IN SEARCH OF THE IDEAL BIOMATERIAL

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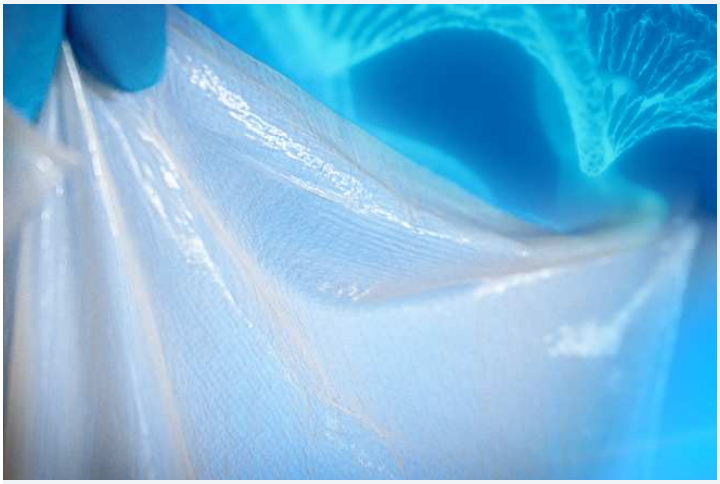
Organ function loss due to injury, congenital defect, resection or disease often poses a risk of death. For this reason, science is continuously searching for new methods for the treatment and regeneration of damaged tissues and organs.

Increasing demand for transplantation organs resulted in intensive progress in disciplines that enable the development of organ substitutes, as biomedical tissue rejection remains a significant obstacle despite the advances in immuno-suppression. Selecting the appropriate material for implant fabrication is one of the key issues in the design of an advanced regenerative medicine treatment process. Will bacterial cellulose become a versatile biomedical engineering medium for soft tissue repair and organ tissue replacement? Are there any prospects for the chemically pure, pyrogen- and endotoxin-free biocellulose material manufactured by *Bowil Biotech Sp. z o. o.* to become the ideal biomaterial for implantation? Is there a chance to continue commercialization of this material for applications in modern reconstructive surgery?
This article attempts to answer these and other questions asked by physicians, scientists and investors interested in the development of large-scale systems intended for manufacturing this versatile biopolymer.

INTRODUCTION

Biomaterials are materials accepted by the human body, permanently connecting with living tissue and supplementing or replacing organ tissues to take over their functions. Various synthetic materials, such as metals, polymers or ceramic materials are used for implantation, however, they exhibit limited physical properties and are difficult to manipulate. They also undergo calcification and degradation or corrosion over time. All synthetic materials are also thrombogenic and create a risk of thromboembolic complications, therefore continuous anticoagulation treatment is required following implantation.
Other serious disadvantages of biomaterials include the risk of cross-contamination, intimal hyperplasia, fibrous reactions and tissue encystations. Tissue or organ transplantation between individuals of the same or different species is often highly problematic and associated with many limitations.

Transplantations of the patient's own tissues are performed in a limited scope – mainly as part of skin, bone or stem cells transplantations – and not always allow for full implant functionality.
Allotransplantations are associated with a number of bioethical issues (definition of death, consent to transplantation, organ trafficking) and are prohibited by some countries and religions due to ethical or cultural reasons. The availability of dead donor organs is still insufficient to meet the needs and is associated with a long and complex procedure meant to reduce transplant rejection risk. On the other hand, xenografts are often preserved with the use of cytotoxic chemicals and carry the risk of transmitting a zoonotic disease from the donor to the human body; they also ignite various fears and concerns. Moreover, breeding genetically engineered animals is seen as an abuse of animal rights and is morally unacceptable for many people.



Materials used for implantation may also be of natural origins and be manufactured in biological systems and living organisms by means of biotechnological processes. Interest that the medical and scientific community takes in such materials is increasing due to their non-toxicity, structures similar to those present in the human body and better integration with the body when compared to synthetic materials.
Society perceives these materials not only as free from any ethical concerns, but also environmentally friendly. From among the natural biomaterials, bacterial polymers, including biocellulose (bacterial cellulose, nanocellulose, microbial cellulose), attract special attention.

This biopolymer is produced by non-pathogenic bacteria (mainly of the *Komagataeibacter genus*) by means of aerobic fermentation. By controlling the synthesis methods, bacterial cellulose can be grown in various shapes and forms, adjusted to exhibit certain functional features.

Molecular formulas of biocellulose and plant cellulose are identical, but the former one exhibits significantly different properties such as:

- three-dimensional, cross-linked, nanofibrillar structure
- ultra purity (without hemicellulose and lignin)
- high absorbency and liquid-holding capacity
- flexibility, resilience and plasticity
- high mechanical and fatigue strength
- selective gas and liquid permeability
- biocompatibility

Due to these properties, bacterial cellulose stands out from among the other known biomaterials and is widely described as the perfect material for biomedical purposes. Possible uses include: tissue scaffolds, drug delivery systems, wound dressings and implants [1,2,3,4]. The nanofibrillar cross-linked structure of biocellulose is similar to that of collagen networks in the extracellular matrix and exhibits an extraordinary impact on cell adhesion and proliferation. Such properties are highly desirable for tissue scaffold production [5].
Biocellulose material may have similar applications to those of collagen, but it has the advantage of not exhibiting immunological reactivity [6]. Sample applications of biocellulose implantation in animals include: bony tissue [7], cartilage [8], skin [9], dura mater [10], tympanic membrane [11], cornea [12], abdominal wall [13], blood vessels [14].
The potential uses of biocellulose in modern surgery seem enormous, but is there a chance for this material to be commercialized as a universal medium for the repair or replacement of tissues or organs in humans? Let's take a closer look at features of bacterial cellulose as the perfect biomaterial that may be placed inside an organism and will provide certain functions in the long term.

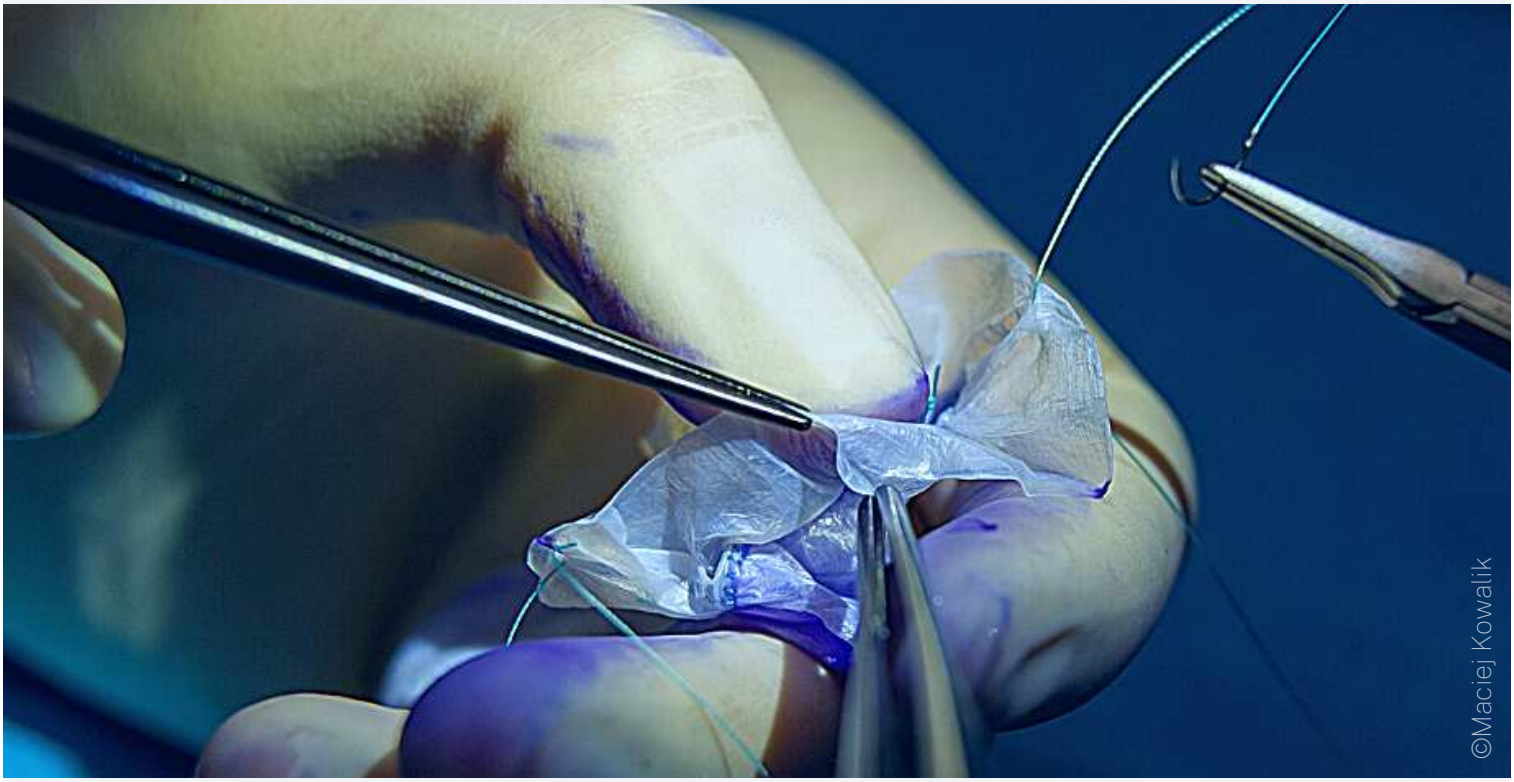
The perfect implantation material

Biomaterials designed for the treatment, repair and support of damaged biological structures should exhibit biological and structural features similar to those of the native extracellular matrix, permanently connecting with living tissue or taking part in its regeneration.

Materials intended for implantation are expected to support cell adhesion and exhibit perfect biocompatibility, stability in the physiological environment, appropriate wear resistance and good manipulative properties during suturing. The possibility of achieving the desired material form and shape, as well as repeatability of the manufacturing process are also important.

Structural stability, no resorption

Preservation of functional properties and the lack of biocellulose resorption was confirmed by numerous implantations of this material in animals. Bacterial cellulose was also incubated in a simulated body fluid and physiological saline, revealing no significant changes in the structure or dry matter. It was confirmed that biocellulose surface significantly hinders bacterial adhesion and colonization [15]. Bacterial cellulose is not a bioresorbable material; it is also not digested nor metabolized in the human body. Active decomposition of this material is not possible, as the human body cannot synthetize the appropriate enzymes that would hydrolyze cellulose.



Biocompatibility, correct healing-in

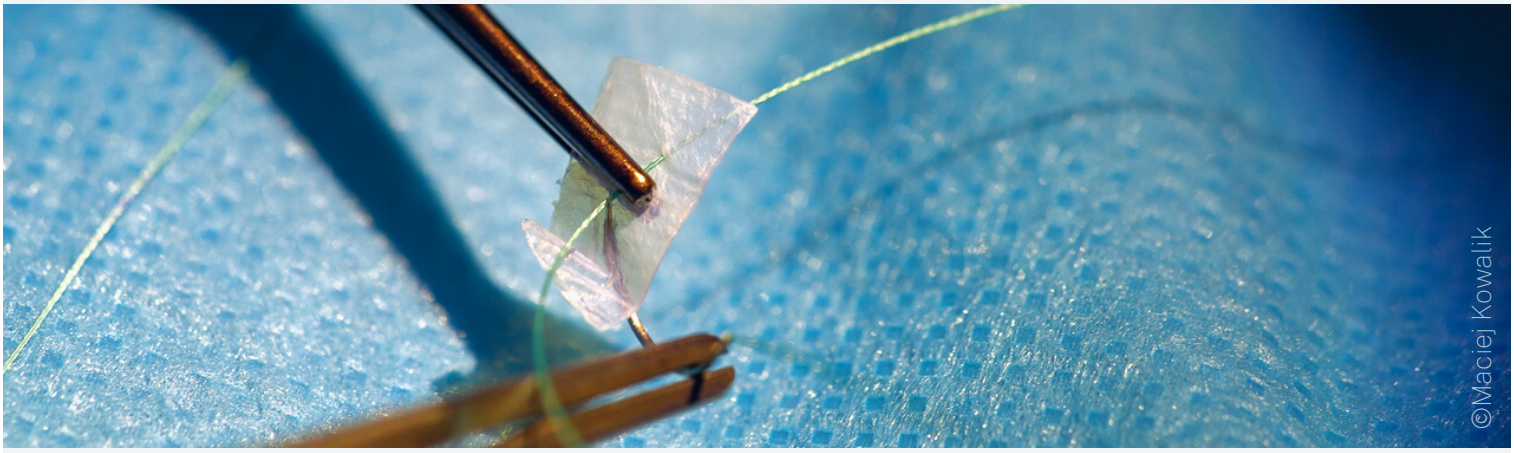
By implanting bacterial cellulose into various organ systems, it was demonstrated that it induces a mild inflammatory response with an intensity comparable to that achieved in control animals and reactions to synthetic prosthetic devices. These changes were considered part of the normal post-surgical procedure healing process [15]. No edema, exudation or fibrous capsule around the implantation sites nor acute local inflammatory responses were observed in the studies, even in the case of a long-term implantation [16,17]. Observations included new blood vessels around the implanted material, cell migration to the material and formation of a homogenous endothelial cell layer on the implant surface [18,19]. Fibroblasts ingrowth and viability on the implant were documented in dura mater surgeries. After several dozen days, collagen fibers also appeared and fibrous connective tissue proliferation continued [20,21].

Biocompatibility in the human body

Clinical studies of patients undergoing duraplasty confirmed the safety and efficacy of an implant made of biocellulose and implanted onto the dura mater. There were no reports of inflammatory mediators activation or cases of cerebrospinal fluid fistula, hemorrhages, behavioral disorders or neurological effects. These studies have also shown superiority over xenografts in terms of exposure to prions or other infectious agents [22]. The biocellulose dura mater substitute used in the studies – *SyntheCel®* – is the only commercially available implant. In humans, bacterial cellulose was also used in myringoplasty procedures, leading to complete regeneration and closure of the damaged tympanic membrane [23].

Correct immunological response of the body

Lack of an excess immunological response of the body was demonstrated in rabbits by placing biocellulose in the conjunctival sac and on the dorsal skin. No cornea, iris or skin irritation was identified; no local inflammatory reactions in the form of erythema, edema or other effects were reported [25]. Bacterial cellulose exhibits weaker immunostimulating properties than collagen and induces one of the weakest immunological responses among polysac-charides [28]. Results of immunohistochemical testing demonstrated poorcell adhesion characteristics, enabling implants to exhibit biological inertness *in vivo* without causing a severe inflammatory reaction [29], neurotoxic nor toxic effects [10].



No genotoxic effects such as mutagenicity, carcinogenicity or teratogenicity

Studies into cell DNA integrity in the presence of biocellulose did not reveal any modifications of fibroblasts or morphology of the studied cells [24]. The potential to induce gene mutation was also verified as part of testing that included: Ames test, chromosomal aberration test, unscheduled DNA synthesis assay and progressive mutation assay. Bacterial cellulose did not cause an increase in the number of mutations and no significant increase in the number of cells with chromosomal aberrations was observed [25,26]. No embryotoxic or toxic effect on reproduction was demonstrated *in vivo* in fertilized rats. No serious anomalies in the course of pregnancy, stillbirths, congenital malformations or visceral deformations were observed; no significant differences in fetal anatomy were identified [27].

Hemocompatibility, low thrombogenic potential

Biocellulose implants did not cause plasma coagulation, did induce a slower coagulation cascade than synthetic prosthetic devices and did not disrupt hemostasis [19,30]. No tendencies for platelet aggregation nor local thrombus formation were identified in operated animals despite the fact they did not receive anticoagulation agents. Presumably the smooth surface of the material prevents clots formation. For this reason, authors of the studies suggest that it is possible to perform biocellulose implantation without antiplatelet therapy [31]. Moreover, *ex vivo* testing with the use of human peripheral blood and a test developed by Schima did not reveal any biocellulose hemolytic activity. It was also found that bacterial cellulose is impermeable to blood (its pores are significantly smaller than the smallest blood cell) [15].

Wound healing support

Biocellulose dressings provide a moist environment within the wound (the material can contain >95% of water) and therefore create the appropriate conditions for healing and proper autolytic dead tissue debridement process: sterility, temperature, slightly acidic physiological pH, access to oxygen and thermoregulation. Moreover, these dressings stimulate keratinocytes proliferation and fibroblasts growth, regulate enzymatic activity and facilitate migration of nutrients and epidermal cells to the wound bed. This enables the correct course of re-epithelization, granulation tissue formation, collagen synthesis and angiogenesis processes [32,33,34].

Appropriate mechanical strength

High tensile strength of biocellulose and its resistance to deformation ensure long implant survivorship in the body, especially in the case of intense or cyclic stress. These parameters strongly depend on the water content in the material and on the method of its removal [35,36]. Changing the biosynthesis method or substrates, adding other compounds or post-production modifications of native biocellulose allow to obtain a material with the specified durability and rheological properties [37,38]. When compared to natural tissues, biocellulose exhibits similar flexibility but significantly higher tensile and tear resistance [5,15].

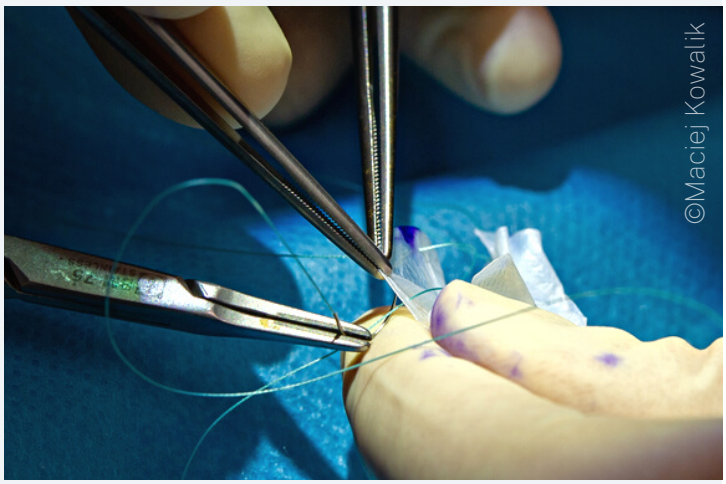
Sterilization process tolerance

Thermogravimetric analyses of bacterial cellulose showed revealed that its degradation temperatures range between 327°C and 370°C, while no material weight loss was demonstrated in temperatures of up to 150°C. Biocellulose is suitable for thermal sterilization, which does not significantly impact the material's mechanical properties [40]. Sterilization using gamma irradiation performed under standardized conditions (≥25 kGy) does not cause macroscopically detectable integrity changes of the biocellulose hydrogel and is safe for its functionality. Sterilization with the use of standard methods eliminates the need to use glutaraldehyde which contributes to calcification of biological implants.

Perfect compliance to surgical suturing

High plasticity and softness of biocellulose ensures good integration with the patient's tissues. The material does not fray and is easy to manipulate, cut and puncture with a needle.

Bacterial cellulose provides high suture retention durability, which is crucial for an implantable material to be able to secure and maintain the implant position in the body. Biocellulose is a multidirectional material, therefore it does not require taking the cutting or suturing direction into account. It also does not crease nor fold [39]. Its wet and soft form ensures perfect adhesion even to irregular tissue. On the sutured tissue, biocellulose creates a seamless lamination and thus prevents implant migration and promotes collagen deposition [29].



Sustainable manufacturing process without the use of animal-based materials

As a vegan material, bacterial cellulose does not carry the risk of exposure to prions or other infectious agents of animal origin and does not give raise to concerns of an ethical nature. The process of obtaining biocellulose by means of aerobic fermentation is carried out with the use of harmless and environmentally safe technologies. Renewable resources, including sugar industry by-products, distillers grains or biomass may be used as a source of carbon.

Additionally, as a biodegradable material of biological origin, bacterial cellulose is suitable for composting and it decomposes into natural, non-toxic monosaccharides occurring naturally in the environment [40,41].

SUMMARY

From among the biomaterials used for implantation, natural polymers such as bacterial cellulose offer a very high utility potential.

Due to their better integration with the organism when compared to synthetic materials, non-toxicity and structures similar to those present in the human body, there is growing interest in such materials.

The unique properties of biocellulose, such as biocompatibility and stability in the body, as well as the appropriate mechanical and manipulation properties make it perfectly suitable for applications in tissue engineering and reconstructive surgery. Bacterial cellulose is not only a very functional alternative for synthetic materials, but also an ecological alternative, manufactured in an environmentally friendly biotechnological process. The potential of this “customized” material may be used in many disciplines for tissue reconstruction, implantation of its various forms in the human body, as well as for innovative practices in regenerative medicine and modern surgery.

The chemically pure, pyrogen- and endotoxin-free biocellulose material is manufactured by **Bowil Biotech Sp. z o.o.** Our plant is the world’s only industrial scale manufacturer of CelMat® medical devices and biosynthesized cellulose that meets the standards set forth for bioimplants. Advanced technologies, rigorous quality control and comprehensive supervision over the manufacturing process guarantee user safety and efficacy of the manufactured medical devices.

Bowil Biotech is an innovative biotechnological company that industrialized many years of Polish scientific and technical studies. As it focuses on the development of new applications for biocellulose, the company is active in the international arena by carrying out R&D projects in the field of medicine, biotechnology and cosmetology. Bowil Biotech builds lasting business relationships in an ethical and reliable manner, having regard to the impact of its own initiatives on quality of life, the environment, as well as public and economic interests.

We look forward to our cooperation.

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