

APPLICATION OF BACTERIAL NANOCELLULOSE MEMBRANES FOR EPITHELIAL TISSUE REPAIR

APLICAÇÃO DE MEMBRANAS DE NANOCELULOSE BACTERIANA PARA O REPARO DE TECIDO EPITELIAL

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ABSTRACT

Objective: With the increasing growth of populations prone to wound-healing complications there is an urgent need for novel strategies to both prevent and treat this substantial biomedical burden. One therapeutic approach of particular relevance to wound healing is tissue engineering (TE). This is considered a promising biomedical technology, which aids and increases the repair and regeneration of deficient and injured tissues. Bacterial Nanocellulose (BNC) synthesized in abundance by *Gluconacetobacter hansenii*, is reported to function as a scaffold for the regeneration of a wide variety of tissues, showing that it could eventually become an excellent platform technology for medicine. Previous studies say that BNC has a distinctive nanofibrillar structure that may become a perfect matrix as an optimal wound healing environment. **Method:** Therefore, based on fundamental concepts of tissue engineering, this review addresses the broad application of BNC membranes as an engineered scaffold, aiming to contribute for a better understanding of its potential application for epithelial tissue repair.

Keywords: Bacterial nanocellulose. Wound healing. Tissue engineering.

RESUMO

Objetivo: Com o crescimento das populações propensas a complicações na cicatrização de feridas, existe uma necessidade urgente do desenvolvimento de novas estratégias para prevenir e tratar este problema substancial na área da saúde. Uma abordagem terapêutica de particular relevância para a cicatrização de feridas é a engenharia de tecidos (ET). Esta é considerada uma tecnologia inovadora, a qual auxilia na reparação e regeneração de tecidos danificados. A nanocelulose bacteriana (BNC), sintetizada em abundância pela bactéria *Gluconacetobacter hansenii*, é conhecida por funcionar como um excelente *scaffold* para a regeneração de uma ampla variedade de tecidos, evidenciando que poderia vir a ser uma tecnologia biomédica promissora. Estudos anteriores relataram que a CB possui uma estrutura nanofibrillar distintiva a qual é capaz de formar uma matriz que proporciona um ambiente ideal para a cicatrização de feridas. Portanto, com base em conceitos fundamentais da engenharia de tecidos. **Método:** Esta revisão aborda a ampla aplicação da membrana de CB como *scaffold*, com o objetivo de contribuir para uma melhor compreensão da sua potencial aplicação para o reparo de tecido epitelial.

Palavras-chave: Celulose bacteriana. Cicatrização de feridas. Engenharia de tecidos.

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INTRODUÇÃO

The morbidity and mortality from chronic ulcers of varying etiology represent a significant health care problem⁽¹⁾. Although advances in multidisciplinary wound care have improved clinical outcomes, the economic and social impact of non-healing wounds continues to grow⁽²⁾. With the increasing growth of populations prone to wound-healing complications there is an urgent and unmet need for novel strategies to both prevent and treat this substantial biomedical burden⁽³⁾.

One therapeutic approach of particular relevance to wound healing is tissue engineering (TE), a concept described over 20 years ago⁽⁴⁾. TE has emerged at the intersection of numerous disciplines to meet a global clinical need for technologies to promote the regeneration of functional living tissues and organs. It employs the principles from the fields of materials science, cell biology, transplantation, and engineering in an effort to treat or replace damaged tissues⁽⁵⁾. It is considered a promising biomedical technology, which aids and increases the repair and regeneration of deficient and injured tissues⁽⁶⁾. In the field of reconstructive medicine the development of new innovative matrices for skin repair is in urgent need and clinicians have been looking for better skin substitutes for clinical application⁽⁷⁾.

Many researchers all over the world have been fascinated by the chance of creating a skin-like substitute without any further harm to the patients⁽⁸⁾. In this perspective, our laboratory is currently focused on the development of biologically and physiologically competent skin substitutes by using bacterial nanocellulose (BNC) biomembranes as scaffold for development of new strategies for tissue repair⁽⁹⁾. BNC is a natural cellulose with nanofibrous structure and it is biosynthesized by by certain bacteria, including the bacterium *Gluconacetobacter hansenii*, formerly known as *Acetobacter xylinum*⁽¹⁰⁾. In addition to its common use in medical applications as wound dressings, temporary artificial skin and artificial blood vessels, BNC is currently expanding its

use as a biomaterial with 3D nano-network for scaffold preparation in tissue engineering field⁽¹¹⁾.



Figure 1: Biomedical applications of BNC-based materials.
Source: Prepared by the author

It is clear from previous studies that materials derived from BNC can provide a promising future for biomedical application⁽¹²⁾. Therefore, based on fundamental concepts of tissue engineering, this review addresses the broad application of BNC biomembranes as an engineered scaffold, aiming to contribute for a better understanding of its potential application for epithelial tissue repair, especially as a skin substitute.

TISSUE ENGINEERING

The past three decades have seen the emergence of an endeavor called Tissue Engineering (TE) in which scientists, engineers, and physicians apply tools from a variety of fields to construct biological substitutes that can mimic tissues for diagnostic and research purposes and can replace (or help regenerating) diseased and injured tissues⁽¹³⁾.

In the context of Regenerative Medicine (RM) TE has been addressed for many years as one of the most important topics in medicine in the twenty-first century. TE can be broadly classified into therapeutic applications, where the tissue is either grown in a patient or grown outside the patient and transplanted, and diagnostic applications, where the tissue is made *in vitro* and used for testing drug metabolism and uptake, toxicity, pathogenicity, etc.⁽¹⁴⁾. While the first clinically relevant TE efforts

were mainly concerned with the generation of bioengineered skin substitutes, subsequently applications have been continuously extended to a wide variety of tissues and organs⁽¹⁵⁾. The main targets are those tissues that are prone to injury, disease, and degeneration. Corresponding organs that have been the targets of tissue-engineered equivalents are listed in Table 1.

Table 1: Overview of tissue engineered organs.

Tissue	Function	Approach	Challenges
Skin	Barrier for the body	Matrix implanted to guide regeneration; implants with autologous or allogeneic cells	Lack of appendages, slow process for growing cells, slow vascularization
Cornea	Transparent barrier for the eye	Matrix implants; extracellular matrix generated by cells cultured ex vivo	Maintain transparency and barrier properties of the matrix
Liver	Detoxification, production of liver-specific proteins	Hepatocytes from xenogeneic, allogeneic or stem cell-derived sources, or immortalized hepatoma seeded in implantable matrices, extracorporeal bioreactor systems	Cell source, maintenance of hepatic function, high cell density, vascularization of implants
Pancreas	Secrete insulin to maintain glucose homeostasis	Free or encapsulated islet transplantation	Choice of transplantation site, vascularization, cell source, immune rejection
Cartilage	Critical component of joints	Matrix implanted to guide regeneration; implants with autologous or allogeneic cells	Slow process for growing cells, control of cell differentiation, host integration, long-term durability
Heart	Provides blood circulation	Materials, including decellularized organs, seeded with progenitor and stem cells differentiated into cardiomyocytes	Tumorigenicity, control of cell differentiation, electrical integration
Kidney	Regulates body fluid volume and pH, metabolite excretion	Stem cell-derived nephrons cultured ex vivo	Replicating glomerular selectivity while retaining high hydraulic permeability

Tissue	Function	Approach	Challenges
Neurons and spinal cord	Send electrical stimuli to control bodily functions	Materials shaped as tubes for axonal guidance and regeneration, sometimes used in combination with anti-inflammatory strategies; neural stem cells	Reconnecting proper axons, controlling proinflammatory environment, preventing scar tissue formation

Source: Adapted from Berthiaume et al., 2011⁽¹³⁾

Most TE utilizes living cells, and supplying enough cells is an important issue. The process starts with a source of cells derived from the patient or from a donor. Cells may be immature, in the stem cell stage, or cells that are already capable of carrying out tissue functions. Stem cells possess high proliferative capacity and ability to differentiate into multiple lineages, which makes them attractive for deriving large cell quantities⁽¹³⁾. An important feature for effective tissue engineering is the cellular environment that mimics some critical aspects of the in vivo setting through proper control of the materials and mechanical setting. Persuading cells to form tissue is inherently an engineering process as they need physical support (typically in the form of some sort of 3D scaffold) as well as chemical and mechanical signals provided at appropriate times and places to form the intricate hierarchical structures that characterize native tissue⁽¹⁴⁾.

An important feature of TE includes the scaffold, which provides an architecture where seeded cells can organize and develop into the desired organ or tissue prior to implantation. Scaffolds are porous, degradable structures fabricated from either natural materials (collagen, fibrin) or synthetic polymers (polyglycolide, polylactide, poly(lactide coglycolide)). They can be sponge-like sheets, gels, or highly complex structures with intricate pores and channels fabricated using new materials-processing technologies⁽¹⁴⁾.

Scaffold provides an initial biomechanical profile for the replacement tissue until the cells produce an adequate extracellular matrix during

the formation, deposition, and organization of the newly generated matrix: the scaffold is either degraded or metabolized, eventually leaving a vital organ or tissue that restores, maintains, or improves tissue function. Properties of biocompatible scaffolds can be considered from different aspects including optimal nutrient and waste transport, delivery of bioactive molecules, material degradation rate, cell-recognizable surface chemistries, mechanical unity, and the ability to promote signal transduction pathways⁽⁵⁾. The success of tissue organization and development mostly depends on these properties, because they can eventually induce cell adherence, nutrient/waste transport, cell differentiation, cell viability, and matrix organization.

Indeed, a powerful tool to provide additional exogenous stimuli for the engineered tissue construct to achieve long-term success is the bioreactor. Bioreactors have gained increasing attention as a vessel in which various parameters can be precisely controlled. It can be designed to provide the desired conditions for the cells to regenerate functional tissue⁽¹⁶⁾.

TE and development of complex tissues or organs are still a challenge in twenty-first century and researchers are practically focused on four main points which need optimization: biomaterials, cell sources, vascularization of engineered tissues, and design of drug delivery systems⁽¹⁷⁾. Biomaterials and cell sources should be specific for the engineering of each tissue or organ. On the other hand, angiogenesis is required not only for the treatment of a variety of ischemic conditions, but it is also a critical component of all tissue-engineering strategies. Therefore, controlling the dose, location, and duration of releasing angiogenic factors via polymeric delivery systems, the utility of a variety of biomaterials in tissue regeneration will be dictated.

TE is an emerging technology with some encouraging early clinical applications. The potential implications for patient treatment and economic impact are enormous. Close interdisciplinary communication and cooperation among chemists, materials scientists, biologists, and clinical scientists is crucial⁽¹⁸⁾.

STATE OF THE ART ON ARTIFICIAL SKIN

The skin, the largest organ of the human body, is organized into an elaborate layered structure consisting mainly of the outermost epidermis and the underlying dermis. Epidermis consists of layers of keratinocytes separated from the dermis by the basement membrane. The dermis is composed of the predominating extracellular matrix (ECM) [collagen, elastin, and glycosaminoglycans (GAGs)] and the fewer cellular constituents of mainly fibroblasts. A subcutaneous adipose-storing hypodermis layer and various appendages such as hair follicles, sweat glands, sebaceous glands, nerves, lymphatics, and blood vessels are also present⁽¹⁹⁾. These multiple components of the skin ensure survival by carrying out critical functions such as protection, sensation and regulation.

Loss of integrity of large portions of the skin due to injury or illness may result in significant disability or even death⁽²⁰⁾. Most skin wounds can heal naturally, but extensive and irreversible wounds necessitate coverage using skin substitutes to aid repair and regeneration. With the increasing growth of patients with dysfunctional wound healing, there is an urgent need for novel strategies for tissue repair. TE offers the potential to create functional skin, and the synergistic efforts of biomedical engineers, material scientists, and molecular and cell biologists have yielded promising therapies for non-healing wounds.

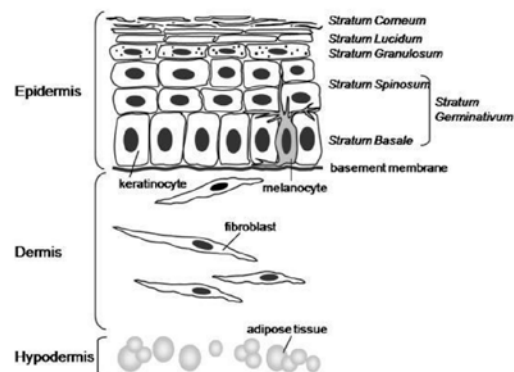


Figure 2: Representative figure of skin draw showing its components and layers.
Source: Brohem et al., 2011⁽¹⁹⁾

As cells making up human skin tissue grow within an organized three-dimensional (3D) matrix surrounded by neighboring cells, standard monolayer (2D) cell cultures do not recapitulate the physiological architecture of the skin. Engineering skin substitutes have relied upon the creation of 3D scaffolds as ECM analog to guide cell adhesion, growth, and differentiation to form skin functional and structural tissue. 3D scaffolds can not only cover wound and give a physical barrier against external infection as wound dressing, but also can provide support both for dermal fibroblasts and the overlying keratinocytes for skin tissue engineering. A successful 3D scaffold must have a highly porous structure and good mechanical stability. High porosity and optimally designed pore size provides structural space for cell accommodation and migration and enables exchange of nutrients between the scaffold and environment⁽²¹⁾.

Several types of substitutes like cultured allogeneic and autologous keratinocytes, allogeneic and autologous composites, acellular matrices, matrices based on biological substances such as collagen/hyaluronic acid, and matrices seeded by different cell types (keratinocytes, dermal fibroblasts, stem cells) already exist⁽³⁾. A representative figure of an artificial skin can be seen in Figure 3.

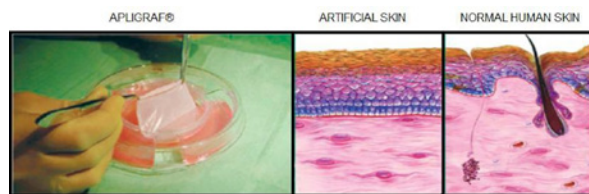


Figure 3: Bilayered skin substitute, Apligraf®.
Source: Adapted from Zhong et al., 2010⁽²²⁾

The ideal tissue-engineered skin product should offer: protection - by establishing a mechanical barrier to microorganisms and vapor loss; promotion - by delivering components, cytokines, and growth factors, which can promote and enhance natural host wound healing responses; and provision - of new structures, such as dermal collagen or cultured cells, that are incorporated into the wound and persist during wound healing⁽²³⁾. Several products have been

used to treat extensive acute wounds (especially burns) as well as to promote healing of chronic nonhealing wounds such as diabetic ulcers and venous ulcers. Some of tissue-engineered medical products that are available in the market are listed in Table 2.

Table 2: Representative skin substitutes approved by the U.S. Food and Drug Administration.

Product/ manufacturer	Scaffold material	FDA-approved indications	Competitive advantages
Alloderm® LifeCell corporation, USA	Human acellular lyophilized dermis	Burns/full- thickness wounds	Not rejected; 2-year shelf life
Apligraf® Organogenesis Inc, USA	Bovine collagen	Venous/ diabetic ulcers	Mimics function of dermis; cryopreserved product
Dermagraft® Advanced BioHealing, USA	Polylactic acid/ Polyglycolic acid, ECM	Diabetic foot ulcers; ulcers secondary to epidermolysis bullosa	Mimics function of dermis; cryopreserved product
Epicel® Genzyme Biosurgery, USA	-	Deep partial- thickness and full-thickness burns; congenital nevi	Autologous cells; no rejection, high incidence of permanent take
Integra® Integra NeuroSciences, USA	Polysiloxane, bovine cross linked tendon collagen	Deep partial- thickness and full-thickness burns	Two layers; good barrier function; used in>10,000 patients; moderate shelf life
OrCel® Ortec International, USA	Bovine collagen sponge	Split-thickness donor sites; mitten hand deformity surgery of epidermolysis bullosa	Mimics cytokine expression of healing skin; 9-month shelf life cryopreserved
TransCyte® Advanced BioHealing, USA	Silicon film, nylon mesh, porcine collagen	Full and partial- thickness burns	1.5-year shelf life; frozen

Source: Adapted from Priya, S. G. et al., 2008⁽²⁰⁾

Recent development in skin substitutes research aims gradually to establish a fully functional skin substitute which could mimic skin not only by its structure, but which could be capable to assure also its revascularization, reinnervations, and replacement of skin appendages. Skin replacement has been a challenging task for surgeons ever since the introduction of skin grafts by Reverdin in 1871⁽²⁰⁾. It represented the earliest attempt at engineered tissues, and by the

1990s commercial development of these products had begun⁽²⁴⁾. The first tissue-based therapies developed were skin grafting techniques. The first synthetic skin substitute was developed in 1962; however, the first successful tissue-engineered skin products were made in the late 1970s and early 1980s^(25,26). Interestingly, this product which was a matrix for regeneration of the dermis (Integra[®]) was allowed for sale only in 1996⁽²⁷⁾. In 1998, a medical device containing allogeneic cells (Apligraf[®]) was approved by the U.S. Food and Drug Administration (FDA) as a skin substitute that could be used for the treatment of venous leg ulcers⁽²⁸⁾.

One of the main limitations of engineered skin substitutes is slow revascularization and in some instances poor attachment to the wound bed. Tissue-engineered skin also lacks several important structures and cell types, including sebaceous glands and sweat glands as well as melanocytes and dendritic or Langerhans cells. Although it is difficult to recreate human skin entirely, if cell science, molecular biology, genetic engineering, material science and clinical expertise join their efforts to develop optimized techniques, further advances will lead to the successful production of tissue-engineered products resembling natural human skin in a near future.

BACTERIAL NANOCELLULOSE (BNC) BIOMEMBRANES

Several biopolymers have been investigated for applications such as scaffolds, among which stands out the BNC⁽²⁹⁾. Cellulose is well known as one of the most abundant biodegradable materials in nature and has been the topic of extensive investigations in macromolecular chemistry⁽¹²⁾. Cellulose is a linear homopolymer of glucose ($C_6H_{10}O_5$)_n with n ranging from 500 to 5000, and it is a widespread polymeric material in nature⁽³⁰⁾. It is insoluble in water and it is degraded by specific enzymes⁽³⁰⁾. Cellulose-based polymers have wide applications in tissue engineering, controllable delivery systems, blood purification, sensor,

agriculture and water purification⁽¹²⁾. Figure 4 represents the chemical structure of cellulose:

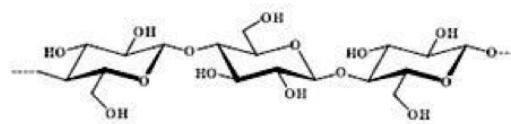


Figure 4: Chemical structure of cellulose.
Source: Prepared by the author

BNC is a promising natural cellulose with nanofibrous structure and it is biosynthesized by certain bacteria, including the bacterium *Gluconacetobacter hansenii*, formerly known as *Acetobacter xylinum*⁽¹⁰⁾. This bacterium constructs a cellulose film between the culture medium and the gaseous surface, which has a dense layer on one side and a thick gelatinous (and porous) layer on the opposite side⁽³²⁾. In addition to its common use in medical applications as wound dressings, artificial skin and artificial blood vessels, BNC is currently expanding its use as an excellent biomaterial with 3D nano-network for scaffold preparation in tissue engineering field.

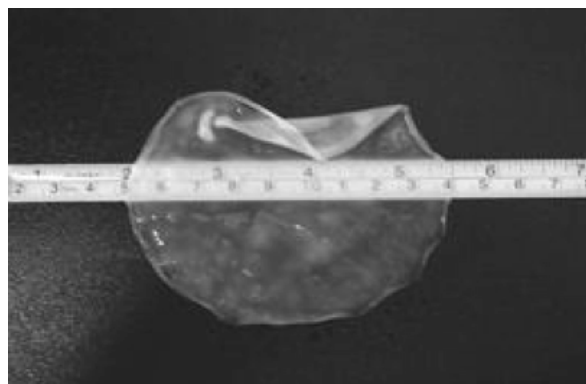


Figure 5: Hydrated micrograph of BNC membrane.
Source: Picture from Intelab research

According to previous studies, BNC has a great potential to be used as a substrate in tissue engineering because of their properties including high capacity to retain water (hydrophilicity), high crystallinity, high tensile strength, biocompatibility and nanofiber-network conformability, which significantly differ from the structure of plant

cellulose⁽³³⁾. This nanofibrillar structure has proven to be a viable matrix to improve tissue repair and has been also used in a wide range of applications as a scaffold for treatment of second- or third degree burn ulcers, for artificial micro vessels and for tissue engineering of cartilage⁽³²⁾. Some of the physical and mechanical properties of BNC which characterize it as an ideal wound dressing material are listed in table 3:

Table 3: Properties of BNC membranes and its correlation with an ideal wound dressing.

Ideal wound dressing	BNC
maintain a moist environment at the wound/dressing surface	high water holding capacity; high water vapor transmission rate
provide physical barrier against bacterial infections	nanoporous structure does not allow any external bacteria to penetrate into the wound bed
highly absorbable	partially dehydrated membrane is able to absorb fluid up to its original capacity
sterile, easy to use, and inexpensive	membranes are easy to sterilize (by steam or γ -radiation) and package. The estimated cost of production of 1 cm ² is \$0.02
provide easy and close wound coverage, but allow easy and painless removal	high elasticity and conformability
provide porosity for gaseous and fluid exchange	highly porous material with pore sizes ranging from several nanometers to micrometers
nontoxic, nonpyrogenic, and biocompatible	biocompatible, nonpyrogenic, nontoxic
provide high conformability and elasticity	high elasticity and conformability
provide mechanical stability	high mechanical strength

Source: Adapted from Czaja *et al.*, 2006⁽³⁵⁾

In order to improve the positive features of BNC for tissue repair, it can also be modified through incorporation of several composites such as collagen, gelatin, alginate, benzalkonium Chloride, poly ethylene glycol (PEG), cotton gauze and *Aloe vera* as well as through incorporation of bioactive molecules, changes in porosity and crystallinity^(34,11). Modified BNC could function as scaffold for regenerating a variety of tissues, which may possibly make an interesting biomaterial for medical devices and consumer products.

When considering the unique properties of BNC, its commercialization for wound care seems

very promising. The BNC topical applications are effective due to the cellulose's water holding ability and water vapor permeability. Also, it molds very well to the surface of the skin, providing a conformal covering even in usually difficult places to dress wounds, such as areas on the face as shown in figure 6:



Figure 6: Application of commercially available BNC biomembranes for burn care.

Source: Adapted from Fu *et al.*, 2013⁽¹¹⁾

The first efforts to commercialize BNC on a large scale were initiated by Johnson & Johnson in the early 1980s. This company pioneered in exploratory investigations on the medical application of BNC in the treatment of different types of wounds but the commercialization was not launched⁽³⁵⁾. BNC has been tested and successfully used as a wound dressing, especially in burn cases⁽³⁶⁾. Studies have shown that burns treated with BNC coverings healed faster than traditional treatments and had less scarring⁽³⁷⁾. This technique has been so successful that commercial microbial nanocellulose products have been developed. The table 4 summarizes some of the commercially available BNC membranes.

Table 4: Commercially available BNC membranes for tissue repair.

Product	Applications	Manufacturer/ Country
Biofill ®	Temporary skin substitute; burns, skin ulcers, cover surgical incisions, traumatic injuries and abrasions	BioFill Produtos Biotecnologicos, Brazil
XCell ®	Venous and chronic ulcers	Xylos corporation, USA
Gengiflex ®	Treatment of periodontal diseases	USA
Bioprocess ®	Venous leg ulcer	BioFill Produtos Biotecnologicos, Brazil
Dermafill ®	Chronic and burn wounds	Cellulose Solutions, USA
Bionext ®	Temporary skin substitute; burns, traumatic wounds, ulcers and chronic wounds in diabetic patients	Bionext Biological Products, Brazil

Source: Extracted from Fu *et al.*, 2013⁽³⁸⁾

There have been several publications and reports on the successful use of BNC in the clinical field. In 1990, Fontana et al. first reported the application of nanocellulose pellicles of varying thickness, as temporary skin substitutes⁽³⁹⁾. The product, called Biofill®, has been used for several skin injury treatments such as basal cell carcinoma/skin graft, severe body burns, facial peeling, sutures, dermal abrasions, chronic ulcers, and both donor and receptor sites in skin grafts. The use of BNC as a permanent skin substitute has been the focus of many researchers but so far there is no report in the literature about its clinical implementation. Taking into account the successful application of BNC membranes for tissue repair, it configures a promising and attractive biomaterial to be used in investigations for the development of artificial skin.

CONCLUSION

Understanding the biology of tissue repair is essential for the improvement of tissue-engineered medical products. Thus tissue engineering techniques bring a new element of rational design and development to therapeutic medicine. One of the main requirements of any biomedical material is that it must be biocompatible, which is the ability to remain in contact with living tissue without causing any toxic or allergic side effects. Some time ago humans have used one form of cellulose or another in medical applications and wound care products. Now, through the serendipity of better understanding a novel form of nanocellulose assembled by bacteria, scientists are positioned to make good use of such material. Because of its unique properties, BNC has been shown to be a highly effective wound dressing material, by improving healing process of burns and chronic wounds. The application of BNC as a permanent artificial skin is not reported in the literature yet, opening the field for further investigations taking into account its promising application for wound repair in the tissue engineering field.

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