BACTERIAL NANOCELLULOSE AS A PLASTIC MATERIAL FOR CLOSURE OF DEFECTS OF THE DURA MATER: LITERATURE REVIEW

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Objective. To analyze publications devoted to the possibility of using bacterial nanocellulose as a plastic material for defects in the dura mater associated with spine and spinal cord pathology.

Material and Methods. The PubMed database was searched with keywords "bacterial cellulose properties" and "bacterial cellulose". The search was limited to articles published in English- and Russian-language journals in 2009—2019. The limitation was caused by the need for up-to-date evaluation of the properties of bacterial nanocellulose. The search with keywords "bacterial cellulose properties" returned a list of 963 articles and with key words "bacterial cellulose" — a list of 3908 articles. The Google search engine was also used, in which articles were found actually reflecting properties of bacterial nanocellulose without which complete understanding of its nature is impossible. After assessing the found data, 76 articles were selected that reflect this issue to the fullest extent. More than fifty percent of the reviewed articles were published within the last 10 years. Evidence level: IV; recommendation grade: C, though randomized trials with evidence level Ib and recommendation level A are used.

Results. Implants made of bacterial nanocellulose are able to perform the function of the extracellular matrix by providing a barrier function, creating conditions for the circulation of metabolites and oxygen, and preventing the achievement of excess cell concentration.

Conclusion. The use of bacterial nanocellulose as an implant for closure of the dura mater defects associated with the spinal cord pathology is a promising direction in neurosurgery, since nanocellulose does not cause adhesions to the nervous tissue and performs a barrier function.

Key Words: spinal cord, bacterial nanocellulose, bacterial cellulose synthesis, cultivation conditions, properties of bacterial cellulose, plastic material.

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According to Yu.A. Zozulya, Yu.V. Kushel, Itzkovich et al. [1–3], spinal cord tumors account for 1.4–10.0 % of all tumors of the central nervous system (CNS). Ostrom et al. [4] informed that in the USA in 2007–2011, primary neoplasms of the spinal cord and cauda equina made up 6.0 % of all tumors of the CNS; Helseth and Mork [5] declared that meningiomas were the most common among all extramedullary tumors (25–46 % of cases).

In many cases, resection of spinal cord neoplasms,especially meningiomas with largest area of matrix causes the formation of dural defects which cannot be tightly sealed with the patient's own dura. A need arises to cover it with a plastic material in order to prevent secondary CSF leak or pseudoprotrusion of spinal cord and root, infectious complications of the spinal cord and meninges. According to our data [6], secondary CSF leak and pseudoprotrusion of spinal cord and root occur after the resection of spinal cord tumors in 2.3 % of cases. Similar complications may occur after surgery in patients with a complicated spinal trauma in the early and late periods of the spine and spinal cord injury when using posterior approaches to the spinal cord.

It's frequently required to repair dural defects in patients with congenital spinal hernias. The CDC studies [7] revealed congenital meningomyelocele in 35 cases per 10,000 of newborns, and Orioli et al. [8] – 1.4 cases (Brazil).

The most efficient method is sealing CSF spaces with duraplasty using own tissues of the patient (autografts). At the same time, autografting for such purposes is limited because of the method complexity. Moreover, using a part of the femoral fascia is an additional injury-risk factor for the patient, which can lead to prolonged surgery duration. Nowadays, the use of allografts (preserved cadaveric dura) is prohibited due to the risks of transmitting infections and viruses, as well as difficulties in preserving and storing the material.

Synthetic materials are widely used for dura mater plasty, but their use is far from being perfect. Nearly one hundred years of efforts have failed to create synthetic materials with ideal properties able to substitute the dura mater. The main problems in using implants are as follows:

1) allogeneic graft tissues, in particular, xenograft tissues, may cause implant rejection;

2) dura mater and surrounding tissue adhesion: after the plasty, the implants exhibit different degree of adhesion, mainly associated with inflammatory response, physical and chemical properties of the material (the lower the protein and fat content in the material, the lower

the degree of adhesion and inflammatory response);

3) development of aseptic inflammation;

4) allografting and xenografting may cause expansion of viruses and prions among people and animals;

5) nascent granulation tissue that regenerates and covers the graft material may cause bleeding (a breaking between the material and neocapillaries covering the matrix is possible; capillaries are fragile, therefore the graft displacement may cause bleeding and formation of subdural hematoma in the peridural space);

6) development of liquorrhea due to unreliable sealing of the dura defect;

7) development of syringomyelia may be caused by the tethered spinal cord syndrome resulting from its adhesion;

8) too large collagenous implants are lysed, while new neodural tissue need more time to form.

In this connection, it is still very important to synthesize a modern material for dura mater plasty. The authors consider film-like bacterial nanocellulose (BNC) with a thickness similar to that of the human dura mater to be the optimum material.

The objective of the study is to analyze publications devoted to the possibility of using bacterial nanocellulose as a plastic material for the dura mater defect repair.

Material and Methods

PubMed database was searched through with the keywords "bacterial cellulose properties" and "bacterial cellulose". The search was limited to articles published in English- and Russian-language journals from 2009 to 2019. The limitation was caused by the need for the foremost information on bacterial nanocellulose properties. The search with the keywords "bacterial cellulose properties" returned a list of 963 articles and a list of 3,908 articles with the keywords "bacterial cellulose". Google search engine was also used to find articles directly regarding bacterial nanocellulose properties and contributing to its complete understanding. After assessing the found data, we selected 76 articles. More than fifty percent of the reviewed articles were published within the last 10 years. Evidence level: IV; recommendation grade: C, though randomized trials with evidence level Ib and recommendation level A were used.

Results

BNC is known for more than half a century. It is a linear chain of a polysaccharide with the same chemical structure as plant cellulose has. The genus *Acetobacteraceae* is the most efficient producer of bacterial nanocellulose. Acetobacter xylinum was given its current name *Glucoconacetobacter xylinum (G. xylinum)* in 2006. According to Brown [9], *G. xylinum* from the genus *Acetobacteraceae* is a gram-negative aerobic bacillus bacterium, which has been identified for the first time in 1886 for vinegar production.

In 2012, Yamada et al. [10] proposed the new classification. In particular, they distinguished the new genus Komagataeibacter and identified its new combinations on the basis of their taxonomic and genetic characteristics. The following type species of the genus were identified: *Komagataeibacter xylinus, Komagataeibacter hansenii, Komagataeibacter europaeus, Komagataeibacter oboediens, Komagataeibacterinter medius, Komagataeibacter swingsii, Komagataeibacter rhaeticus, Komagataeibacter saccharivorans, Komagataeibacter nataicola, Komagataeibacter sucrofermentans, Komagataeibacter kakiaceti, Komagataeibacter kombuchae, Komagataeibacter maltaceti, and Komagataeibacter medellinensi.*

Komagataeibacter xylinus is a gram-negative bacillus producing acetic acid during fermentation and being the most active producer of bacterial nanocellulose. Hypothesis put forward by the authors of this article and Williams et al. [11] explains the main goal of BNC production by the bacteria: 1) to hold bacterial cells in the static culture, a film is formed on the surface of the medium in order to provide bacteria with oxygen; 2) to provide cell protection from X-ray radiation or harsh sunlight; 3) to protect cells from the penetration of heavy metal ions; 4) to transport nutrients by diffusion; 5) to retain moisture; 6) to produce cellulose by bacteria (protective mechanism against drying and penetration of other bacteria).

The strain of bacteria is one of the most important factors affecting BNC production. It is the strain, medium, conditions and cultivation techniques that are responsible for porosity, thickness and the mechanical characteristics of BNC.

Methods of BNC production. According to Watanabe et al. [12], BNC can be produced by two methods of cultivation: under static and agitated culture conditions, as well as by using bioreactors. Under static culture conditions, the film is produced in a sheet form and grows as a thickening peripheral roller at the walls of the container, and its center is thinner because the bacteria adhere to the container walls and produce more cellulose in that very place.

Under agitated culture conditions, BNC grows as sphere-like granules. Hu et al. [13] proved that cellulose spherules produced at a rotational speed of 200 rpm were hollow, at the same time spherules produced at a rotational speed of 150 rpm were solid. Cellulose cultivated under agitated culture conditions exhibited a lower mechanical strength in comparison with cellulose cultivated under static culture conditions. According to Ruka et al. [14], cultivation under agitated culture conditions leads to lower yields of cellulose in comparison with that under static culture conditions. The static cultivation method requires greater areas than the agitated one.

Cultivation conditions. Castro et al. [15] found out that bacteria *Komagataeibacter xylinum,* using various sources of carbohydrates, were able to produce cellulose extracellularly at temperatures between 25 and 30 °C, and pH from 3 to 7. These data were proved by Gromet et al. [16]. According to their opinion, the optimum synthesis of cellulose takes place under static conditions between 28 and 30 °C, and pH from 4 to 6. *Komagataeibacter xylinus* is able to efficiently

synthesize cellulose from many carbohydrate substrates. Mikkelsen et al. [17] have demonstrated that the most efficient production occurred with the use of glucose, sucrose, and glycerol, which are the basic sources of carbohydrates. Keshk et al. [18] consider that glucose metabolism leads to the accumulation of gluconic acid (GA) and simultaneous pH reduction. The synthesis of cellulose decreases due to GA accumulation at the culture pH of less than 4. When all of glucose is oxidized at pH 4–6 in the culture, bacteria start to metabolize GA. When bacteria consume GA, a gradual increase of pH in the culture is observed. According to Hwang et al. [19], the synthesis of cellulose and the cell fission will resume only after the pH level rises above 4. This trend is observed in agitated culture, when the oxygen level in the culture medium increases, because bacteria are obligate aerobes. The oxygen dissolved in the culture and its concentration can sufficiently impact the rate of cellulose synthesis. According to Tantratian et al. [20], low-oxygen cultures are not able to grow sufficient quantities of cellulose.

According to Mohammadkazemi et al. [21], the BNC yield and characteristics are influenced by the following important factors: cultivation method, source of carbohydrates, a strain of bacteria, acidity and temperature, as well as the type, quantity and composition of the culture medium.

Process and mechanism of BNC fiber formation. Molecules of cellulose are synthesized inside bacteria. El-Saied et al. [22] have proved that BNC is formed as a result of extracellular secretion of nanofibers produced by various bacterial species. A protofibril is the basic unit of a microfibril. Lee et al. [23] have demonstrated that protofibrils are extruded through small pores (50–80 pore-like sites on the surface of a bacterium) as thin ribbon-like axes by one from the pore and are intertwined with each other. After the extrusion, protofibrils under the influence of β-1-4-glucan bonds are crystallized and combined into microfibrils. A protofibril has a certain crystalline configuration that depends on the genome of bacteria and synthesis conditions. According to Brown et al. [24], the bacterium controls the arrangement of protofibrils, when the terminal cellulose synthesizing complexes line up along the cell membrane of the bacterium, and protofibril crystallization takes place. Ross et al. [25] have found microfibrils (ribbons with the width of 20–50 nm) to be freely formed from ribbon-like protofibrils consisting of 1,000 glucan chains in the exact hierarchical sequence. Benziman et al. [26] consider that the mutual orientation of the bound glucan chains and the orientation of sequential bindings of the chains is the result of the tightly coupled crystallization and polymerization *in vivo*, that explains the property of a less stable structure of crystalline modification of cellulose I. One cell of the bacterium produces a microfibril of cellulose from 10–100 protofibrils. According to Brown et al. [27], each protofibril is assembled into a linear terminal complex consisting of three subunits (cellulose synthesizing sites), and each subunit contains at least 16 catalytic subunits of cellulose synthase. Microfibrils associate into ribbons, and intertwined ribbons form a film. In the native state, the BNC film is a network of swollen intertwined ribbons.

So, protofibrils, because of their nanosized properties, form pathways for cell growth and tissue formation, and, on microscopic and cellular level, ensure a scaffold and semi-rigid nanostructure, which is similar to self tissues of the organism.

Difference between BNC and plant cellulose, and BNC purity. In comparison to plant cellulose, ultrathin BNC exhibits a high degree of purity and a higher degree of crystallinity, better ability to absorb liquids, greater strength of the fiber structure, and nanoscale dimensionality. The studies of Jonas et al. [28] prove that although BNC is chemically identical, but differs from plant cellulose by the degree of polymerization; the degree of polymerization of BNC is 2,000–6,000, and that of plant cellulose varies between 13,000 and 14,000. Krassig et al. [29] in their study have identified the extent of BNC polymerization as 2,700.

The main chemical formula of cellulose is as follows:

$C_6PH_{10}P + 2O_{5P}+1 \approx (C_6H_{10}O_5)P$ or $(C_6H_{10}O_5)n$,

where p is the degree of polymerization; n is the number of links in the chain.

According to Krassig et al. [29], cellulose is a polysaccharide with a linear chain consisting of molecules of D-glucose (is an elementary unit of cellulose) linked by β-1,4-bonds. The molecular mass (m_0) of the basic unit of glucose is 162 mmol/l, the molecular mass of a cellulose polymer – $Mr = m_0P + 18 \approx 162$ P, where P is the degree of polymerization. According to Klemm et al. [30], the elemental composition of the mid layer of BNC contains 44.71 % Carbon, 6.68 % Hydrogen, and 1.47 % Nitrogen, while the purification with NaOH solution changes elemental composition to the following: 44.16 % Carbon, 6.56 % Hydrogen, and 0.25 % Nitrogen.

According to Yamanaka [31], one of the advantages of BNC is the absence of lignin, pectin, hemicellulose, and other biogenic products, which are usually associated with cell walls of plants. After chemical and thermal purification of bacterial polysaccharide, BNC contains 100 % pure cellulose and becomes transparent.

Bacterial cellulose is identical to plant cellulose in molecular formula and polymeric structure, but these two forms differ in arrangement of glycosyl units in the elementary units of crystallites, which leads to a higher crystallinity of bacterial cellulose.

Fiber and pore sizes. Haigler [32] proved the protofibril size to be approximately 0.0015 μm in diameter. Iguchi et al. [33] determined the diameter of protofibrils to be approximately 0.002–0.004 μm. These protofibrils form a ribbon-like microfibril with a length of about 0.08 μm. Microfibrils are combined into ribbons. Nanofibers of BNC are ribbon-like structures with the diameter of about 0.1 μm and the length of about 100 μm. The fibers are combined into a network. The BNC network is a porous material with nanosized pores. When the ribbons superimpose each other, the pore area and the BNC thickness

increase. Grande et al. [34] determined the average pore size (mesh) of dried BNC to be 0.523 ± 0.273 μm, and the orientation (the average angle formed by the segments and the x axis) of nanofibers to be 85.64° ± 0.56°.

Favi et al. [35] studied the morphology of lyophilized and completely dried BNC. The diameter of fibers of lyophilized BNC was 0.032 μm with the standard deviation of 0.01085. The diameter of fibers of BNC dried at the critical point was 0.029 μm with the standard deviation of 0.00828. The pore size of lyophilized BNC was 0.254 μm with the standard deviation of 0.07665 μm. Under hydration, the pore size decreases sufficiently because of fiber swelling and additional hydration shell around the fibers. So, the problem of porosity of bacterial cellulose implanted into a human organism is still unsolved for the time being. For example, the erythrocyte diameter is 7 μm, the size of the human fibroblast is within 10–40 μm. It is a problem for cells to penetrate into pores of native BNC, because the pore size is less than that of the cells. According to Uraki et al. [36], BNC prevents the penetration both of cells and bacteria because of its nanoporous structure. This feature is very important, because BNC implants are supposed to be barriers for pathogenic bacteria.

The formation of native cellulose occurs from the bottom upwards, i.e. the lower layer adjoins the upper layer. According to Klemm et al. [37], the lower surface is more porous, so human chondrocytes can penetrate deep into the membrane up to 70 μm; the upper surface is solid and compact, so it completely prevents the cell migration within the network. Tang et al. [38] note that, the BNC porosity varies between 92 and 94 % (a fraction of the volume of voids over the total volume) because of extremely small volume fraction of cellulose nanofibers. This feature ensures high level of inertness and biocompatibility (because the content of any substances in BNC implants besides water is insufficient and depends on the purity of cellulose). Porosity is an important factor for delivering nutrients and oxygen, because they are needed for cell proliferation. It is also important in tissue engineering, because the pore size does not let the cells penetrate deep into the scaffold. Low molecular weight proteins, carbohydrates, water and oxygen easily penetrate through the pores.

Currently, the behavior of proteins and molecular aggregates in porous structures including BNC has not been properly studied. Most probably, the formation of aggregates inside BNC leads to the accumulation of some types of protein molecules, lipids, and polysaccharides, thus, the protein and lipid compositions of BNC-based implants may differ from the composition of blood plasma, liquor or extracellular fluid.

BNC hydrophilicity. Due to its hydrophilic nature, BNC vastly encloses water within the network of nanofibrils, so the material behaves as a hydrogel. Klemm et al. [39] point that naturally hydrated BNC contains more than 99 % of water and less than 1 % of whole cellulose. Wet film hydrophilicityis explained by the structure of pores and tunnels, and depends on the total area of inner and exterior surfaces of native cellulose's pores. According to Klemm et al. [30], the moisture retention by native bacterial cellulose approximates to 1,000 %. For a comparison, air-dried bacterial cellulose can contain 106 % of water, lyophilized bacterial cellulose – 629 %, and cotton fibers – 60 %. The control of porosity can be used to change water retention potential and water recovery rate of BNC. Both parameters (water retention potential and water release rate) determine the utility of BNC as a bandaging material. The moisture content in bandaging material enhances wound repair processes, because cells grow faster and regenerate better while under moist conditions.

BNC strength. A peculiar characteristic of bacterial cellulose is its mechanical strength. This polymer is known to contain three types of intermolecular interactions: hydrogen bonds (forces with low interaction energy), Van der Waals forces, and covalent bonds. In cellulose, stable β (1 □ 4) covalent bonds appear between monomeric glucose residues and determine the linear alignment, thus promoting the formation of two intramolecular hydrogen bonds in each glucose residue. Festucci-Buselli et al. [40] have described the following intra- and intermolecular hydrogen bonds: one hydrogen bond links the hydroxyl group in the position 6 of the glucose portion with the hydroxyl group in the position 2 of the neighbor glucose portion. The other hydrogen bond links the hydroxyl group in the position 3 with the hydroxyl group in the position 5. Intermolecular hydrogen bonds connect different cellulose chains by the interaction between the hydroxyl groups in the positions 3 and 6 *(Fig.)*. Weak energy of hydrogen bonds is compensated by their enormous number due to a high degree of polymerization of cellulose, totally, it can exceed the energy of covalent bonds in the macromolecule. Van der Waals forces act on considerably greater distances than hydrogen bonds, but their energy is considerably lower. Hydrogen bonds, Van der Waals forces, and covalent bonds in BNC are of great importance. They are responsible for the physical features of cellulose (conformation of macromolecules, phase and relaxation states, submolecular structure) and influence all the properties of cellulose, including physical, physico-chemical, and chemical ones.

Higher mechanical properties of BNC as compared to plant cellulose are due to a more pronounced microstructure. Sugiyama et al. [41] believe that microfibrils are also linked by interfibrillar hydrogen bonds like in plant cellulose. Nevertheless, the density of interfibrillar hydrogen bonds is higher, because microfibrils are considerably thinner, which leads to a greater contact area. According to Klemm et al. [39], single BNC fibers exhibit mechanical strength comparable to that of steel or Kevlar. Mechanical properties of a hydrogel are partially determined by the water content and water excretion under compression. The water retention capacity of the BNC hydrogel depends on structural composition of the network of fibers.

So, the mechanical properties of BNC are determined by numerous interfibrillar bonds appearing as a result of the formation of H-bonds, covalent bonds, and Van der Waals forces between macro-

molecules of cellulose on the surfaces of fibrils and fibers, resulting in a high strength of BNC.

Biodegradation. Biodegradation (hydrolytic decomposition of cellulose) can be catalyzed chemically (for example, by acid degradation) or enzymatically. Cellulose degrading enzymes, which are called cellulases, naturally occur in some types of fungi and bacteria and allow them to convert cellulose into low molecular weight compounds, including monomers. The degradation is caused by hydrolase attack on the β (1 □ 4) bonds. Thus, it's very important to prevent any contacts of such fungi and bacteria with BNC when the products made from it are being synthesized.

Martson et al. [42] have studied biodegradation of viscose cellulose in the Cellspont product. When this product is produced, it is treated with a diluted sulfuric acid and sodium hypochlorite solution. Micropores in the walls of pores have been increased in 16 weeks after the implantation. This material was not completely decomposed after 60 weeks of the implantation in rats. This method can be used for BNC, too.

In its native form, cellulose consists of amorphous and crystalline parts. According to Beguin [43], hydrogen bonds in crystalline parts hold single molecules, and the physical nature of BNC decreases the accessibility of hydrolytic enzymes. According to Miyamoto et al. [44], cellulose with a higher degree of crystallinity is more stable in tissues.

Cells of mammals do not have cellulase enzyme necessary for the degradation of cellulose. According to Mendes et al. [45], they have not found changes in the structure of native BNC after the implantation in Swiss Albino mice within the period of 90 days. It can be concluded on the basis of this experiment that BNC-based scaffolds are not decomposed in normal cell cultures, which makes them promising to be used as scaffolds for long-term three-dimensional cell cultures. Li et al. [46] have described an enhanced *in vitro* degradation of BNC by periodate oxidation. After this treatment, BNC becomes biodegradable in water and phosphate-buffered saline at 37 °C, meanwhile the original structure of the BNC network remains intact. Native cellulose is not dissolved in physiological saline.

Sterilization. Barud et al. [47] have confirmed BNC to be thermally stable up to 200 °C. Thermal degradation starts within the range of 200–400 °C. BNC is sterilized with dry heat (>140 °C) or by autoclave treatment (121 °C). Sterilization of BNC in the native or lyophilized state occurs without any changes in the structure of the network. The latter aspect seems to be very important for a long-term storage and stability of BNC-based materials. Gamma sterilization carried out under standardized conditions (⩾25 kGy) does not cause macroscopically detected changes in the BNC intactness. The sterilization as a method of struggle with micro- and fungal flora allows long-term storage of medical products.

Bioactivity of BNC cells. Bioactivity means that the biomaterial directly affects physiology and morphology of living cells, through the control of their adhesion, migration, proliferation, differentiation, and release of the extracellular matrix which contribute to the formation of a new tissue. The studies of Petersen et al. [48] confirm that in terms of the cells, an important feature of BNC is the structure of its nanofibrils that resembles the structure of extracellular matrix components, in particular, collagen. BNC and collagen have similar diameters (<0.1 μm), they both are polymers functioning mainly as mechanical support structures.

Bodin et al. [49] prove that BNC mimics self tissues of the organism, provides a good matrix for in vitro seeding of cells and follow-on use in tissue engineering. BNC supports an efficient cell adhesion and prevents dedifferentiation because of the increased surface area and possibility of three-dimensional seeding. The porous structure of BNC allows for mass transfer of nutrients and oxygen, thus supporting the cell survival. BNC nanofibers can participate in the orientation of molecules of the extracellular matrix deposited by cells. When chemical substances enhancing cell adhesion are added, proliferation of these cells might not be increased. BNC may support the growth of endothelial, smooth muscle cells and chondrocytes, and it does not cause any toxic effect on them. Svensson et al. [50] have demonstrated that BNC exceeds plastic and calcium alginate as a substituent of cartilaginous tissue, and promotes the migration and

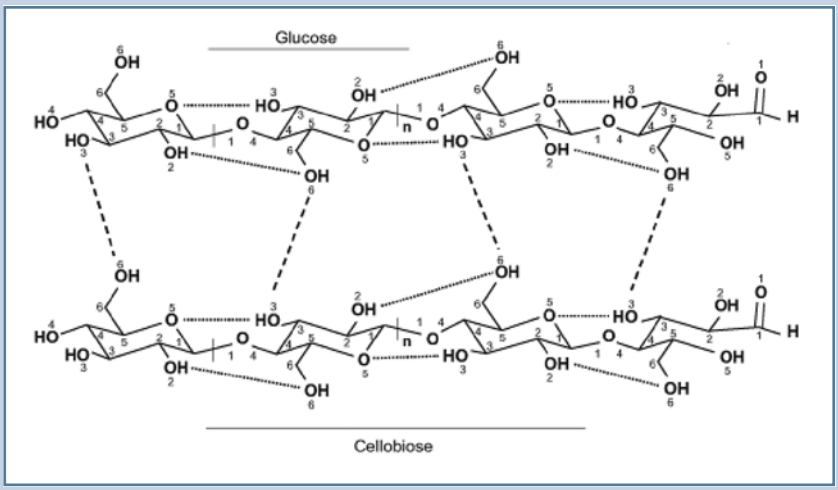

Fig.
Intra- and intermolecular hydrogen bonds in the network of cellulose I: dashed lines show intermolecular hydrogen bonds; dotted lines show intramolecular hydrogen bonds [40]

proliferation of chondrocytes. According to Zahedmanesh et al. [51], BNC porous tubes may be potentially used as vascular grafts. The tubes were seeded with smooth muscle cells of bovine aorta and endothelial cells of cattle, these cells were cultivated in the BNC lumen and proliferated in the confluent layer of the cells. Andersson et al. [52] seeded porous BNC scaffolds with articular chondrocytes obtained from adult patients, as well as with neonatal articular chondrocytes. The performed analyses showed that chondrocytes proliferated within the porous BNC. So, this new biomaterial can be used for regeneration of cartilaginous tissues.

Kim et al. [53] have found a BNC and gelatin composite to exhibit good adhesion of fibroblasts and proliferation. Biocompatibility has been improved in comparison with the pure BNC, and the created scaffolds have been bioactive.

In order to obtain greater porosity and surface area of BNC, Gao et al. [54] used sublimation drying. BNC confirmed its perfect biocompatibility with mesenchymal stem cells obtained from synovial fibroblasts.

Souza et al. [55] used adipose tissue-derived stem cells incorporated into the cellulose membrane. They determined that the integration between the adipose tissue-derived stem cells and BNC membrane was satisfactory; the composite delivered the cells into the damaged tissue. The accepted stem cells participated in the process of the wound regeneration and, depending on the composition of cells, contributed to the cell growth and wound repair.

Krontiras et al. [56] made two- and three-dimensional porous scaffolds of BNC-alginate composite. In order to obtain a porous structure, the BNC and alginate mixture was exposed to freeze drying, that caused a cross-linking of the neighboring molecules. The studies demonstrated that cells on two-dimensional surfaces were scarcely distributed. The cells grown in macroporous 3D scaffolds contained more cells growing in clusters. Scaffolds with relatively lower alginate content had greater porosity and hydrophilicity. The study demonstrated three-dimensional cultivation of adipocytes in macroporous BNC scaffolds to be a promising method for producing adipose tissues as an *in vitro* model for adipose biology and metabolic diseases. Favi et al. [35] have proved BNC to be cytocompatible with equine-derived bone marrow mesenchymal stem cells, supporting cellular adhesion and proliferation, osteogenic and chondrogenic differentiation; and the cells seeded on BNC are viable and metabolically active.

Numerous examples of bioactivity of various cells on BNC scaffolds support a promising opportunity of further BNC use as a scaffold.

Bacterial cellulose and cell adhesion. Cell adhesion cannot be achieved with native BNC. Chu et al. [57] have demonstrated that plasma treatment improves biocompatibility of cells, their physical and chemical properties and optimizes biocapacity. The experimental data of Pertile et al. [58] have proved that the plasma treatment of the surface of BNC improves adhesion of microvascular and neuroblast cells, but does not affect fibroblasts.

Pertile et al. [59] used lesser-sized peptide molecules from signaling proteins of the extracellular matrix, such as integrin-ligand according to the following sequence: isoleucine-lysin-valine-alanine-valine, fused to a cellulose binding module with the aim to improve cell adhesion to the surface of BNC. These recombinant proteins were adsorbed on BNC, thus they improved the adhesion of neuronal and mesenchymal cells.

Andrade et al. [60] have improved the affinity of fibroblasts for BNC. They have managed to coat BNC nanofibers with a cellulose binding module, combining with the sequence of amino-acids glycine-tyrosine (a glycine-arginine-glycine-asparagine-tyrosine complex is formed). The results demonstrate that fibroblasts exhibit efficient interaction with BNC because of this complex.

The above-mentioned studies confirm that various peptides improve cell adhesion.

Biocompatibility of bacterial cellulose. Biocompatibility of BNC means that, being in contact with living tissues, it does not cause any toxicity or immunospecific adverse effects. A good biocompatibility was described by Klemm et al. [30] for tubulous hollow BNC implants used as vascular grafts. Perfect results of BNC biocompatibility were demonstrated in rats after subcutaneous implantations within the period of 12 weeks. A total absence of fibrous capsules and macrophages was revealed microscopically, indicating the absence of the organism reaction to short- and long-term implantations.

Mendes et al. [45] evaluated tissue reaction to the presence of the BNC membrane after subcutaneous implantation in mice. The authors analyzed histologic parts of the membrane and surrounding tissues on the 7th, 15th, 30th, 60th and 90th days after the surgery, and did not find any signs of rejection during the whole period of the study. Polymorphonuclear leukocytes and lymphocytes observed on the 7th, 15th, and 30th days after the surgery proved a mild inflammatory response. At the same time, on the 60th and 90th days after the surgery, no inflammatory response was observed.

According to Mormino et al. [61], biocompatibility can be improved by changing the BNC pore sizes. For this purpose, porogens increasing the BNC network porosity were used. Backdahl et al. [62] used starch particles and paraffin wax as pore agents in order to control pore size and interaction with BNC. When starch or paraffin was chemically removed, there appeared areas for various cells to adhere. The studies of Backdahl et al. [62] demonstrated that muscle cells were able to adhere inside the pores and to contract.

Neurosurgical usage of BNC in experimental models and clinical practices. Studies in duraplasty with BNC attract special attention. Mello et al. [63] pointed to a low reaction of BNC as a dural implant in dogs. Xu et al. [64] used BNC to repair dural defects in rabbits. The histologic examinations demonstrated on the 30th day, that BNC was enveloped with connective tissues; the formation of a new periosteal bone tissue was observed on the 360th day. The new bone was difficult to separate from the surrounding

tissues. In this study, BNC was compared with acellular bovine pericardium graft NormalGEN. The results demonstrated that the implanted BNC caused a less pronounced inflammatory response of the organism. This was proven by the low expression level of IL-1 , IL-6, TNF- , inducible nitric oxide synthase, and cyclooxygenase-2, their indices on the 7th–14th days were reliably lower than in the group with the NormalGEN graft ($P < 0.05$). This study proves that BNC can be used to repair dural defects in rabbits, moreover, it does not adhere to spinal cord surface.

Lima et al. [65] performed duraplasty with BNC in 40 Wistar rats divided into two groups: control group, where a synthetic dura Preclud made of inert expanded polytetrafluoroethylene was used; and study group, where BNC was used. The animals were followed up for 120 days. No infectious complications, CSF leak, delayed bleedings, behavior disorders, seizures, or paralyses were observed. The BNC membrane showed a good biocompatibility, and the absence of immune reactions of the organism to its transplantation was observed.

Sanchez e Oliveira Rde et al. [66] created an antenatal model of meningomyelocele during the 74th–77th gestation day in ovine fetuses. All the animals were divided into three groups: control group, where the animals did not receive prenatal corrective surgery, and two study groups, where the animals received corrective surgery using an acellular dermal matrix AlloDerm (Group A) and BNC (Group B).

Group A consisted of four animals, Group B contained six animals. The defects were closed on the 100th gestation day with the implant, subsequently the fetuses were kept in uterus until term. The sheep were put out of the experiment on the 140th gestation day. The fetal spine was macro- and microscopically analyzed. At microscopy, adherence of the material to the skin and nerve tissue was analyzed.

In all cases in Group A, the ingrowth of blood vessels of the recipient's tissue into AlloDerm implant was detected. The implant was adhered to the skin with unclear boundaries. No clear boundaries between the implant and the spinal cord were seen, which confirmed the adhesive process.

In Group B, the Nexfill implant was covered with fibroblasts and connective tissue on the histological section. The fibroblasts formed a new cell layer resembling a new dura mater. Neither the connective tissue nor the collagen penetrated the layers of Nexfill. Moreover, neither proliferation of the blood vessels, cellular ingrowth, nor adhesion to the Nexfill surface was noticed. These characteristics described for Nexfill differed sufficiently from the characteristics observed in Group A, they were statistically significant ($P = 0.029$; F-test). The authors observed adhesion, multiple cell migration, and proliferation of vessels in AlloDerm on all sections. Moreover, these signs were not observed in animals of the Group with BNC ($P < 0.05$).

So, the study of Sanchez e Oliveira Rde et al. [66] has demonstrated that BNC prevents the adhesion of the brain matter, meanwhile the same cannot be said of the use of the dermal matrix. On this basis, the authors consider that BNC reduces the risk of the formation of the tethered spinal cord syndrome because of the absence of the adhesive process.

After the experimental works with the use of BNC in order to close myelomeningocele using rabbit [67, 68] and ovine [69–73] models, the scientists proceeded to clinical research study.

There are two studies devoted to the pathology of the spinal cord in the form of meningomyelocele, i.e. MOMS and CECAM. MOMS was carried out by Adzick et al. [74], it was registered at the web-site: ClinicalTrials.gov under no. NCT00060606. During this study (from February 2003 to December 2010), the randomized trial involving 158 patients with already diagnosed antenatal fetal pathology in the form of meningomyelocele was carried out. Eighty born children were operated on this pathology in the postnatal period, 78 children were operated in the prenatal period.

The open fetal surgery started with the dissection of the neural placode from the surrounding tissue. The dura was identified, separated from the placode, and then the dural defect was closed with a fine running suture. If there was insufficient dura for closure, Duragen was used. If it was impossible to obtain skin closure, relaxing incisions were performed or AlloDerm was used.

The prenatal surgery has led to the improvement of composite score for mental development and motor functions in 30 months ($p = 0.007$), and the improvement of several secondary outcomes and movement of the operated-on children in 30 months. At the age of 12 months, the proportion of infants who did not have evidence of myelocele was higher in the antenatal surgery group (36 %) than in the postnatal surgery group (4 %).

The infants in the prenatal surgery group demonstrated a higher level of functional recovery of the lower extremities, that was two or more levels better than expected (32 % vs. 12 %; $P = 0.005$). The children in the prenatal surgery group had more chances to walk without orthotics than children in the postnatal surgery group (42% vs. 21 %, $P = 0.01$).

Pedreira et al. [75] reported the results of their Phase I CECAM trial in treatment of fetal myelomeningoceles located at the level from L1 to S5 vertebrae. The dural defect was closed with a BNC patch without saturation. In this case, the neural placode was released with endoscopic scissors by circumferential incision across the transition zone. Then, the skin was dissected to put a BNC patch over the defect and to suture. BNC was used in this case. The skin over the patch was closed using a single running stitch with a 2.0 surgical suture (nonabsorbable polypropylene). The neurological status and MRI control were carried out 3, 6, and 12 months after the surgical treatment.

This research notes the decrease in the number of relapses of myelomeningocele and postnatal motor deficit, and 86 % of patients after the endoscopic surgery had no reappearance of myelomeningocele and CSF leak. The motor function of the patients involved in this trial was the same or even better in 85% (6 out of 7) of the treated patients in

comparison with the postnatal surgery in the MOMS study (67 % of cases of those treated intrauterine, 46 % in the postnatal surgery group).

The study of Pedreira et al. [75] demonstrates that the antenatal surgery of myelomeningocele can be performed through the percutaneous endoscopic approach using a BNC transplant and a single layered closure of the skin. The authors consider that the surgical technique and BNC could lead to a sealed dural closure, exclude reappearance of myelomeningocele, and improve motor functions of the patient.

A special attention should be paid to a prospective, randomized, double blind controlled trial carried out on duraplasty; it is deposited on the web-site: ClinicalTrials.gov no. NCT00859508 [76]. The trial involved 99 patients. Sixty-two patients were operated with the use of a BNC implant, 37 patients (control group) with the use of 15 Duraform implants (made of bovine tendon collagen), eight dural regenerative matrices DuraGen II (made of bovine tendon collagen), ten dural graft matrices DuraGen (made of bovine tendon collagen), two dural regenerative matrices Durepair (made of bovine fetal skin collagen), one synthetic material (dural substitute Preclude), and one regenerative matrix DuraGen Plus (made of bovine tendon collagen). The studies were carried out from February 2006 to January 2009. BNC was used in the form of the SyntheCel product registered in the USA and approved by FDA.

The intraoperational data have demonstrated no sufficient differences between the patients with the implanted BNC and the patients of control group in terms of the timing of surgery, volume of blood loss, and intraoperational complications or inpatient stay in hospital ($P \geqslant 1.260$).

The paper demonstrates that the BNS implant is as good as implants of the control group ($P = 0.206$) within the period of six months. The infection process in the zone of the surgical intervention was 6.5 % in the BNC group and 5.4 % in the control group ($P = 1.0000$). There were neither CSF fistulas nor pseudomeningocele in six months in 96.6 % (57/59) patients with the implanted BNC and 97.1 % (33/34) in the control group.

Conclusion

The most important properties of BNC are as follows:

– ultra-thin reticular structure resulted from high-crystalline orientation of cellulose protofibrils;

– insolubility of native BNC;

– high elasticity and transparency (after purification) due to nanoaffinity of fibers;

– hydrophilic properties due to the presence of multiple pores;

– hydrogele-like properties, because at least 95 % of the weight is water, the greater part of which is not bound with the polymer and can be squeezed by soft pressure;

– exclusive chemical purity because of the absence of hemicellulose, pectines and lignin, which are associated with plant cellulose;

– biocompatibility determined by a high purity of the material and absence of toxicological effects on living tissues;

– extremely high tension strength determined by the ultra-thin reticular structure and expressed in high values according to Young's modulus.

BNC implants capable of acting as intercellular matrix, thus creating conditions for circulation of metabolites and oxygen and, simultaneously, preventing the achievement of excess cell concentration, can be widely used in various fields of medicine in the future.

Judging by the presented analysis, it is evident that BNC is necessary for the duraplasty. The existing preclinical and clinical trials confirm prospects of BNC studies in the neurosurgery as implants to repair dural defects in case of pathology of the spinal cord and meninges. Further research in this field would contribute to better understanding the aspects of duraplasty, as well as assist in obtaining and studying BNC-based composite materials and estimate their potential usage, making it possible to choose materials for different situations (hyperproduction of CSF, large-scale dural defects, infectious complications, etc.) and increasing the effectiveness of duraplasty with decreasing the number of complications.

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