConkey agar and coated with a thin layer of the same medium. Petri dishes with samples were incubated at 37 °C for 10 h and covered by the bacterial culture and then covered by the medium. After successful inoculation, the Petri dishes were incubated at 37 °C.

After 18 h of incubation the diameter (ø) of the growth inhibition zone around the sample was measured, using the following criteria for antibacterial activities inhibition zone: ø–0–5 mm—absence of antibacterial effect; ø–5.1–14.9 mm —bacteriostatic effect; ø–≥ 15 mm—bactericidal effect.

2.3 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 h 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 Eagles 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 h, 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. After a further 24 h, 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.

2.4 Cell viability

Alamar Blue (AB) assay was used to assess cell viability on days 3, 7 and 14 after seeding. Media was removed from each well and washed with PBS. 1 ml of Alamar Blue™ solution was added to each scaffold and incubated for 2 h. Two aliquots of 200 µl of Alamar Blue™ solution were collected from each scaffold and read at a wavelength of 570 nm in a colorimetric plate reader (Bio-TEK, UK) to obtain baseline values of colorimetric absorbance.

2.5 Animals

20 rabbits of the chinchilla breed (aged 5–6 months with body weight 3–3.5 kg), were used for the experiments and housed in vivarium conditions. Housing of the animals and all experimental procedures were carried in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986); Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. The procedures were approved by the Institutional Ethic Committee.

Animals were divided into two groups:

I group–control (10 animals)–duraplasty using the fascia lata autograft.

II group–experiment (10 animals)–duraplasty using the chitin-chitosan membrane.

2.6 Surgical procedure

For the duraplasty we used chitin–chitosan material made from 200 kDa because of the results of the previous experiments. They have shown antibacterial properties and significant better cell viability.

The duraplasty procedure was as follows (Fig. 2): after general anesthesia (Ketamine 7 mg/kg and thiopental 10 mg/kg) and peripheral vein catheterization the animals' heads were shaved from the supercilial 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 nose and it was perpendicular to the transverse incision. The triangular skin flaps were retracted.

The transverse incision was made at 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. Two holes (at the distance of 0.5–0.7 cm from each other) were drilled using a trepan with a sharp point of 0.5 cm diameter. Using the Luer's and Liston's 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 (Vicryl 5/0, Ethicon Endo-Surgery). Suture material was not used for the chitosan-based membrane as it had good adhesion

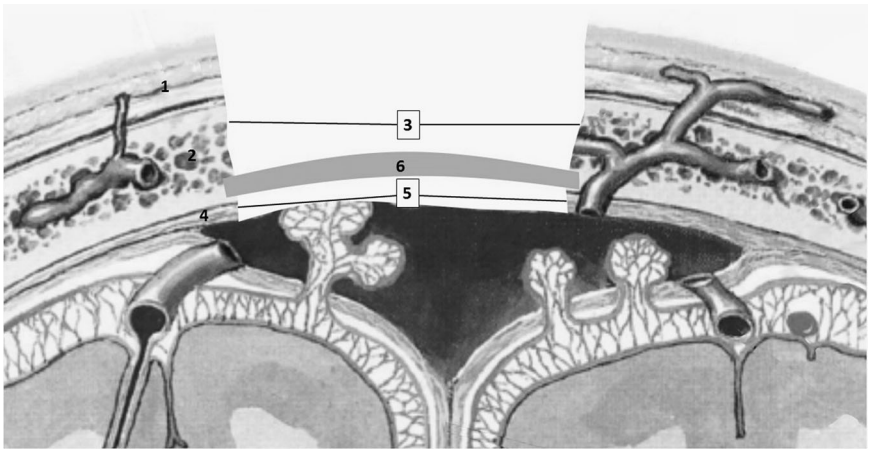

Fig. 2 Scheme of operation procedure. Skin (1) flaps were retracted under the bone (2) of temporal region and trepanation opening (3) was created up to the dura meter (4). Dura matter defect (5) was made and replace by the chitin–chitosan membrane or fascia lata (6)

properties. The graft was bigger in size than the defect, and was placed between the undamaged dura mater and internal bone surface. The smooth surface was placed on the brain side and the rough surface touching the periosteum of the skull.

Myorrhaphy, suturing of the muscles, was conducted after the operation without closure of the bone defect. Duraplasty was conducted following the same technique in the opposite side of the head. Finally, we closed the wound with simple interrupted sutures and applied an aseptic dressing.

2.7 Histological analysis

Histological samples from the surgical sites were prepared 2 weeks and 2 months after the performed operation. Animals were killed by narcosis overdose (ketamine, 70 mg per 1 kg), the dura mater with surrounded tissues was taken and fixed in 10% formaldehyde and then dried in alcohols of elevated concentrations and set into paraffin wax. 12 µm thick sections were prepared and stained with hematoxylin and eosin. The specimens were visualized using a light microscope (Olympus, Japan).

2.8 Microbiological analysis of cerebrospinal fluid (CSF)

Cerebrospinal Fluid (CSF) was extracted using a sterile syringe from the cisterna magna before the animal euthanasia. 2.0 ml of CSF was centrifuged at $1000 \times g$ for 10–15 min for bacteria sedimentation. The supernatant was removed with a Pasteur pipette and the sediment was vigorously mixed. Two drops of sediment were used to prepare the Gram stain and one drop for the primary culture media. A quantitative microbiology culture report was generated to provide the number of potential pathogens per ml, including the identification of each pathogen to the genus and/or species level.

3 Results

3.1 Antibacterial properties of the chitin-chitosan film

In vitro experiments indicated that the chitin–chitosan films displayed antibacterial activity against all types of bacteria examined. The antibacterial effect strongly depends on the molecular weight of the chitin–chitosan material. Samples made from 200 molecular weight showed a bactericidal effect, while 500 and 700 kDa were bacteriostatic (Table 1).

3.2 Cell culture

Cells were viable in all scaffolds on 3 days after seeding. There was no significant difference in RR fluorescence between the smooth surface of scaffolds with different molecular weights. RR fluorescence from materials of 500 kDa weight with a rough surface was significantly higher compared to samples made from chitosan 200 kDa ($p = 0.009$) and 700 kDa ($p = 0.01$). Also there was a significant difference in cell number between rough and smooth surface in scaffolds made from chitosan 500 kDa ($p = 0.004$). It is likely that the rough surface has a higher surface area for cell attachment.

On the 7th day after seeding there was significantly higher cell viability on all chitin–chitosan samples both smooth and rough surfaces. More rapid growth was observed on the smooth surface and in this period there were no significant differences between the cell viability on the smooth and rough surfaces of scaffolds made from chitosan with different molecular weights. Cell viability on the 14th day after seeding was not significantly different from the previous timepoints, nor were there any significant differences between smooth and rough surfaces. However, the smooth surface of the 200 kDa chitosan supported cells better than the smooth surface at the other molecular weights. (Fig. 3).

Table 1 Antibacterial activity of chitin–chitosan films

No	The molecular weight of the grafts	Diameter (ø) the inhibition zone (mm) (M ± m)			
		S. aureus	*S. pyogenes*	*P. aeruginosa*	*K. pneumonia*
1	200 kDa	16.5 + 0.5	16.4 + 0.5	17.3 + 0.5	16.0 + 0.5
2	500 kDa	14.7 + 0.5	15.3 + 0.5	13.3 + 0.9	14.0 + 0.5
3	700 kDa	13.1 + 0.3	12.3 + 0.8	12.3 + 0.7	12.0 + 0.5

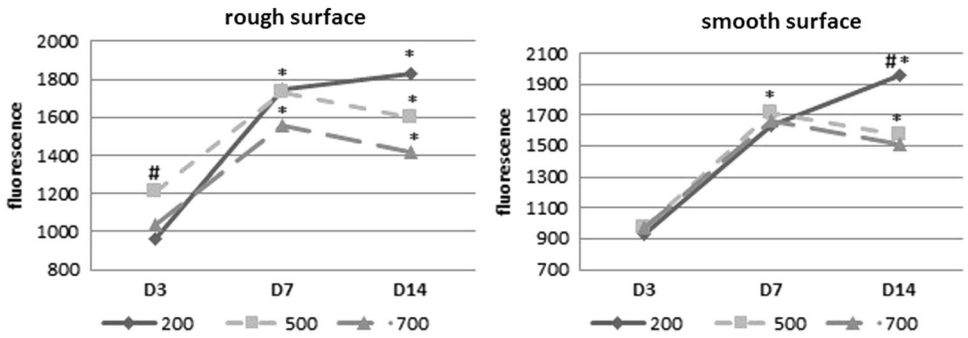

Fig. 3 Cell viability of MLO-A5 cells on chitin–chotosan materials made with varying molecular weights of chitosan (200, 500 or 700 kDa). Mean ± SD of the fluorescence of the media after the resazurin reduction assay, which is an indicator of metabolic activity. $N = 5$. * Significantly different from day 3. # Significantly different from the other molecular weights of chitosan

3.3 Postoperative results

After two weeks of duraplasty using the fascia lata, the incision was visible, but both skin and muscles were adherent. While accessing the implant, we separated tissues using sharp blades as the external surface adhered firmly to the temporal muscle (Fig. 4a). There were no signs of inflammation across the graft surface when muscle fibers were separated. In two animals, fibrin was deposited onto the membrane surface providing evidence of an inflammatory reaction. The fascia seemed rather deformed (Fig. 4b). The fascia lata could be easily separated from the dura mater between sutures. One animal had minor cerebrospinal spinal fluid leakage as the consequence of inappropriate defect closure caused by the tearing of the stitches.

The edges of the autograft, where they were sutured, were thickened due to excessive fibroblast proliferation and connective tissue formation. During sample harvesting for histological analysis we observed adhesion scars. The adhesion scars, which were difficult to separate, formed across the whole fascia lata surface that had attached to the dura mater in four out of five animals.

After two weeks there was no evidence of inflammation in the post-operative wounds due to the application of the chitin-chitosan membrane. Adhesion across the temporal muscle did not occur in the animals from the experimental group, because an additional chitosan membrane was placed above the defect. We did not observe any deposition of fibrin or connective tissue growth across the graft surface. The chitosan substitute was semitransparent which enabled viewing of the condition of the dura mater surface (Fig. 4c). The implant was attached firmly to the edges of dura defect, where connective tissue grew. This explains why no animals had cerebrospinal fluid leakage. Absence of the massive reaction of connective tissue provided evidence that that chitin–chitosan material was biocompatible. These properties would allow such a chitosan–chitin substitute to be used for severe skull defects. While harvesting material for the histological analysis, no adhesion scars were observed in any of the animals, further confirming that the applied material was did not induce a negative tissue response.

Two months after the fascia lata was placed in the dura mater, the incisions were almost invisible. The temporal muscle adhered firmly to the autograft and there was significant growth of connective tissue across the autograft surface. Thus, the autograft surface was covered completely with conglomerates of the connective tissue (Fig. 5a). Cerebrospinal fluid leakage did not occur in any animal demonstrating that the subdural space was closed tightly. When the piece of dura with implant was removed adhesion scars were observed in the majority of samples (four out of five animals). The newly-formed vessels had adhesion scars that formed across the brain surface, which attached to the fascia lata. The adhesion scars could be separated using a sharp blade (Fig. 5b). Formation of the adhesion scars caused alterations in cerebrospinal fluid flow.

Two months after the chitin–chitosan membranes were implanted, it was observed that the muscles could be easily separated from the bone defect due to the application of the additional membrane (Fig. 5c). Connective tissue grew across the duraplasty site. There were no visible differences between the dura mater and connective tissue that replaced the substitute. Chitosan integrated into the newly formed connective tissue, the subdural space was closed tightly and there was no evidence of cerebrospinal fluid leakage. While

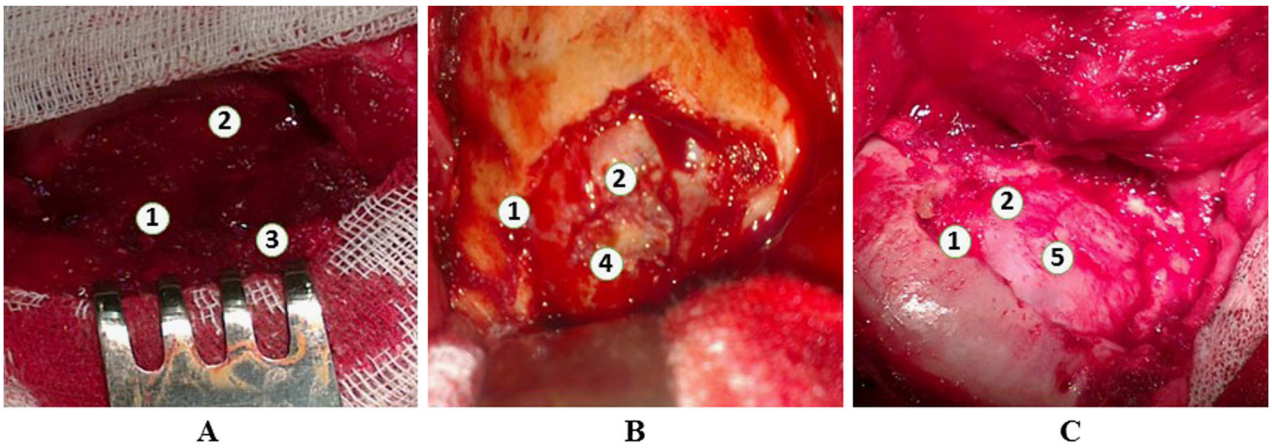

Fig. 4 Postoperative results in two weeks after dural closure with fascia lata autograft (**a** and **b**) and with chitin–chitosan graft (**c**). 1—The edges of bone defect, 2—The edges of dara mater defect, 3—adhesion of temporal muscle to fascia lata autograft, 4—the fascia lata autograft covered by fibrin, 5—the chitin–chitosan graft

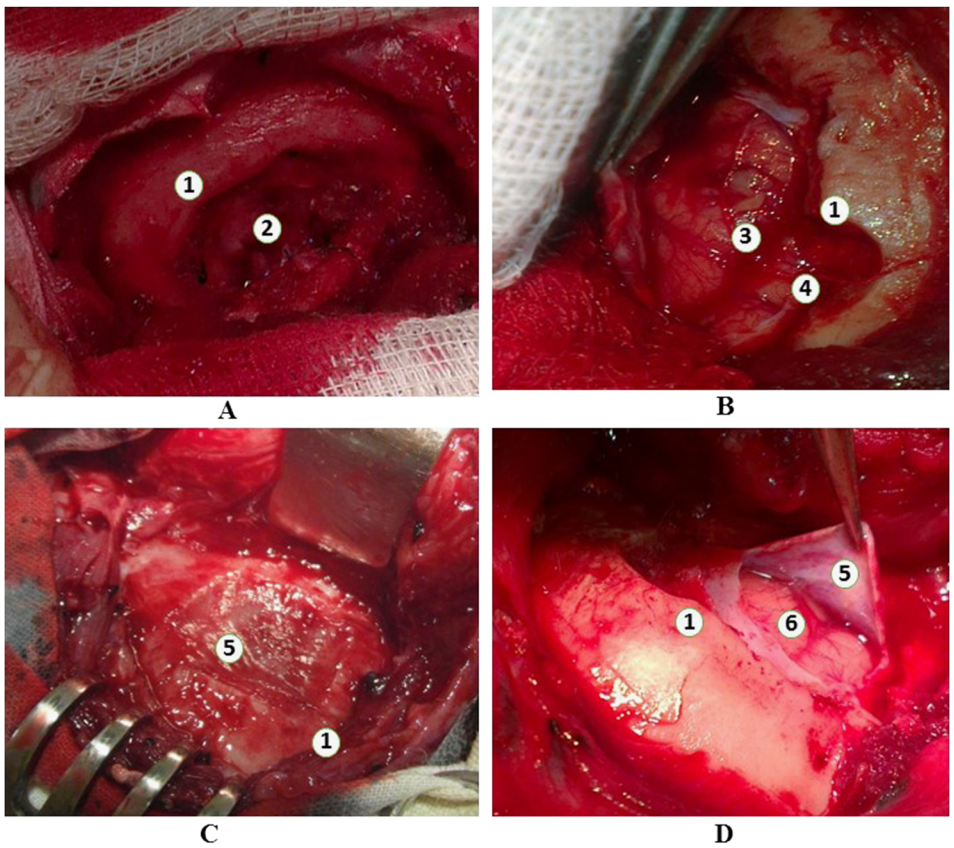

Fig. 5 Postoperative results in two months after dural closure with fascia lata autograft (**a** and **b**) and with chitin-chitosan graft (**c** and **d**). 1—The edges of bone defect, 2—the autograft in conglomerate of soft tissue, 3—the autograft, 4—brain injury and bleeding from the vessels after autograft removing, 5—the chitosan–chitin graft and the native dara mater, 6—the surface of the brain with vessels

harvesting material for histological analysis, no adhesion scars were visible in animals and there was no cerebrospinal fluid leakage. (Fig. 5d).

3.4 Histology

The histological analysis confirmed that 2 weeks after implantation in all samples there was excessive connective tissue growth across the fascia lata surface. Excessive fibroblast proliferation occurred also across the internal surface of graft due to its contact with CSF. At the edges of the defect vessels adhered to the layer of connective tissue, but the number of vessels was minimal. The thickness of the fascia lata graft did not change within two weeks. Significant mixed cell infiltration occurred across the areas, where the stiches were placed, as a consequence of foreign

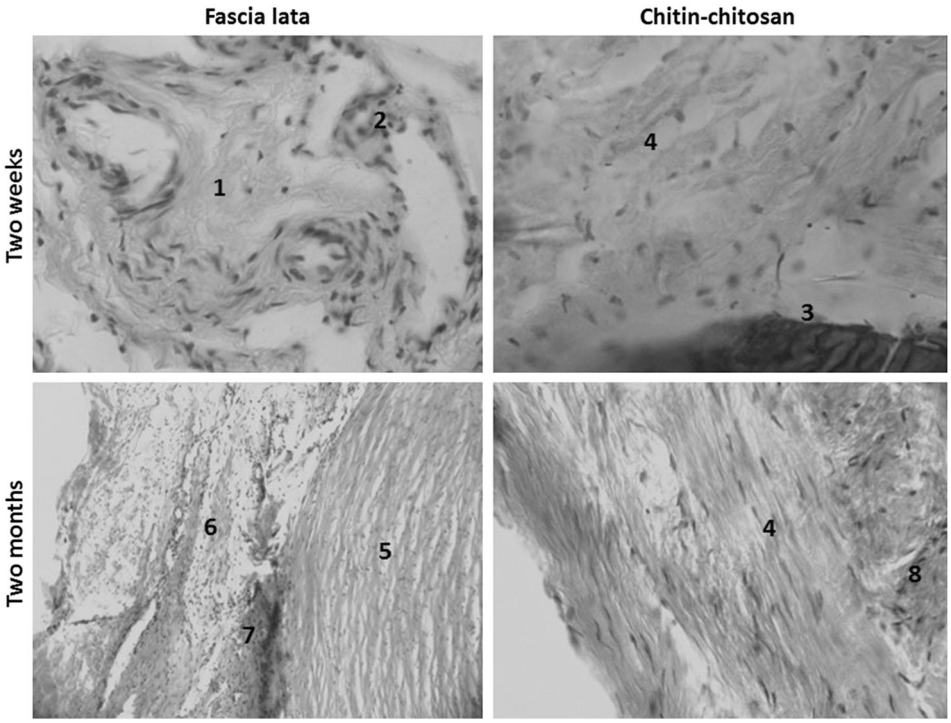

Fig. 6 Histological micrographs of fascia lata autograft and chitin–chitosan graft in different terms after the duraplasty. Hematoxylin and eosin staining, zoom—200. 1—destruction of collagen fibers, 2—cell infiltration around the stiches, 3—remnants of the chitosan–chitin membrane, 4—the newly-formed connective tissue, 5—the fascia lata autograft, 6—connective tissue growth at the top of the autograft, 7—cell infiltration, 8—the edge of dara mater

material implantation and aseptic inflammation development (Fig. 6). Thus, the new tissue formation and vessel growth in the newly formed connective tissue occurred after two weeks, but biodegradation of the autograft did not occur.

Biodegradation of chitin–chitosan graft started at the edges of samples within two weeks after implantation. The edges of the material were thinner (by 10–15%) compared to central zone and replaced with connective tissue. Collagen fibers that formed the connective tissue were parallel to the dura mater and implant and grew across the dura mater surface. Vessels adhered to the undamaged edges of dura controlling connective tissue growth.

The histological analysis of samples removed two months after duraplasty confirmed that the fascia lata was completely covered with connective tissue from both sides (external and internal surfaces). There were a lot of vessels with medium diameter in the newly-formed tissue. The thickness of connective tissue was almost equal to the thickness of the graft. Two months after the operation, the autograft was covered with the connective tissue and there were adhesion scars perhaps due to bioactivity of the fascia lata.

Histological analysis in animals with chitin–chitosan grafts indicated that connective tissue grew at the site of substitute, which was similar to the dura mater in structure. Connective tissue was present as a layers of collagen fibers orientated parallel to the dura mater. The chitin–chitosan membrane remained across the central area of graft and was surrounded by connective tissue. The membrane was thinner than the control membrane confirming that biodegradation had occurred. However, complete degradation had not occurred over the time of the experiment therefore it would be necessary to conduct a longer study to observe complete degradation of the chitin–chitosan membrane. The newly-formed tissue was vascularized as evidenced by the presence of small and medium diameter vessels.

3.5 Bacteriology of SCF

Bacterial growth was reported in one case (10%) of the animals in group I (dura mater graft) 2 weeks after the duraplasty. Coagulase-positive staphylococcus—*Staphyloccus aureus* were isolated from the CSF at 3×10^3 CFU/ml.

Microorganisms were not identified in the CSF at 2 weeks in any animals that were treated using the chitin–chitosan membrane. Postoperative results showed that the subdural space was tightly closed and chitosan possessed antibacterial properties, as demonstrated by previous experimental and clinical investigations [21] and the in-vitro bacteriological tests. Slow biodegradation of chitosan-based materials [26] releases oligomers that stimulate fibroblasts. It is likely that enzymes contained in cerebrospinal fluid catalyze the degradation of the membranes near the edges of dura mater. Chitosan oligomers stimulate migration of fibroblasts that released collagen fibers to replace the chitin–chitosan graft [24]. At the same time, growth of capillaries occurred in the dura mater leading to connective tissue formation at the site of defected

dura mater. Additionally, the presence of chitosan prevented growth and multiplication of microorganisms that could infect the post-operative wound.

2 months after duraplasty no bacteria were found in CSF in any group.

4 Discussion

First of all, the ideal dural graft must be non-toxic and not cause a pronounced inflammatory response. Homologous fascia lata and pericardium have biological activity and can improve the healing process [27]. Autografts do not induce any immunological reaction and are available at no cost. However, it is difficult to obtain a graft of the needed size and shape for large defects. Furthermore, surgical collection of the fascia lata is associated with additional morbidity [28], it also prolongs surgical and anesthetic time and causes pain at a donor site [27]. Cadaveric dara mater has been widely used for several decades; however there are many reports of transmission of Creutzfeldt-Jacob disease [29] and severe immune responses [27]. Synthetic grafts are widely used (800,000 dural substitutes are utilized in the USA every year) but have a number of significant disadvantages such as chronic inflammatory response; acting as a nidus for infection; causing delayed haemorrhage or neural compression by in folding; sometimes provoking a pseudotumoural fibrocellular scar with compression of brain parenchyma and poor handling characteristics [30, 31]. Collagen based grafts do not induce an immune response but the potential transmission of bovine spongiform encephalopathy is still present, when natural collagen is used [3].

In our experiments there was no evidence of inflammatory or immune responses to the implantation of the chitin–chitosan membrane. Additionally, chitosan appeared to have a bacteriostatic effect and it application prevented postoperative bacterial contamination [21].

Tissue-like elasticity without permanent deformation is also an important feature of these materials. The cadaveric dura, the pericardium and the fasciae (both temporal and fascia lata) are not sufficiently compliant, especially after sterilization. The majority of biological (collagen) and synthetic materials are not elastic enough. The chitin–chitosan membrane has satisfactory elasticity and can simulate surfaces of any complexity. Sandoval-Sanches et al. [25] showed that bilayer chitosan membrane was more elastic compared to collagen matrix materials. There are no studies that are devoted to comparisons of the elasticity of synthetic and chitosan-based materials.

The membrane described here possesses properties that make it applicable for duraplasty of different regions of the skull. Firstly, the membrane can be easily removed where necessary because it does not adhere to the brain or the tissues above. Previously adhesions caused by fascia [32], cadaveric dura and pericardium, bovine dura [30], galea-pericranium [33] and collagen biomatrix have been described [28, 34, 35]. However, we did not observe any signs of adhesion in experimental animals with the chitin-chitosan materials, while the rabbits (80%) with fascia lata grafts did have signs of adhesion. Moreover, there were no reported signs of adhesion in other experiments with mono and bilayer chitosan substitutes [24, 25]. Another advantage of the chitin–chitosan membrane was its transparency which allowed viewing of the subdural space which will be a helpful feature if reoperation is needed.

Many discussions of duroplasty improvement are devoted to consideration of resorbable dura grafts [36]. If a material is resorbable, it should be completely replaced by the dura-like tissue at the end of healing process. The material described here was slowly resorbable and the resorption rate will depend on the amount of chitin added as chitin is less resorbable than chitosan [20]. These properties would allow completion of the graft replacement later in the healing cycle compared with chitosan-only materials and prevent cerebrospinal fluid leakage. During graft replacement connective tissue growth is not excessive and does not provoke adhesion or scar formation.

Our proposed chitin–chitosan membrane is also an economical alternative to dara mater grafts due to the accessibility of chitin as a second most abundant polysaccharide found in nature after cellulose [15].

5 Conclusion

1. Chitin–chitosan materials have strong antibacterial effect and can support cell viability independently of chitosan molecular weight.
2. Application of the chitosan-based membrane as a dura mater replacement prevents adhesion scar formation and bacterial inflammation both in the early and late postoperative periods and ensures tightness of the subdural space even without suturing.
3. Application of the chitin–chitosan materials is more effective to close the dura mater defect compared to classical duraplasty using fascia lata [37–40].

Acknowledgment The team of authors thank for Robert Owen, PhD student at the University of Sheffield, INSIGNEO Institute for silico Medicine for help with the cell culture experiments. M. Pogorielov was funded by Ukrainian Ministry of Education and Science for research visit to The University of Sheffield (2014 yr.).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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