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BACTERIAL NANOCELLULOSE AS A MICROBIOLOGICAL DERIVED NANOMATERIAL

ABSTRACT

Bacterial nanocellulose (BNC) is a nanofibrilar polymer produced by strains such as *Gluconacetobacter xylinus,* one of the best bacterial species which given the highest efficiency in cellulose production. Bacterial cellulose is a biomaterial having unique properties such as: chemical purity, good mechanical strength, high flexibility, high absorbency, possibility of forming any shape and size and many others. Such a large number of advantages contributes to the widespread use of the BNC in food technology, paper, electronic industry, but also the architecture in use. However, the greatest hopes are using the BNC in medicine. This text contains information about bacterial nanocellulose, its specific mechanical and biological properties and current applications.

Keywords*: nanomaterials, bacterial nanocelullose, mechanical properties, application in medicine*

Nanomaterials

Nanomaterials its specific properties owes its size. The most striking feature of nanomaterials is a large area boundaries chapter. Depending on the kind of material are the outer surfaces, as in the case of nanoparticles, nanotubes or nanofibers, in nanocrystalline materials are the internal surfaces of the section: the grain boundaries or interphase boundaries. This causes their strong chemical reactivity and a tendency to agglomerate, while diffusion occurs much faster than in the microcrystalline materials. This results include low thermal stability of these materials, much faster overlap of various kinds of phase transformation or facilitated movement of the grains with respect to each other at elevated temperature. At the same time reducing the grain size, according to the Hall-Petch relationship, it causes an increase of yield stress as well as tensile strength or hardness [1,2].

Nanomaterials can be divided by origin into 3 groups: - natural - anthropogenic (a side effect of human activity), - designed. Natural nanoparticles come mostly from thermal processes, eg. Forest fires, volcanic eruptions (eg. volcanic dust) or from the oxidation of minerals, erosion of rocks or evaporation (eg. sea salt formed during the evaporation of a drop of sea water). Anthropogenic nanomaterials are a side effect of human activity. Formed, for example. During the combustion of coal (carbon black) or during welding, or vulcanization of rubber. Nanoparticles may also arise during the mechanical working of materials by cutting,

sawing and grinding. Nanomaterials designed and produced in a targeted manner by man include fullerenes, nanotubes, liposomes, dendrimers or nanofibers.

The range of applications of nanomaterials is still expanding. Particularly high hopes associated with their use in medicine, especially in transplantation. In the currently used implants observed problem is the lack of precise adherence to tissue surfaces, which could be solved by introducing free zones eg. carbon nanofibers or nanotubes [3]. These are particularly preferred material because of their lack of interaction with human tissue. Because of their construction, they also may be introduced into the interior of a bactericidal substance, for example active particles of silver, which further reduces the risk of inflammation. Nanotubes are also used to fill cavities in bone caused by trauma. Studies in mice by Samuel Strupp from Northern University have shown that carbon nanotubes in the presence of fractures within a faster treatment of the injury, as osteogenic cells more easily deposited on such a backbone rebuilding the damaged structure. Using nanomaterials, also they managed to get very encouraging results in the case of a damaged spinal cord in mice. After 6 weeks, due to the self-bonding nanomaterials in a new, larger structure, followed by rebuilding the spinal cord, so that the returned feeling in the legs of mice. Creation of nanostructures in pure titanium increases its strength to the level of melt strength Ti6A14V, what eliminating the problem of negative impact on the human body of alloying elements. Nanoparticles are also used as drug carriers. Depending on the disease entity is introduced into the interior of another medicament [4-7]. Big hopes are using nanoparticles to combat cancer [8-9]. Researchers in the US succeeded in developing a kind of nanogenerator which recognizes the antibody by the tumor cells, and then it lead atoms of radioactive element, for example actinium or a chemotherapeutic agent in tumor cells. They inhibit angiogenesis or destroy cells by radiation. The tumor cells will stop mitosis, resulting in the necrosis. This method is also effective against bacterial infections, which used to detect the fluorescent signal is bacteria. Antibiotics - located in the liposome envelope, via the bloodstream enter the site of infection, and then because of infrared signal they disintegrate releasing the drug substance in a particular place and time. Antibiotic removes only microbes, not affecting healthy cells [10]. Experiments conducted in mice show the high efficacy of such treatments [11]. One of the many nanomaterials used in medicine is also nanocellulose.

Nanocellusose- bacis information

Cellulose is one of the most important naturally occurring polymers, that is a critical material for many large-scale production. For centuries, cellulose from wood is used as the main source of energy. The cellulose fibers are first obtained by chemical reaction of the cellulose (isolated from wood) solution in the mixture of copper hydroxide and aqueous ammonia. This method began to produce rayon and viscose, and then pharmaceutical products, building materials, membranes and coatings. Modern production methods are based on enzymatic, chemical and physical reactions. Production of nano-size cellulose allows for calling it nanocellulose. Considering features, method of production and processing conditions, nanocellulose was classified in 3 main groups: microfiber cellulose (MFC), crystalline nancellulose (NCC) and bacterial nanocellulose (BNC) [12].

MFC was first discovered by Sandberg in the early 80s of the last century [13,14]. MCF production based on the high-pressure homogenization timber, sugar or tomatoes, leading to the formation of fibers and microfibrils release (Fig. 1a). In aqueous they adopt gel form and have pseudoplastic and thixotropic properties (Fig. 1b). The disadvantage of this method proved to be the high production costs resulting from the need for large amounts of energy, more than 25 000 kWh per 1 ton of MFC. Reducing energy consumption resulted in the development of a new method for the production of MFC, consisting of pre-machining of the source of cellulose (by physical, chemical and enzymatic methods) before undergoing a process of homogenization. Isolation of cellulose from cell wall of plants [15], and its stabilization with glucuronic acid residues which bind the cellulose led to easy delamination [16,17], which also has one of the production methods MCF. The main areas of MFC application are currently: cosmetics, pharmacy, medicine and paper industry [12].

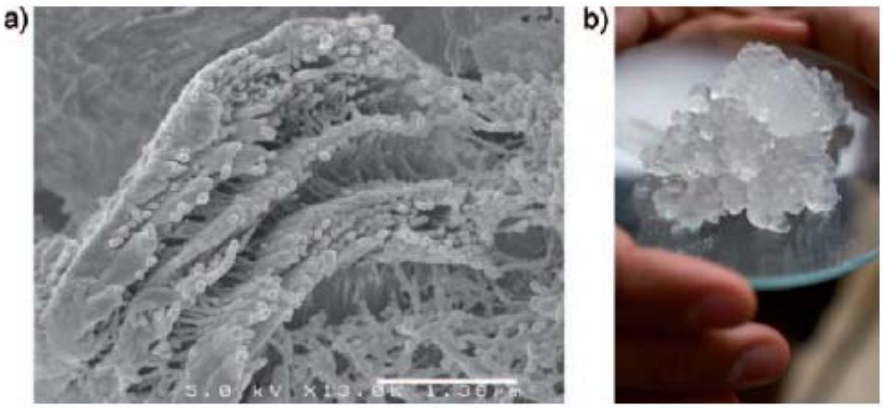

Fig. 1. a) Cell wall of plant cellulose b) MCF hydrogels [12]

Nanocrystalline cellulose is produced by the removal of amorphous parts of purified wood, cotton or algae, by acid hydrolysis. Production of NCC is based on a technique involving homogenization in a magnetic field, which results in formation of regular single domain NCC [18]. The main applications of NCC are: paper industry and food packaging production [19].

Bacterial nanocellulose- basic information

Bacterial nanocellulose (BNC) incorporates meaningful structural elements and properties of the well-known plant cellulose [20–26] with the special features of nanoscale materials. Fig. 2 shows the molecular formula of BNC, that is a repeated connection od D-glucose (dextrose) bulding blocks. The remarkably hydroxyl group-functionalized, lineal rigid chain homopolymer- polymeric dextrose- is characterized by wide chemical modifying capacity, strong hydrophilicity and relevant biocompatibility [27–32]. The molecular structure imparts cellulose with its typical properties such as degradability, chirality, and wide chemical changeability inaugurated by the high donor reactivity of the OH groups. It has ordered and disordered regions, in which the ordered domains are largely crystalline and the disordered molecules hold privileged course parallel to the chains in a very fine fibril or fiber like strand that is called microfibril and they form surface disorder on the microfibrils. Broad hydrogen bond networks give cellulose a plurality of partially crystalline fiber structures and morphologies [33].

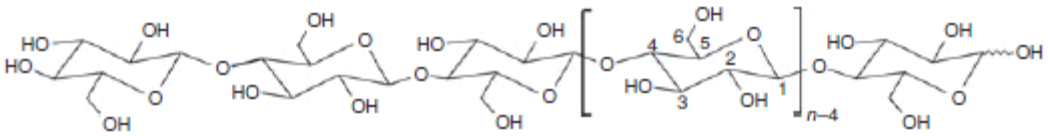

Fig. 2. Molecular structure of cellulose (n = degree of polymerization) [34,35]

The nanofibers of BNC are structures of around 100 μm in length and 100 nm in diameter. These ribbons are made up of bundles of cellulose microfibrils of 2–4 nm in diameter [36,37]. In its native state, BNC is a water-swollen network of cellulose nanofiber. Fig. 3 shows a SEM image of a native BNC network. Grande et al. have used image analysis to measure the morphological properties of dried BNC networks. The average distance between junction points is 0.523 ± 0.273 μm, while the average angle formed by the segments and the x-axis of the nanofibers is 85.64 ± 0.56° [38].

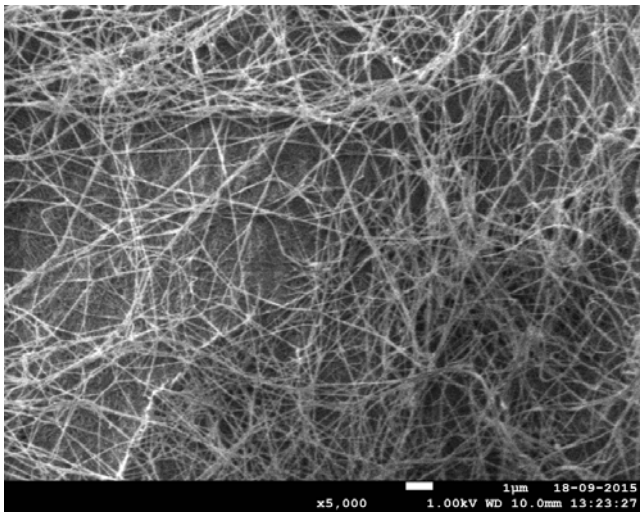

Fig. 3. SEM micrograph of a bacterial cellulose sample showing a coherent 3-D network formed by cellulose fibers

Never-dried BNC has special mechanical properties with a stress-strain behavior that takes after tender tissue [39]. The tensile strength of the BNC pellicle has been measured at 2 MPa, which is an excellent value when taking into consideration the 99% water content. BNC also has great mechanical properties in the dry state, that shows table 1. The stress at break of BNC single fibers is analog to steel [40]. BNC is therefore well suited for use as a reinforcing factor for polymer composites and paper [36]. Proper to the high modulus of elasticity in combination with a big internal loss agent, BNC also is a better material for loudspeaker membranes and headphone [37]

Table 1. *Mechanical properties of bacterial cellulose in dry state [41]*

Material	Young's Modulus (GPa)	Tensile Strength (MPa)	Elongation (%)
Bacterial cellulose	15-35	200-300	1.5-2.0
Polypropylene	1-1.5	30-40	100-600
Polyethylene terephthalate	3-4	50-70	50-300
Cellophane	2-3	20-100	15-40

Production of bacterial nanocellulose

Production of BNC is based on placing the bacterial strain (Gluconacetobacter xylinus most often) in a solution comprising nutrients including glucose as a carbon source (Tab. 2). Solution should be respectively oxygenated, having a pH below 5 and the optimal temperature for the process of manufacture (25- 290C). These parameters determine the efficiency of BNC production, which is synthesized and secreted as exopolysaccharide in contact with air. Furthermore manipulation of the process parameters, for example glycerol additive, can help to form the desired nanofiber network that determines the appropriate properties (Fig. 4).

Table 2. *Selected bacterial strains BNC producing and the conditions needed for the desired culture. Own study based on [42]*

Microorganism	**Carbon source**	**Additive**	**Cultivation time**	**Efficiency (g/l)**
A.Xylinum BRCS	glucose	ethanol, oxygen	50 h	15.30
Acetobacter sp. V6	glucose	ethanol	8 d	4.16
Acetobacter sp. A9	glucose	ethanol	8 d	15.20
Gluconoacetobacter xylinus IFO 13773	glucose	lignosulphonate	7 d	10.10
Lactobacillus mali JCM1116	saccharose	-	72 h	4.20
Acetobacter xylinum NUST4.1	glucose	sodium alginate	5 d	6.0
A.xylinum BPR2001	fructose	agar	56 h	12.00

Fibers created by bacteria cellulose having a diameter in the nanometer range and they pass into the aqueous medium and then combine in the ribbons to in the next stage create characteristic three-dimensional network (Fig. 2). Produced BNC is a white, artificial leather, highly hydrated and flexible membrane [35]. This membrane is subjected to a further purification process. The bacteria and residues from the culture medium are removed by heating in 0.1 M sodium hydroxide solution at boiling point for 10 to 120 minutes (depending on the thickness of the resulting cellulose film) or by repeated washing it at the appropriate pressure. The final stage of BNC production is material compressing (molding), packaging and sterilization. Fig. 5 illustrates process of BNC creating by the Gluconacetobacter bacteria.

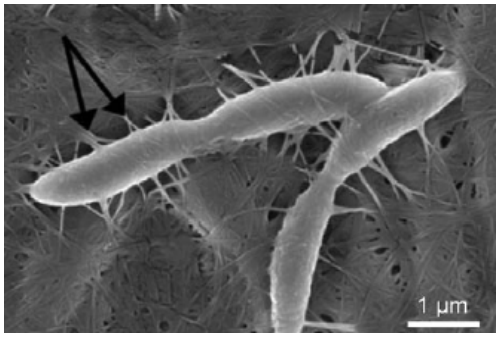

Fig. 4. Gluconacetobacter bacteria involved in the process of creating nanofibers of cellulose [35]

The shape of the resulting cellulose hydrogel can be controlled by selecting appropriate synthesis conditions. The first of these is the type of bacterial culture. Figure 3 illustrates BNC formed by different strains of Gluconacetobacter two types called- fleece with different network structures.

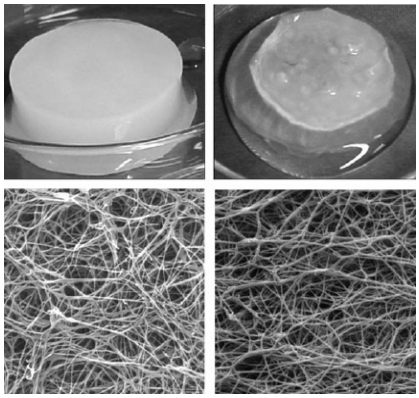

Fig. 5. Cellulose fleece caused by a bacteria Gluconacetobacter DSM 1466 strain (pictured left), and ATCC23769 strain (pictured right) [43]

For obtaining a suitable network nanocellulose structure also affect the water-soluble additives such as glycerol, β-cyclodextrin, polyethylene glycol (PEG 400), or polymerizable monomers which, after the synthesis process can be separated in the purification process. This is a method of in situ forming, which is not only complex addition of compounds to the medium, but also the final processing of the bacterial nonocelullose synthesized [35]. The formation of the hydrogel may take place in a dynamic or static, so that you can give it the shape of a sphere, film, or other forms such as pipes. Moreover, the deposition surface of the bacterial cellulose can significantly affect its shape, as shown in Fig. 6. In the static method among others is achieved membrane in the form of a lobe, that is prepared in a polypropylene container (Fig. 6 a). Using a dynamic method can give spherical shapes of cellulose (Fig. 6 b). Matrix technology allows to obtain tube, which finds its application in the production of vascular implants (Fig. 6 c) [35].

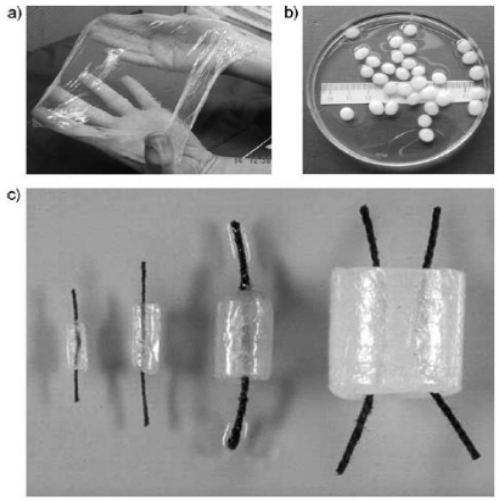

Fig. 6. The forms of bacterial nanocelullose hydrogel [44]

Properties of bacterial nanocellulose

Bacterial cellulose is a biomaterial having unique properties such as: high chemical purity, good mechanical strength, high flexibility, high absorbency, the possibility of forming

any shape and size, inertness and non-toxicity, biocompatibility, biofunctionality and hipoalergenicity [45, 46].

Such a large number of advantages contributes to the widespread use of the BNC in food technology, paper, electronic industry, but also the architecture in use (Tab. 3). However, the greatest hopes are using the BNC in medicine.

Table 3. *Application of BNC in various industries. Own study based on [47]*

Field	Application
medicine	dressing material (artificial leather), vascular implants, dental implants
cosmetology	emulsion stabilizer (creams, tonics), a component of artificial nails
fabrics	textiles, materials with highly absorbent,, materials for the production of tents and camping equipment
paper industry	paper with special characteristics, repair old books, durable banknotes
environmental protection	ultrafiltration water, sewage treatment, absorption of oil pollution, toxins
food	edible cellulose type "nata de coco"
acoustics	membrane speaker
science	protein immobilization, chromatography, the component of the culture medium

Applications of bacterial nanocellulose in medicine

BNC thanks to its valuable properties, contributed to the medicine development in the world. It has been proved that the BNC does not have the cytotoxic or genotoxic properties, so BNC materials are widespread in medicine. The most important applications of BNC rare:

- bioactive implants cartilage - remove proteolytic enzymes, cytokines and reactive oxygen species, protecting the body against the formation of inflammation and protecting it against carcinogenesis; BNC connection with collagen contributes to a more selective reduction of proteases and interleukins, resulting in the antioxidant activity; examples of bioactive implants: cartilage for example: septum of the nose, ear, intervertebral discs (Fig. 7)
  Claim for cartilage material adequate to do the transplant is incomparably greater than the possibility of its purchase. Exaction autologous cartilage for transplantation, it is possible only in a some scope. The major restriction is the age of the patient - the cartilage in children is weakly expanded, while adults often occur degenerative changes. They are looking so materials suitable for prothesis cartilage of a specified mechanical strength and complete unconcern receiver (no immune response and allergic reactions). Predestined to bacterial cellulose for broader use in reconstructive-restorative surgery.

Fig. 7. Examples of cartilage implants, ear, nose and facial bioactive made from the BNC [48]

- prototypes of blood vessels - the tube with a length of 5-25 cm or longer with properties depending on the production conditions, characterized by good stability and mechanical strength and resistance to water, other liquids, ions, small particles; they may be sterilized by conventional methods; most often in medicine can be found as a neurotubes. A typical example of a tube with BNC is shown in Fig. 8.
  The material must base on both: blood pressure of the living body and mechanical strains during microsurgical preparation and anatomizing. The natural BNC has mechanical properties, including tear resistivity and shape retention, which are better than many artificial materials. In collation with organic layers, like polypropylene or cellophane, BNC processed into a film or sheet show excellent mechanical strength. Fulfillment mismatch between the surrounding native tissue and synthetic graft has been reported as a main agent in final misfortune of the currently used cardiovascular graft replacements. Thus, expending biomaterials that display near mechanical properties as the tissue it is replacing is significant target in biomedical equipment design.

Fig. 8. Tube, acting as a blood vessel, made of BNC presented on a red glass, symbolizing the blood flow [49]

- dressing materials - in the form of patches or large lobes; biocompatible, sterile, porous, flexible; a kind of "water jacket" that allows wounds to breathe, heal, prevent the formation of scabs and scars; they reduce pain, protect the skin from infection and do not cause a loss of body fluids; can also be used as protective clothing for miners or employees of emergency services (fire-fighters, soldiers), who often are exposed to burns in contact with the fire [50]. Examples of application of dressing materials to treat skin burns illustrated by Fig. 9.
  High mechanical strength, appreciablel transmission for gases and liquids and low vexation of skin advisable that the coat of BNC was usable as an artificial skin for interim covering of sores. Company Biofill® produce BNC for wide applications in

surgery and dental implants. Second and third degree of burns, ulcers and others cases are successfully treated by Biofill® which is temporary substitute for human skin. Advantages of Biofill® are: prompt pain relief, close adhesion to the wound, faster healing, reduce post-surgery trouble, revised exudates retention, facility of wound control (transparency), reduced infection rate, reduced costs and time of treatment. limited elasticity in areas of great mobility is the only one disadvantage of BNC [51].

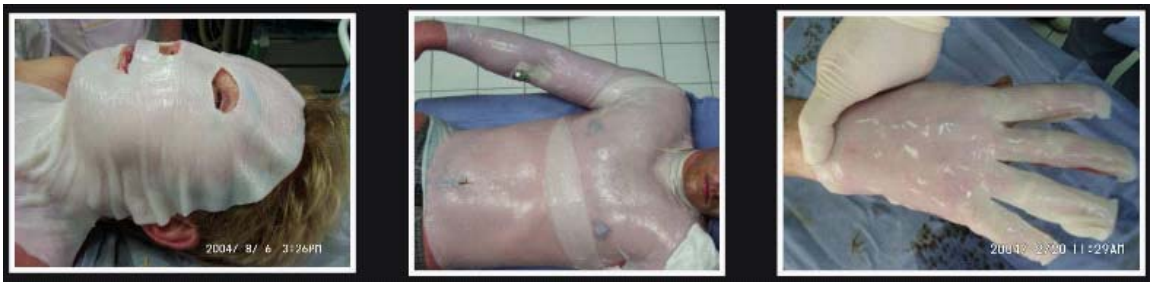

Fig. 9. Dressings made with BNC (CelMat) that are imposed on burned tissue [51]

- surgical implants - can be, for example. tracheotomy tube (reconstructive surgery) (Fig. 10.), artificial heart valves, blood vessels in the form of tubes or neurotubes (regeneration of nerves). As indicated previously conducted studies, BNC covered with epithelial tissue specific to the organ in which it is implanted. These features are particularly important for bioimplants in the circulatory system where inflammation is responsible for the degenerative changes, and the possibility of covering the body's own tissues can bring a negligible effect thrombogenicity.

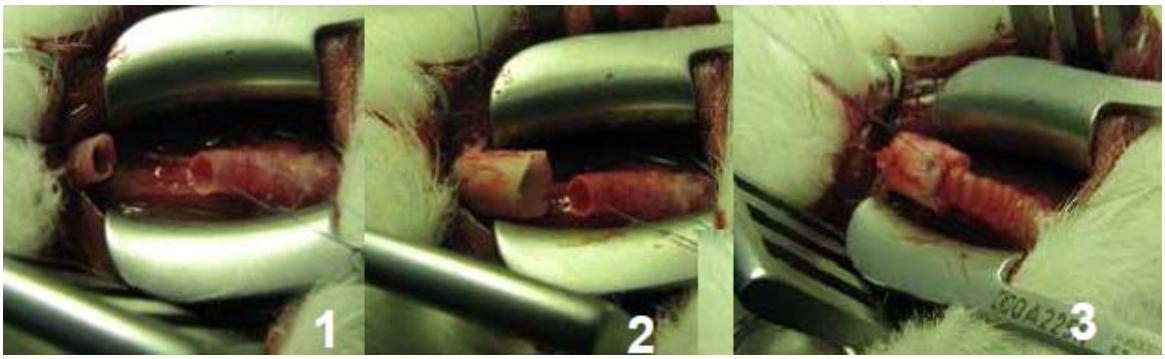

Fig. 10. The reconstruction project of tracheotomy tube [48]

Forecasting future

Easy and unlimited access to cheap and reliable material which is BNC which can perform implants in the circulatory system can bring invaluable effect of increasing the number and shorten the operation. In addition, it is expected to reduce the cost of certain cardiac surgery, up to 20% and increase the quality of life of patients. Production of high-quality biomaterials and pericardium patches with BNC in Poland by domestic entrepreneur will affect the economic development in the biotechnology sector. Potential Application BNC covers a much wider area than cardiac surgery. Determination of the mechanical properties of

BNC and BNC interaction with human and animal organisms (biocompatibility and biodegradation) will consider additional uses BNC. These may include bandages and prostheses for animals and humans, materials in plastic surgery and dentistry, laboratory equipment for testing of biological materials, including various kinds of containers and packaging, substrates for diagnostic tests, and other applications. An additional advantage of the products and waste BNC is that like any cellulose, they will biodegrade in a natural environment Expected future applications of bacterial cellulose go beyond the above-described excellent dressings or implants. Bacterial cellulose is a potential material for other areas of life. Probably soon it will be used in the production of electronic paper, what means that BNC will be use as lightweight, flexible and durable mobile displays [52]. Nanocellulose fibrils constitute in this case, the bonding matrix metal ions and dyes that could be reversibly changing color through voltage change of the electric field between the transparent electrodes. BNC is also a potential material for the production of so-called electroactive polymers, because of the large amount of hydrogen bonds, which stabilize the structure of the cellulose [53]. Controlled hydrolysis of BNC gives mixtures celo-oligosaccharides with a certain degree of polymerization and crystallinity, which may be additive for paper and materials for the production of glues, composites or membranes [54]. Bacteial cellulose is curently use in food industry. The Philippine dessert, Nata de Coco had a major impact on the global outlook for expansion of microbial cellulose production. BNC can be use aa a diet drinks, that have the plasma cholesterol-lowering effect [55]. Monascus-bacterial cellulose complex, which combined the properties of bacterial cellulose and Monascus fungi, showed the potential to be a new foodstuff as vegetarian meat or seafood replacement. Growth of Monascus mycelium did not impart a flavor to this new product creating it a good base as a flavor-added food. The color and texture of the complex were like liver or lean meat. It also provides high fiber content, limited calories and healthy nutrients. Moreover, the waste broth from the fermentation could be further used as a source of water-soluble pigments.

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