Functionalized Bacterial Cellulose-based Biopolymers for Pharmaceutical Applications: Current Research Trends and Challenges

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Abstract

Over the last decade, bacterial cellulose (BC) has been gaining a lot of attention as a platform material for diverse industrial applications. Because of its unique attributes like ultra-high purity, hydrophilicity, crystallinity, surface area, and porosity, BC could be used as biomaterials, wound dressing agents, tissue scaffolds, and vascular grafts in pharmaceutical industries. Despite its well-defined properties, its applicability and ability to compete with other high-performance biomaterials get restricted due to delimitations like lack of antibacterial properties, optical transparency, conductivity, magnetic properties, and stress-bearing capability. It is necessary to understand the current state of the art of these biomaterials and the existing challenges to facilitate their real-time implementation. Thus, the chapter highlights the existing biomedical applications of BCs, delineating their limitations. It further provides an overview of the bioprocessing strategies during the production (in-situ modification) and ex-situ compounding with chemical/biological active substances that enhances the physiochemical and surface properties to tailor-made these biomaterials to improvise their efficacy during biomedical application. The techno-economic issues associated with different physicochemical approaches of functionalization were also detailed. Advances and prospects to overcome the technical issues have been discussed, facilitating the application of the novel biomaterials to revolutionize the biomedical market.

Keywords: *Bacterial cellulose; Biomedical applications; Bioactive compounds; Functionalization*

1. Introduction

Over years much emphasis is being laid on the development of biomaterials from renewable resources, driving the paradigm of research towards more sustainable and greener technologies to develop a circular bio-economy. Cellulose is said to be the most abundant biopolymer found on earth with a myriad of biological sources like plants, tunicates, fungi, algae, and several non-pathogenic, aerobic bacteria of the genera *Sarcina*, *Rhizobium*, *Agrobacterium* and *Gluconaacetobacter* (formerly *Acetobacter*) **(Ullah *et al.*, 2016)**. The formation of bacterial cellulose pellicle floating in an acetic acid medium was first noticed and reported by Brown in the year 1886 **(Brown, 1886)**. It was produced by *Bacterium xylinum*, which was later retitled as *Acetobacter xylinum*, then given the name of *Gluconacetobacter xylinus*, and since the last two years again renamed as *Komagataeibacter medellinensis* **(Römling & Galperin, 2015)**. These bacteria are often found in rotten fruits and vegetables, fermented foods (for example, kombucha, nata de coco, vinegar) and have the capacity to produce acetic acid by oxidizing alcohols, aldehydes, and sugar in the presence of oxygen. *Gluconoacetobacter* and *Komagateibacter* genera extracellularly release the cellulose into the fermentation medium for their protection against desiccation and ultraviolet light (UV) damage, also known as bacterial cellulose (BC) **(Raghavendran *et al.*, 2020)**. The highly porous network of BC helps in the exchange of nutrients and oxygen, necessary for the survival of bacteria. The unique range of properties presented by these bacteria-derived cellulose has been exploited for several applications in both native and composite forms, as discussed in the subsequent sections.

Komagataeibacter (formerly *Gluconacetobacter*) *xylinus* is the most commonly exploited, gram-negative obligate aerobic bacteria used for the commercial production of BC as it can utilize a wide variety of carbon and nitrogen substrates **(Saxena & Brown, 2016)**. The traditional synthetic medium for growing acetic acid bacteria is the Hestrin and Schramm (HS) medium which consists of glucose, peptone, and yeast extract. The yield of BC can be increased by adding methanol in the HS medium and small amounts of endoglucanase in the production culture. The production costs can be reduced by using cheaper substrates such as agricultural wastes like rotten fruits and vegetables, industrial by-products like tea processing waste, corn-steep liquor, food-agro residues, molasses, and date syrup, and petrochemical and wood processing wastes. BC can be produced through static, agitated, or stirred fermentation operations, with each producing varied forms of cellulose. For the production of BC, under

static conditions, aerosol, horizontal lift bioreactors, and rotary biofilm contractors have been developed to provide a large surface area as the BC is synthesized at the air-liquid interface. Fibrous suspensions, spheres, and pellets are formed under agitated and stirred fermenter conditions. BC produced under static conditions has superior mechanical strength, higher yield and less branching as compared to BC synthesized under stirred and agitated conditions **(Gregory *et al.*, 2021)**. Thus, based on the targeted application and required properties, the most suitable operation mode can be chosen to alter the BC microstructure as per requirement. BC produced via static operation was used for wound dressing and scaffold material in biomedical applications, whereas the pellicles synthesized under agitated operation were used in adsorption of heavy metal ions, oils, and organic solvents along with immobilization of enzymes. The downstream processing of BC involves harvesting manually or by filtering the culture medium followed by mild alkali treatment to remove cell debris and other contaminants.

In the year 2019, the global BC market was approximately USD 299 million and is expected to reach around 777 million by 2027. Although most of the research is still being conducted at the lab-scale level using the biopolymer, only some of the industries like Xylos Co. (USA) have been able to commercialize BC under the name Prima Cel™ for wound dressing along with Biofill® and Gengiplex® developed for tissue regeneration. A German company, Fzmb GmbH, has established itself as the largest producer of BC for cosmetic and biomedical applications **(Ullah *et al.*, 2016)**. The present chapter emphasizes the physicochemical properties of the versatile BC, which makes it suitable for use in a variety of biomedical applications. Further out of the pool of diversified applications researched so far, a few have been discussed in detail. Lastly, the development of BC composites to overcome the limitations and an overview of the techno-economic challenges faced has also been provided.

2. Physiochemical properties contextualization of bacterial cellulose

Researchers have already established novel ways of producing microbial or bacterial cellulose in both lab and pilot scale. For widening the utilization of the sustainable BC based material, its intrinsic properties need to be enhanced for its valorization in the paper, textile, food, pharmaceutical, materials and packaging, biomedical, and cosmeceutical industries, among others. It is a natural, homogenous polymer with a linear arrangement of D-glucopyranose units connected with β linkages and has an average degree of polymerization of about 9000-10000 **(Gallegos *et al.*, 2016)**. The operon which regulates the multistep BC synthesis called

*bcs*ABCD and its related genes were first identified in *K. xylinus.* They help in encoding the required proteins and enzymes, which help in developing the 3D network of cellulose through linear polymerization of glucose units **(Portela *et al.,* 2019)**. Also, the numerous reactive hydroxyl groups available help in establishing interactions with over 90% of water molecules by forming plentiful inter-and intra-molecular hydrogen bonds, which results in high water absorption and retention, chirality, biodegradability and leaves tremendous scope for functional modification, allowing its use in a plethora of applications **(Gregory *et al.,* 2021)**. Thus, BC offers the possibility of developing custom-modified cellular matrices to introduce new or improve existing properties according to the requirement. It has water-holding capacity of 70-800 times its dry weight, depending on the culture conditions. The structure of BC is almost the same as that of plant cellulose, with the advantage of being devoid of lignin, hemicellulose, pectin, and other biogenic products commonly present in plants **(Rahman & Netravali, 2016).** It is100 times thinner presenting a dense 3D network. Thus, making it highly pure with special physicochemical characteristics due to its super molecular network with simpler harvesting resulting in minimal requirement for downstream processing.

Due to the diverse nature of BC compared to plant cellulose, its applicability is not restricted only to biological sciences and chemistry but also extends to engineering and physics **(Hu *et al.,* 2014)**. It possesses fiber-binding ability, transparency, biodegradability, biocompatibility, and moldability, among others **(Shezad *et al.,* 2010),** making it a versatile biopolymer of commercial interest. Due to its high porosity, it allows the free exchange of fluids, and it has a very high water absorption capacity which helps in maintaining a moist environment. It stands out to be a potential candidate for several biomedical applications due to its ribbon-shaped macro and micro-fibrils forming an ultrafine network providing higher surface area, elasticity, resistance, flexibility along with biocompatibility, non-toxicity, and non-carcinogenicity **(Ullah *et al.,* 2016)** (**Figure 1**). Despite 99% water content, the synthesized cellulose pellicle has excellent stress-strain properties resembling a soft tissue with stress break at 2×10^6 Pa **(Svensson *et al.,* 2005)**. The tensile strength is comparable to Kevlar® and steel. It also possesses exceptional mechanical properties even in dried form, making it suitable for applications requiring high mechanical strength **(Picheth et al., 2017)**. It mimics the extracellular matrix helping in the growth and proliferation of cells **(Dutta *et al.,* 2019)**. For in-vivo applications, it can be made susceptible to degradation through modification, and glucose being its degradation product makes it suitable for bioresorbable purposes. For incorporating additional properties, such as antibacterial and anti-inflammatory activity, good

biocompatibility, enhanced mechanical strength, improved cell adhesion and proliferation, biomimetic capabilities, and a more even distribution of cells, BC-based composites have been fabricated, which consist of a matrix and reinforcement materials for acquiring the intended property.

Fermentation conditions, along with the culture medium composition, greatly impact the arrangement, chemical structure, and viscosity of the microbial polysaccharides, including BC, which in turn may also affect its properties **(Shezad *et al.*, 2010)**. The experiments performed by the above-mentioned authors further revealed that batch cultivation yielded smaller-sized sheets compared to fed-batch cultivation, which gave highly extended and uniform sheets. The cultivation mode has a greater effect on the mass fraction of cellulose I_α and I_β, the size of crystallite and crystallinity index. However, crystallite size and crystallinity index were also influenced by the culture medium. The intrinsic properties of the BC also depend on the source i.e. the synthesizing microorganism. During the biosynthesis of BC, gluconic acid is released, which decreases the medium pH and thus causing a decrease in the synthesis of the polymer. To overcome the issue, **Wulf *et al.* (1996)** created a mutant strain of *Gluconacetobacter xylinus* with a decreased ability to produce gluconic acid, which resulted in a higher yield than wild strains. Yield can also be doubled by restricting the production of glutamate dehydrogenase enzyme in mutant strains **(Shigematsu *et al.*, 2005)**. Moreover, the cellulose (Cellulose II) produced by the mutant strains has an antiparallel arrangement of the glucan chains instead of a parallel arrangement as in cellulose I produced by the wilder type. The change in the arrangement of glucan chains in Cellulose II is due to the average length of the fibers being about ten times the width resulting in the folding of the former. Cellulose synthesized by *G. europaeus* showed different properties compared to the one formed by *G. xylinus* in terms of porosity (higher) and film thickness (lesser). After drying, it showed better absorption capacity (110 times its dry weight) and robust mechanical properties with higher elasticity irrespective of the drying method compared to the cellulose synthesized by *G. xylinus*. It also showed lower hardness, higher penetration depth, and lower Young's modulus in case of room temperature drying and freeze-drying, contrary to the results of supercritical drying **(Zeng *et al.*, 2014)**. *Asaia bogorensis* produced relatively thinner (5-10 nm) films suggesting a difference in organization of synthesis sites in different strains **(Ullah *et al.*, 2016)**. Further, genetically engineered strains of *G. xylinus* with genes from *C. albicans* produced cellulose with enhanced biodegradability. The presence of N-acetyl glucoseamine (NAcG) disturbs the highly ordered

crystalline structure of BC, making it vulnerable to the action of lysozyme, thus reducing its crystallinity and making it more susceptible to biodegradation **(Yadav *et al.*, 2010a)**.

[Insert Figure 1 here]

3. Applications of bacterial cellulose in biomedical industries

In recent years, rapid progress has been made in the field of biomedical devices utilizing both natural and composite polymers due to the increasing need for medical engineering products for wound care, diagnosis of diseases, vascular grafts, regeneration of organs, and drug transportation (**Figure 2**).

[Insert Figure 2 here]

Bacterial cellulose has potential applications individually or as a composite across several biomedicine sectors and permits the development of novel biomaterials due to its ideal structure, biocompatibility, positive cellular interaction, and tissue development, biodegradability, high porosity, sustainability, and moldability into various shapes and sizes satisfying all the criteria laid down by **Davis, (2003)** to be considered as an ideal biomaterial. The following section further discusses the diversified applicability of bacterial cellulose in different fields of biomedical science.

3.1. Wound healing agent

In health-related applications, natural or biobased materials pose several advantages over synthetic ones as their basic molecular structures are already present in the structure of living tissues. As discussed previously, BC has several unique properties making it suitable for biomedical applications. Wound healing is a complex biological process with the aim of restoring the skin barrier function of preventing bacterial infection and hydration. BC has been used as a wound healing agent since the 1980s due to its high clinical potential, specifically for wound and burn care. The mechanical robustness of BC is due to its unique 3D structure consisting of an aggregation of long fibrils with 1.5 nm width resulting in high surface area, elastic property, and resistance, besides being non-toxic, non-carcinogenic, and biocompatible **(Portela e*t al.*, 2019)**. Its ability to maintain a moist environment, absorb wound exudates, reduction in pain, attenuation of thrombogenicity, allowance for fluid exchange, cell adhesion, and proliferation makes it a potential candidate for wound healing and dressing applications. Compared to a dry environment, the wound heals faster in a moist environment maintained by

BC hydrogel due to faster re-epithelization and lesser scar formation, especially helpful in cases of chronic dry wounds with necrotic tissues. The transparency of BC membranes helps in monitoring the rate and extent of wound healing. Various BC-based wound dressing agents are already available in the market like XCell®, which are recommended for treating pressure sores, biopsy sites, ischemic and diabetic wounds, second-degree burns, skin-tear, skin graft donor sites, venous stasis, traumatic abrasions, and lacerations **(Rajwade *et al.*, 2015)**. Nanocell® is used for treating burns, also along with Biofill it can be used for treating ulcers. Gengiflex is used to recover periodontal tissue. Thus, it can also be utilized in the field of dentistry, especially after tooth removal for wound healing, drug delivery for reducing pain and bacterial infections, and also for fabricating bioabsorbable membranes *via* periodate-oxidation.

One major disadvantage of BC is that it doesn't inherently have antimicrobial properties, which can be overcome by loading it with silver nanoparticles **(Yang *et al.*, 2014),** having both bacteriostatic and bactericide effects. BC along with superoxide dismutase and poviargol are used for treating thermal burns from acute radiation **(Legeza *et al.*, 2004)**, and, benzalkonium chloride solution for treating wounds from trauma **(Wei *et al.*, 2011)**. Various composites have also been developed using BC for potential wound healing like BC/chitosan, BC/magnetite, BC/Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) composite scaffold, BC/kaolin, and BC/ poly (2-hydroxyethyl methacrylate) (PHEMA) showing positive results. Chitosan and alginate imposed BC showed better rehydration ability along with quicker wound healing due to the release of N-acetyl-β-D-glucosamine from chitosan instigating fibroblast proliferation and controlled collagen deposition earlier than those treated with native BC or Tegaderm® **(Ullah *et al.*, 2016)**. BC impregnated with silver sulphurdiazine (SSD) not only showed antibacterial activity but also remarkable wound healing ability **(Wen *et al.*, 2015)**. Agarose alone shows weak properties for use as a wound-healing agent but in combination with BC showed good mechanical and swelling properties, thermal stability, and biodegradability **(Portela *et al.*, 2019)**. Polylactic acid (PLA) can also be used as a coating material for enhancing its wound healing and intrinsic properties. BC-RGDC (arginine, glycine, aspartic acid, cysteine peptide)-Gentamicin can be utilized for multipurpose applications, such as wound healing coupled with drug delivery **(Rouabhia *et al.*, 2014)**. Collagen incorporated BC is a novel material that has been developed, possessing both antioxidant and anti-inflammatory properties (**Wiegand *et al.*, 2006)**. Addition of plasticizers like glycerol and polyethylene glycol aided in increasing its porosity, toughness, and water absorption/retention capacity. Incorporating cellulose-degrading enzymes into the BC composites helped in making it bioresorbable, and as it is made

of simple glucose units, thus the degradation products are not toxic **(Hu & Catchmark, 2011a)**. However, a challenge that is often faced in the practical applicability of the product is that due to variation in pH of the wound or tissue, cellulose might lose up to 90% of its activity. In order to overcome the issue and maintain optimal pH conditions, **Hu & Catchmark, (2011)** impregnated the BC with buffer ingredients. They achieved positive results with a 67% increase in glucose release as compared to bufferless BC, and due to the proper pH microenvironment, the activity of the enzyme was also retained. By genetically engineering bacterial strains **Yadav *et al.* (2010b)** biosynthetically incorporated N-acetyl glucosamine (NAcG) into the BC matrix, making it biodegradable *in vivo*. The degradation product of BC being harmless glucose units, these composites may be ideal for many wound care and tissue engineering applications with the purpose of bioresorbability.

The outstanding qualities of BC can also be used in treating burn injuries which require expensive treatments, longer hospital stays, and are difficult to manage. Traditional treatment procedures involved the removal of the necrotic tissue, similar to the case of cancer lesions such as basal-cell carcinoma or skin lesions related to chemotherapy and radiation therapy. All the above-mentioned skin conditions require a perfect dressing that will maintain a moist environment, reduce pain and healing time, enhance tissue regeneration, and prevent infection, all of which are met by BC. Despite the intrinsic features of BC and the scope for development, not many products have made it to the market. Most of the commercial producers are mainly concentrated in the United States of America, Brazil, and Poland (**Portela *et al.*, 2019**).

3.2. Drug delivery materials

The major hurdle faced by topical drug formulation is its loss due to contact with garments or surfaces **(Almeida *et al.*, 2014)**. Various polymers, including BC, have been studied extensively in drug delivery applications. The drug release property of BC has been enhanced by fabricating various nanocomposites. Due to its porous 3D network, BC membrane hydrogels can work as excellent carriers for precise drug delivery applications without loss due to the superior retention capacity **(Rajwade *et al.*, 2015)**. It can be modified for sustained release of the drugs and modulated for bioavailability of the drug during percutaneous administration. It has the additional advantage of absorbing wound exudates and sticking to the uneven skin surface. It can be used for both topical and transdermal drug delivery systems verified through 24 h patch tests **(Almeida *et al.*, 2014)**. Usually, the simplest method of incorporating drugs in

BC membranes is via immersion in the drug solution followed by lyophilization for maximum absorption of the drug. Anti-inflammatory drugs, such as Ibuprofen and Diclofenac, and antimicrobial drugs, such as Gentamycin and Amoxicillin, are most commonly loaded into the bacterial cellulose membranes **(Swingler *et al.*, 2021)**.

BC hydrogels can also be used for oral drug delivery systems by modifying them to become pH and thermo-responsive. They were synthesized using lyophilized BC and acrylic acid (AA), and showed a lower drug release rate at reduced pH levels, which can be exploited in a gastric environment for sustained drug release. The addition of acrylic acid improved its swelling and increased its water transmission rate **(Mohamad *et al.*, 2014)**. Monolayer and multilayer biodegradable films of BC incorporated with sorbic acid (SA) were designed for antibacterial activity against *Escherichia coli* K12-MG1655. The release of SA can be controlled either by fabricating into multilayer BC or by altering the concentration of SA, as at higher concentrations, the dissolution of crystals was less **(Jipa *et al.*, 2012)**. BC modified with hydroxypropyl methylcellulose through in-situ fermentation improved its rehydration and capacity to absorb small molecules making it efficient for smaller drug delivery applications **(Rajwade *et al.*, 2015)**. Post synthesis loading of bovine serum albumin (BSA) by in vitro dissolution or ex vivo permeation showed potential for oral delivery with sustained drug release for 13-14 h directly proportional to irradiation dose **(Amin *et al.*, 2012)**. Another study suggested the use of BC hydrogels facilitating caffeine release for the purpose of cellulite attenuation **(Silva *et al.*, 2014)**. Due to the ability of controlled the drug release of BC, it can also be used for cancer treatment by targeting tumor sites specifically and reducing the exposure of harsh chemicals to healthy tissues.

For transdermal delivery applications, drugs are loaded into partially dehydrated BC membranes along with glycerol which gives a plasticizing effect and facilitates swelling and rehydration making it more suitable. Drug release from BC membrane was evaluated and compared with Ibuprofen and Lidocaine hydrochloride gel, aqueous and PEG400 solution (**Trovatti *et al.*, 2012**). It was observed that the permeation rate was higher for Ibuprofen (lipophilic drug) as compared to other formulations. BC modified membranes with Diclofenac sodium were also explored for *in vitro* studies which showed comparable permeation rates to commercial patches but lower than commercial gel (**Silva *et al.*, 2014**). Enantioselective delivery of racemic propranolol was studied using BC composites with molecularly imprinted

polymeric (MIP) matrices showing successful selective delivery of the drug due to the presence of selective markers (**Bodhibukkana *et al*., 2006**).

Currently, the use of BC in native form or conjugate remains restricted to oral and topical applications (**Yang *et al*., 2017**). Emulsions using drug-loaded BC nanocrystals can be explored for oral and dermal drug delivery. Further research needs to be conducted to widen its approach for developing simpler drug loading methods and comparable drug release profiles for more specific tailor-made applications in the above-mentioned area.

3.3. Tissue scaffolds

Exogenous three-dimensional (3D) extracellular matrices (ECMs) are commonly used as scaffolds in tissue engineering techniques to adapt cells in tissues, regulate tissue framework, and maintain cell phenotype **(Liu *et al.*, 2021)**. Soft tissues encompass articular cartilage, nerves, heart valves, blood arteries, lungs, liver, muscles, skin, and gut, which have a distinct function in the internal organs. Soft tissue engineering is a cutting-edge technique for repairing soft tissue injury by mitigating the limitations of current treatments. Scaffold biomaterials serve a vital role in cell restructuring, angiogenesis, cell migration, mechanical support, and other aspects of soft tissue engineering. BC has stronger mechanical properties than plant-derived cellulose due to its high crystallinity, making it a preferable alternative in the biomedical area, especially when it comes to soft tissue regeneration **(Mbituyimana *et al.*, 2021)**.

Bacterial cellulose, collagen, different proteoglycans, alginate-based substrates, and chitosan are all biological materials that have been employed to make scaffolds for tissue engineering. Natural polymers, unlike synthetic polymer-based scaffolds, are biologically dynamic and often uphold good cell adhesion and growth. An *in vitro* evaluation of bone regeneration nanocomposite produced from BC, Col (Collagen), hydroxyapatite (HA), and osteogenic growth peptide (OGP) or its C-terminal pentapeptide has revealed that Col-based scaffolds have better tissue regeneration **(Bharadwaz & Jayasuriya, 2020)**. Additionally, through sophisticated analysis, BC/Col composites were reported to be composed of needle-like or plate-like apatite crystallites, which mimicked the osteo-calcium phosphate phase thereby providing better bone repair. Furthermore, the presence of peptides in addition to Col may be linked to the nucleation and growth of plate-like apatite crystallite formation **(Saska *et al.*, 2017).**

Wang *et al*. (2017) suggested that B/G (bacterial cellulose/gelatin) scaffolds with immobilized heparin can function as a vascular endothelial growth factor (VEGF) delivery matrix and stimulate angiogenesis throughout tissue regeneration. It was confirmed by using the V-B/G/H0.5 scaffold, where it evidenced greater cell proliferation and migration than scaffolds that lacks VEGF. Also, the use of B/G scaffolds as releasing systems could be applied to other heparin-binding growth factors. A bacterial cellulose/polyvinyl alcohol (BC/PVA) composite hydrogel was utilized to construct a tissue-engineered matrix for corneal repair where its characteristics of BC/PVA were better suited for use than BC hydrogels. Therefore, human corneal stromal cells (hCSCs) were used to test the cytotoxicity of the materials, and BC/PVA exhibited tremendous biocompatibility with cells **(Han *et al.,* 2020)**.

An exopolymer bacterial cellulose film generated from the by-product of kombucha fermentation might be applied as suitable biomaterials for 3D printing and bioprinting. Further, the material ought to possess good printable and mechanical attributes with exceptional biological properties for tissue regeneration **(Pillai *et al.,* 2021)**. Some findings mentioned that 3-dimensionally microporous regenerated bacterial cellulose /gelatin (3DMP rBC/G) scaffolds could be exploited as suitable materials for *in vivo* skin regeneration of 94% within two weeks using a porogen-assisted surface modification technique. It showed improved biocompatibility where scaffolds were regular, with greater porous structure with an adequate quantity of gelatin during *in vitro* studies **(Khan *et al.,* 2018)**. The inclusion of bacterial cellulose in graphene foam increased the biocompatibility, proliferation, differentiation, and growth of neural networks generated from neural stem cell (NSCs) significantly. Primary cortical neurons were grown on bacterial cellulose/graphene (3D-BC/G) that created a dense neural network with higher activity than those grown on graphene. The 3D-BC/G culture system is not only limited to NSCs and primary cortical neurons, however it has the ability to be enhanced for its usage in various fields of regenerative medicine **(Guo *et al.,* 2021)**.

A BC-poly(3-hydroxybutyrate) (P(3HB)) 3D composite scaffold with remarkable dispersion and firm adhesion within BC and (P(3HB)) with sucrose as a porogen was developed with superior load-bearing capabilities, a high surface-area-to-volume ratio, consistent pore diameter distribution, and geometrical similarities to extracellular matrix (ECM). Because of the scaffolds' high porosity, mouse chondrogenic ATDC5 cells were able to infiltrate and migrate deep into the material. Furthermore, improved cell attachment and proliferation, as well as the preservation of the chondrocyte phenotype, were discovered **(Akaraonye *et al.,* 2016; Carvalho *et al.,* 2019).** In a standardized bovine cartilage punch model, researchers

evaluated the regeneration capacity of bacterial cellulose implants laden with and without cells. The 3D perforation enabled chondrocytes to develop and move across the implant network, which improvised the implant's long-term function. By speeding up cell colonization, the cell loading increased its efficiency. The progressive rise in cell seeding, matrix deposition, and chondrogenic differentiation confirmed the progression of cartilage regeneration **(Horbert *et al.*, 2019)**.

3.4. Bio-sensing applications

According to IUPAC (International Union of Pure and Applied Chemistry), a biosensor is defined as an integrated receptor-transducer device that provides semi-quantifiable or quantifiable data using biologically derived sensory components such as proteins, antibodies, enzymes, and whole cells **(Torres *et al.*, 2020)**. In the recent decade, BC-based biosensors have gained a lot of attention due to their low cost, ease of application, non-invasiveness, biodegradability, portability, the scope for miniaturization and automation, light-weight and faster results compared to conventional testing methods (**Koga et al., 2012**). Since BC can be easily reformed and functionalized with carbon nanotubes, conductive materials, nanoparticles, metal oxides, and biomolecules, it makes BC a versatile material for biosensor applications that can transduce signals of optical, mechanical, or electrochemical types (**Torres et al., 2020**). They have the additional advantage of being used *in vivo,* which helps in recording the real-time action of biological signals like proteins and antibodies in response to injury, tissue inflammation, cardiac infarction, infection, and muscle dystrophy. Thus helping in the early detection and diagnosis of diseases and alerting health officials regarding any future complications. Furthermore, BC provides the required matrix for immobilization of the bioreceptor or transducer component in a biosensor. The modified matrix for BC-based biosensors may be prepared either by modifying the culture medium or by adding the material once it is synthesized. After synthesis, the material can be incorporated either by dipping, which preserves its native structure, or by allowing it to act as a template for fixation of other materials or by breaking down the structure into a homogenous suspension with the material to develop a nanocomposite. Based on the type of response, BC sensors can be classified into – electrochemical, resonant, and optical detection. Electrochemical biosensors can be further subdivided into potentiometric, amperometric, and conductimetric.

The use of biosensors in glucose determination has been the most profound since its invention. **Wang *et al.* (2011)** reported the fabrication of BC-Au composites for amperometric detection of glucose by immobilizing glucose oxidase and horseradish peroxidase. When BC was used as an outer membrane in glucose biosensor, it showed better stability as compared to commercially available Cuprophan® for both diluted and undiluted blood samples **(Moniri *et al.*, 2017)**. BC-Ag nanocomposites have been utilized for both analysis of amino acids (l-histidine, l-glutamine, and l-phenylalanine) and also as a substrate for Surface-Enhanced Raman Scattering (SERS) **(Ullah *et al.*, 2016)**. The sensitivity of these novel biosensors has been found to be comparable with that of commercial ones. A biosensor with immobilized horseradish peroxide was fabricated to detect H_2O_2 and achieved a sensitivity of less than 1 µM. In another study, polyaniline was used with BC to enhance its electrical conductivity with considerable improvement from 10^{-8} to 10^{-2} S cm^{-1}, working as an electro-conductive hydrogel **(Shi *et al.*, 2012)**. *In vivo* detection of electrolytes using PVC-based membranes is often riddled with thrombogenic concerns. In order to overcome the issue, **Badr *et al.* (2015)** sandwiched the PVC-based sensors in between native BC or heparin-modified BC membranes, which showed a comparable response in terms of selectivity and limits of detection to PVC-based membrane electrodes with better biocompatibility. Detection of esterase is performed using fluorogenic sensors, where fluorescein-tetraethylene glycol-azide (FTA) is bound to BC using covalent bonds and preventing its diffusion while its *in vivo* use prevents its toxic aftereffects **(Derikvand *et al.*, 2016)**. BC-Prussian blue nanocubes immobilized with lactate oxidase have been developed for detecting lactate **(Aditya *et al.*, 2022)**. Further, inorganic-organic hybrid nanopaper has been developed by functionalizing BC using nanoparticles of oxides of vanadium, titanium, or both **(Gutierrez *et al.*, 2013)**. **Li *et al.* (2016)** developed a biosensor for detecting dopamine levels by breaking down BC pellicles using ultrasound and loading it with palladium nanoparticles. It was further modified using Nafion and laccase, showcasing high specificity and sensitivity. **Fontenot *et al.* (2017)** designed a smart wound-dressing substitute based on nanocellulose composites combined with a protease sensor for chronic wound. Another interesting approach was made by developing chemically modified wound dressing that could detect pathogens or endogenous enzymes like esterase or lipase, which may slow down wound healing. It works on the principle that when these enzymes cleave BC sheets conjugated with 5(6)-carboxyfluorescein-tetraethylene glycol-azide (FTA), it emits a fluorescence that can be detected by a benchtop illuminator **(Derikvand *et al.*, 2016)**. Thus these kinds of successful attempts open the window for its applicability in detecting a wide

range of biomarkers for direct *in vivo* diagnosis. **Yao *et al.* (2018)** developed an optical biosensor using cadmium telluride (CdTe) quantum dots and BC for measuring glucose.

Due to the high porosity, interconnectivity, slow diffusion, and acceptable mechanical properties, BC serves as suitable matrix for the immobilization of biosensor components (**Torres *et al*., 2020**). It can be further developed to hold various biomarkers for the real-time monitoring of health parameters and to overcome current limitations like reusability and to maintain the activity of the enzymes and proteins under sub-optimal conditions.

4. Functionalization of bacterial cellulose for biomedical applications

Due to its unique structure, BC can be functionally tailor-made to suit a variety of target-specific applications through chemical and biological functionalization as shown in **Table 1**. These modifications can be done either during the biosynthesis process by adding into the growth medium called as in-situ method or after the formation of the pellicle, known as the ex-situ method. In-situ alterations can be performed by introducing additives into the culture medium or by changing the carbon source to achieve distinct mechanical and physical properties. On the other hand, in the ex-situ method, the synthesized pellicle can be either immersed into or cast with the active agents re-molded as membrane/films to be used as tissue engineering scaffolds or wound dressing agents or tubular form to be used as artificial veins, catheter, urethra, etc. The micro/nano porous morphological structure of BC allows the activated compounds to easily penetrate and provide the desired functionality. The properties to get easily functionalized because of its cross-linking characteristics facilitates its use as composites. In-situ modification of BC membrane with sodium fluoride by **Sun *et al.* (2020)** demonstrated higher Young's Modulus and tensile strength resulting in bulkier and stiffer fibres. The inference drawn was attributed to the formation of hydrogen fluoride (HF) that interferes with the hydrogen bonding in BC membranes. **Gao *et al.* (2019)** demonstrated that in-situ modification of BC via the addition of glucose modified with carboxyfluorescein would provide fluorescence during UV spectroscopy and confocal microscopy. In-situ modifications are often done with specific chemicals targeted for tissue engineering applications and mostly facilitate extracellular membrane recapitulations **(Gorgieva & Trček, 2019)**. For instance, **Romanov *et al.* (2014)** reported the in-situ addition of hydroxyapatite (HAp) and tricalcium phosphate during the culturing of BC for use in bone tissue regeneration. **Wang *et al.* (2012)** prepared a BC composite with chitosan and heparin as anticoagulation agents for vascular tissue engineering. Apart from biomedical properties, porosity enhancement of BC membrane

could be achieved via the addition of potato starch **(Lv *et al.*, 2016)** and foaming procedure of the medium utilizing mannitol followed by stabilization with surfactant and xanthan to increase the viscosity (**Rühs *et al.*, 2018)**. The disadvantage of in-situ modification strategies is mostly related to the inhibition of bacteria owing to the antibacterial activity of chemical additives, their insolubility in medium, high surface tension, and lack of structural stability of BC membranes **(Gorgieva & Trček, 2019)**.

Ex-situ chemical modification process following post-treatment via chemical reactions involving carboxy-methylation, sulpho-ethylation, phosphorylation, esterification, methylation, oxidation with 2,2,6,6-tetramethyl-1-piperidinyloxy and cationization are summarized by **Gregory *et al.* (2021)**. Ex-situ functionalization with silver (Ag) or zinc (Zn) nanoparticles or oxides is often done to enhance the antimicrobial properties promoting its utilization as a wound-healing agent **(Katepetch *et al.*, 2013; Pal *et al.*, 2017)**. Immersion of BC in polyvinyl alcohol (PVA) **(Millon *et al.*, 2006)** and silk fibroin **(Oliveira Barud *et al.*, 2015)** improves the mechanical strength, non-cytotoxicity and non-genotoxicity of BC membranes. **Zhu *et al.* (2013)** reported the external molding of KBC membranes into interwoven ribbons (20 – 100 nm width) to be utilized as nerve conduits showing its in-vivo application to be biocompatible without any toxicological or haematological effects.

Another aspect to be considered for use as a tissue engineering scaffold for biomedical applications is biodegradability. Though BC is degradable, the lack of cellulase enzyme in the human body, which facilitates the breakdown of cellulose main chain and branches, thereby disturbing its crystallinity, hydrophobic-hydrophilic balance, and morphology, often hinders its degradation in-vivo. Oxidation is an essential approach for stimulating BC degradation, as suggested in the study by **Yang *et al.* (2016)**. To functionalize and enhance the degradability (> 90%), the BC membrane was modified with hydroxyapatite (HAp) and gelatin for utilization as a bone scaffold in the above-mentioned study. Oxidation is accompanied by modification (phosphorylation) of the BC membrane, which further rearranges the bonds and alters the physical/mechanical structure **(Oksman *et al.*, 2016).** Similar to the above-mentioned study, **(Gorgieva *et al.*, 2017)** also reported that periodate oxidation and gelatin biopolymer showed improved biodegradation. Nevertheless, these technological approaches help in producing BC of desirable quality for biomedical applications.

[Insert Table 1 here]

5. Techno-economic challenges of functionalized bacterial cellulose in biomedical application

The field of tissue engineering and regenerative medicines is currently evolving in the domain of material science, where cellulosic biopolymers have currently gained attention because of their ease to use, structural compatibility, and economic feasibility. The technical and economic feasibility is largely dependent on the process conditions of BC formation. Several researchers have optimized the operational parameters concerning BC production during Kombucha fermentation **(Treviño-Garza *et al.*, 2020; Villarreal-Soto *et al.*, 2021)**. However, the detailed techno-economic feasibility analysis is limited. **Dourado *et al.*, (2018)** reported that the economic feasibility is influenced by the low yield and high production costs linked with the bacterial nano-cellulose commercialization**. Ul-Islam *et al.* (2020)** also have reported the majority of costs for BC are linked with the plant direct investments. With a similar conclusion as that of the above-mentioned research, a recent study by **Behera *et al.*, (2021)** projected that increasing the production volume as well as exploring multiple products under biorefinery is expected to improve the overall technical feasibility of the BC production. Bacterial nano-cellulose production kinetics must be addressed for low-cost scale-up and potential biomedical applications. Future research trends must focus on identifying green manufacturing options for biomedical applications from BC to establish an easier market penetration **(Mishra *et al.*, 2022)**. Commercial wound healing agents like Nanoderm^{TM} functionalized with Ag nanoparticles are economical and also have a prolonged efficacy and changing time because of their antibacterial properties **(Zhong, 2020)**. These wound dressing agents are also economical compared to conventional wound healing agents **(*Axcelon Dermacare Inc*, 2020)**.

6. Conclusion

BC is a highly versatile biopolymer with huge potential for fabricating novel, smart and green materials for varied uses in the field of biomedicine. In the quest for green technologies, BC provides the solution as a potential raw material, but it still has to overcome many limitations like low yield and high production costs currently limiting the commercialization in market. Owing to its superior properties in pure form, BC has drawn a lot of attention in the last decade in becoming the biomaterial of choice compared to plant cellulose. BC has intrinsic properties like high mechanical strength, water absorptivity, biocompatibility, porosity, and biodegradability that makes it a pioneer for use as raw material in the field of biomedicine. Efforts should be made for the large-scale production of BC and its nanocomposites under

optimized conditions in a biorefinery approach which will help in enhancing its widespread applications. Future research should focus on overcoming these limitations so that the potential of BC can be utilized to the full extent.

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