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Review

Nanocellulose in biomedical and biosensing applications: A review

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ABSTRACT

Cellulose is abundant in the nature and nanocellulose (NC) in particular is regarded as a credible green substrate to be used in bio nanocomposites for various applications. NC exhibits excellent mechanical reinforcement properties comparable to conventionally used materials due to its high specific surface area and tunable surface chemistry. Additionally, low toxicity, biodegradability and biocompatibility of NC deem it a promising material for use in different biomedical applications. In this review, we highlight the biomedical applications of NC based hydrogels and aerogels/nanocomposites and advancements of their employment in the areas of wound dressing, drug delivery, tissue engineering, scaffolds and biomedical implants. This review also explores the recent use of NC in making biosensors for the detection of cholesterol, various enzymes and diseases, heavy metal ions in human sweat and urine, and for general health monitoring.

Contents

1. Introduction

Research is aggressively stimulated in making use of biodegradable polymers based green nanocomposites in selective sectors (e.g. food packaging and medical sectors) where recycling is not suggested. Environmental Health and Safety (EHS) are two important concerns that nanotechnology can be effectively applied with. Nanocellulose (NC) is obtained from cellulose through mechanical or chemical process. Chemical process includes mechanical breakdown, alkali treatment, delignification, and acid hydrolysis which leads to produce rod-like cellulose nanocrystals (CNCs) with diameter in the range of 3–50 nm and length in the range of 100–500 nm. Mechanical treatment such as grinding and high pressure homogenization generally use to produce cellulose nanofibrils (CNFs) with diameter in the range of 5–50 nm and length in the range of 500–2000 nm [1]. In addition to the plant fibers, wood and biomass, cellulose can be also produced by some kind of bacteria. Bacterial nanocellulose (BNC) are in fact in the category of cellulose nanofibriles and can be converted to CNCs via acid hydrolysis. NC is biodegradable and biocompatible and exhibits high stiffness, strength, surface area, rheological properties, mechanical properties, good cytocompatibility, low toxicity, and low density. Hence, they are quite an excellent and attractive material for biomedical and biosensing applications [2–5]. CNFs, obtained via mechanical defibrillation of cellulose fibers have high aspect ratio (100 or higher) and pose good elasticity/flexibility and mechanical stiffness (modulus ranging from 10 to 50 GPa) [6,7]. CNFs based hydrogels can be formed with low concentrations (~1 wt%) and advantageous for scaffolds as they tend to

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form highly interconnected network [8]. CNCs on the other hand are highly crystalline and are stiff (high compressive modulus of ~150 GPa) [9,10]. These nanomaterials are usually required in higher concentration for forming hydrogels/aerogels. BNC is obtained from bacterial synthesis and has high crystallinity. Unlike CNFs and CNCs, BNC has better purity, the reason being, there are no traces of lignin or hemicellulose in it. This attributes to its wide usage in biomedical applications [11,12]. Even at low concentrations (~0.5–2 wt%), BNC based hydrogel-like films show good mechanical and thermal stability [12].

Bio hydrogels or aerogels are crosslinked polymeric chains forming a 3D network structure, capable of storing large amount of water [13]. For a bio-nanocomposite to be successfully used in biomedical field, it should be biodegradable, biocompatible, must optimum mechanical performance (good flexibility and high elongation at break), porosity, etc. [14]. However, these basic requirements differ based on the type of tissue being operated on (Fig. 1).

Porosity of composite allows the removal of waste and diffusion of essentials nutrients as well as water [16]. In case of tissue engineering, scaffolds, wound healing, etc. specific cells and blood vessels also penetrate through these porous structures and grow throughout, without hindering in the formation of new tissues [17,18]. It has been observed that the mechanical strength of a composite is compromised [19]. Covalent crosslinking of NC with polymers in hydrogels or aerogels, helps in improving the mechanical stability of the biocomposite, at the same time having high porosity [20,21]. Glutaralehyde (GLU) [22], genipin [23], and glyoxal are some known cross-linkers [24]. The advantage of using biomaterials over others is that they allow the progressive decomposition of the material followed by tissue regeneration. These biomaterials developed from NC have been used in scaffolds for tissue engineering, biomedical implants such as urethral catheters, artificial skin, mammary prostheses, in adhesion, barrier, articular cartilage repair, growth of blood vessels, drug release systems, dura-meter and gum reconstruction, testing and diagnostics, etc. [25–28].

Biosensors can be termed as analytical tools or devices, capable of providing quantitative biological information via a biological identification element in spatial or direct contact of a transducer element. Conventionally used equipment for biosensing include inductively coupled plasma atomic emission spectrometry [29], atomic flourescence spectrometry [30], atomic absorption/emission spectrometry [31], etc. These tests require sample preparation, heavy equipments, are not portable and most importantly, they are time consuming. Fabrication of smaller test composites or biosensors, allows equally efficient testing, portability, minimum preparation of samples, etc. Biosensing is among today's emerging technology, offering vast number of choices along with cost and time efficiency, simplicity in a wide ranging of applications. The use of nanomaterials in biosensors increases the available surface area, allowing a greater number of functional groups to be added and arranged onto it. This improve the physical and chemical properties of the composite, which further boosts the specificity and sensitivity of the sensors [32]. In contrast with the conventional sensing methods, it is observed that through biosensing, biochemical information in fields such as biomedical diagnosis [33], food quality control [34], pesticide detection and environment monitoring [35,36] can be swiftly tested. In the recent past, research is aimed at fabricating wearable biosensors, for health monitoring using screen printed electrodes, reusable testing and diagnostic devices, immersive pH biosensors, etc.

In this review, we mention some of the recent breakthroughs in the field of biomedical and biosensing applications using NC based composites (aerogels, hydrogels, etc.) and their crucial properties.

2. Biomedical applications

Low cytotoxicity of NC based composites and high biocompatibility are the two primary reasons for their use in a number of biomedical applications. Hydrogels and aerogels have high porosity which is essential in most biomedical applications, but they lack mechanical stability. The mechanical properties of these hydrogels/aerogels can be tuned to mimic the behavior of specific tissues, while promoting the growth of cells, blood vessels, etc. NC along with cross-linkers, forms a strong network structure without hindering the porosity of bio-hydrogels/-aerogels thereby allowing the passage of essential nutrients. In this section, we mention some of the recent works in the areas related to wound dressing, drug delivery systems, tissue engineering, scaffolds, medical implants, etc.

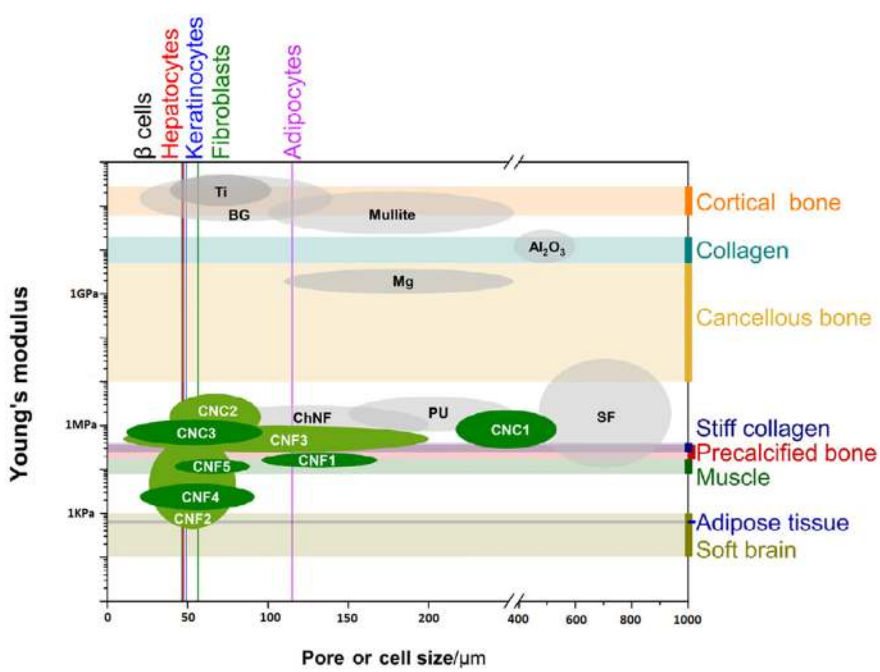

Fig. 1. A plot showing moduli and pore size requirements for different biological tissues. (Reproduced with permission) [15].

Fig. 2. a) Bacterial cellulose membrane for wound healing showed nearly 70% wound closure in 3 weeks; b) 'before and after' photographs of wound healing after about 2 months using BNC membranes.
(Reproduced with permission) [43].

2.1. Wound dressing and drug delivery systems

An ideal wound dressing material is the one which is non-toxic, antimicrobial, maintaining the skin surface moist, absorb the toxins, accelerate healing, and must be able to take out without any trauma to the healed skin. NC-based hydrogels fulfil majority of the requirements and hence recently they have been put in use for wound dressing application [37–40]. The hydrogels also mimic the biological tissue, which can be hydrated because of its porous structure [41,42]. The impact of bacterial cellulose membrane on burn wound and the rate of recovery

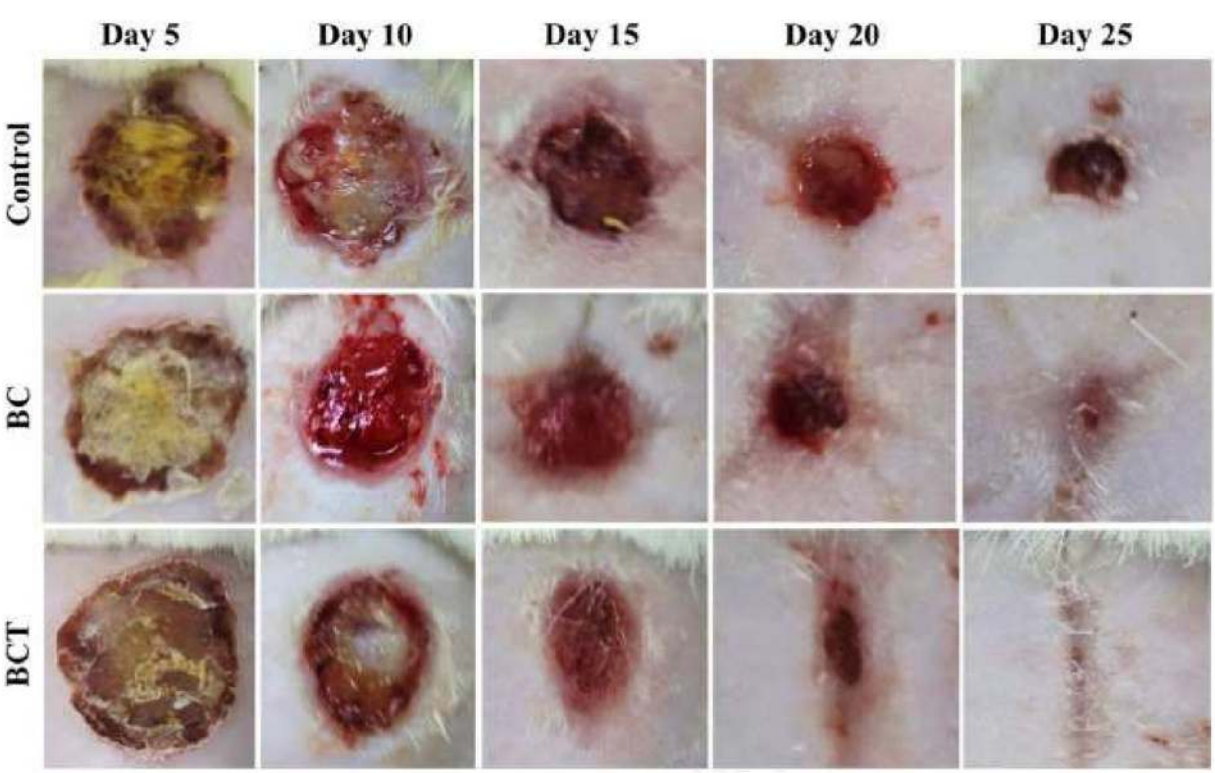

Fig. 3. Wound enclosure on day 5, 10, 15, 20, and 25 for control and BNC, BCT hydrogels.
(Reproduced with permission) [44].

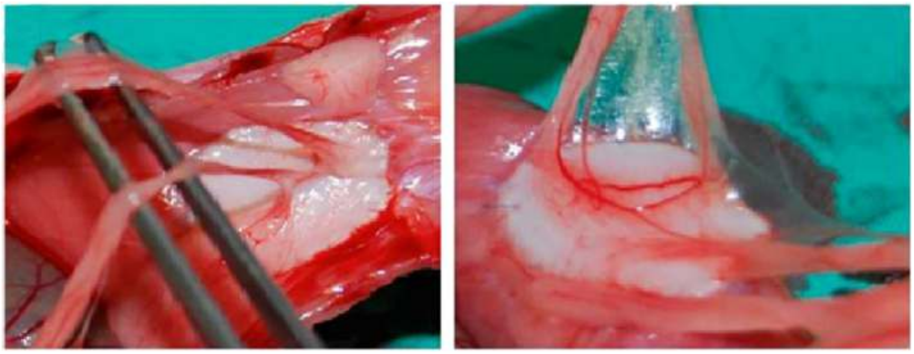

Fig. 4. Adhesions after construction using abdominal wall replacement model after relaparotomy.
(Reproduced with permission) [45].

were assessed [43]. After a time period of only 3 weeks, excellent recovery with approximately 70% wound closure was observed (Fig. 2(a)). Even in case of severe burn wounds, involving loss of skin up to full thickness on fingers and hand, BNC based composites have proven to be efficient (Fig. 2(b)). Complete healing of the wound was successfully achieved in 2 months that too with early reduction in pain. To treat third degree burn wounds, thymol was incorporated in bacterial cellulose hydrogel by Jiji et al. [44]. The nanocomposite hydrogel was reported to have antibacterial activity against burn-specific pathogens. The composite was tested in mouse 3 T3 fibroblast cells for in vitro biocompatibility. With increased cell viability and low toxicity, significant growth in fibroblast cells was observed. Wound closure (Fig. 3) over time was compared for 4 different conditions - control, BNC hydrogel, and thymol incorporated BNC hydrogel (BCT). The better performance of BCT can be clearly observed with the wound nearly closed after 25 days.

Incisional or ventral hernia is commonly treated using a mesh repair for reconstruction. The mesh is likely to cause infections or adhesions. Rauchfuß et al. [45] prepared a bio-based mesh in order to reduce the chances of such infections. Two different models for reconstruction, sublay model, and abdominal wall replacement model, were used. Over an observation period of 90 days, minimal shrinkage of mesh (about 15%) and minimal loss of tear-out force was observed. The adhesions to the abdominal wall after the reconstruction were also found to be moderate (Fig. 4). The authors claimed this study as the first in vivo study which described the use of biocellulose mesh for repairing of ventral hernia and a promising alternative for solving problems associated with modern hernia surgery.

Dermal fibroblasts are vital component for wound healing, particularly the ones related to human skin. Loh et al. [46] made a non-biodegradable composite from BNC and acrylic acid for transfer of human dermal fibroblast (HDF) to wound site and tested it in athymic mice. Wound closure was observed for 14 days (Fig. 5) in 4 different experiments – NT (no treatment), hydrogel-based treatment (H), hydrogel-based treatment with 50,000 HDF cells (HC 50 K) and 100,000 HDF cells (HC 100 K) cultured on the hydrogel. More than 50% of HDF transfer was observed within the 24 h to the wound site. Microstructure analysis of newly formed skin showed no significant difference. The observations from the study provide new perspective to the use of non-biodegradable materials at molecular levels, in wound healing and tissue engineering, accompanied by exogenous cells.

Drug delivery plays a crucial role in consistent supply of drugs or essential components, including antimicrobials, to the wound site. A drug delivery system signifies a technological bioengineered system to give passage to the therapeutic agents, in a better way, to the organs and tissues, the target site. Recent research is aiming at improving pharmacokinetics and optimisation of the substance biodistribution as well, in a human body. NC used in encapsulation helps maintaining stability towards variation in pH, ionic strength, temperature around the target. These factors prove to be crucial for controlled drug release [47]. Due to biocompatibility, biodegradability, and adaptable surface chemistry, high surface area and open pore structure, both CNCs and CNFs have impressive drug delivery capacity. Hence, CNC or CNF based hydrogels are promising materials for drug delivery systems [48–51]. In a recent study, the authors incorporated honey in NC films [52]. The composite film prepared followed first order kinetics for drug release, showing constant release of drugs from the drug delivery system. The concentration of drug was found to gradually increase and remained consistent for about 48 h (Fig. 6). The studies and in vitro tests using artificial sweat, suggested that maximum active ingredients were released from the system. The controlled drug release from the drug delivery system was successfully employed in treating chronic wounds.

A hydrogel was prepared to combine the drug effects of diosgenin and neocymin, by incorporating gelatin cross-linked with genipin into diosgenin-modified NC [53]. High gel yield, good swelling capacity was observed in kinetic and swelling analysis. The composite hydrogel showed antibacterial activity against *Staphylococcus aureas* and *Escherichia coli* determined in the range 50–88%. Additionally, in vitro drug release and compatibility tests revealed more than 90% drug release after 24 h and cell viability of about 80% were achieved. This study is an example of how controlled drug release systems can help in efficient healing of wounds too.

2.2. Tissue engineering and scaffolds

The extracellular matrix (ECM) serves as an essential component in creation of different kinds of cells which serve as the building blocks to the specialized tissues [54]. For a biomaterial to be applicable in 3D cell culture, it must mimic an ECM's properties. This theory holds true in bone tissue engineering(BTE), cartilage tissue engineering, etc. 3D cell culture is not just limited to repairing, replacement or regenerating tissues but also provides a model to investigate drug screening, cancer

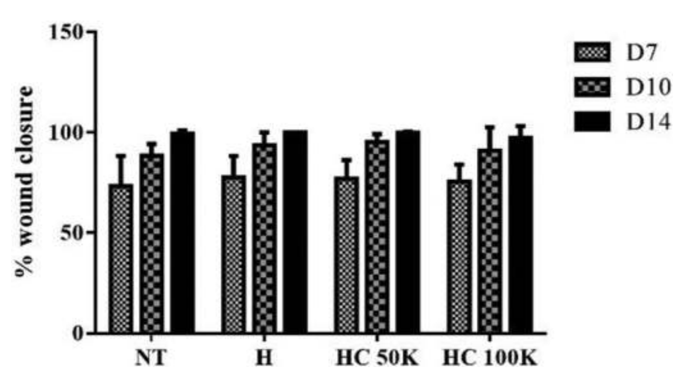

Fig. 5. Wound closure trends over a period of 14 days (D7 – day 7) studied under 4 different experiments.
(Reproduced with permission) [46].

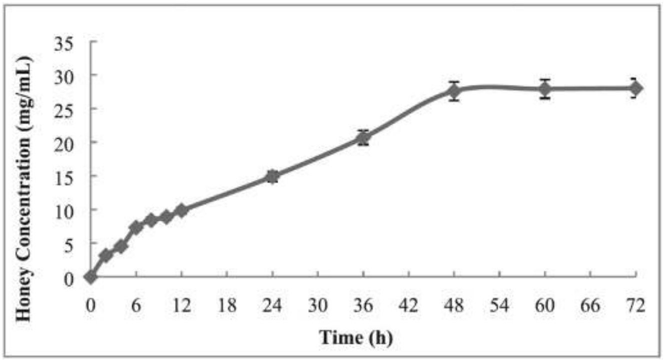

Fig. 6. The controlled release of honey incorporated in cellulose nanocrystal (CNC) films over time.
(Reproduced with permission) [52].

propagation, etc. [55,56]. Mesenchymal stem cells (MSCs) are considered to have high proliferation potential and along with vast differentiation ability. The interaction between the biomaterials and these stem cells, form the basis of tissue regeneration, and hence, efforts are being put into finding various suitable sources of these stem cells [57]. The human oral cavity is a source of tissues which are rich in cells exhibiting MSC-like features, termed as Dental-derived MSCs (D-dMSCs). These cells show good plasticity towards bone tissues and are easily harvestable as well. According to a study, D-dMSC therapy is most utilized in bone tissue regeneration and play a vital role as enhancers, promoters, and playmakers in regenerative medicine [58]. Published in the recent past, the work by Spagnuolo et al. suggests that all MSCs derived from oral tissues are dedicated to differentiate towards precursors of bone tissue and osteoblasts [59]. It goes without saying, that the study involving the culture of such stem cells in vivo must involve certain safety protocols, primarily, the safety issues associated with animal serum. The presence of a quality-controlled and safe supplement for cell culture is essential for establishing a preparatory study for application in regenerative medicine. Marrazzo et al. [60] carried out a comparative study on MSC capacity by monitoring their growth in Fetal Bovine Serum (FBS) and human allogenic Platelet Lysate (PL), based on cell division, metabolic activity and membrane permeability. The results suggested that PL is more suitable supplement for the expansion and differentiation of MSCs in vitro.

3D culture of MSCs is one of the recent works in 3D cell culture using NC hydrogels [61]. CNFs, apart from having high reactivity, have the ability to mimic nanostructured collagen present in bone marrow. Carlstrom et al. [62] blended and crosslinked CNFs with gelatin to prepare scaffolds with a tuned rate of degradation to investigate its potential osteogenic potential for human bone marrow mesenchymal stem cells (hBMSCs) culturing, in vitro. 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation was employed in preparation of CNFs and crosslinking of CNFs with gelatin was achieved by hexamethylenediamine, dehydrothermal treatment (DHT) and genipin. CNFs tend to degrade slowly in vivo which may cause a host to respond with foreign body reaction [63]. Crosslinking of CNFs with gelatin helps in tuning the degradation of CNFs and also in meeting the requirements for BTE while CNF provides for the desired mechanical property of scaffolds. The prepared scaffolds were capable of supporting cell spreading, osteogenic differentiation, and attachment. Osteogenic differentiation of hBMSCs was supported by the Gel-CNF scaffolds without having any adverse biological effects on the cultured cells.

A bilayer scaffold was fabricated by Ghafari et al. [64], constituting of CNFs and polyvinyl alcohol (PVA) for skin tissue engineering application. Dermis and Epidermis are two primary layers of human skin. The fabricated scaffolds had an interconnected porous structure with a definite size in each layer. The scaffolds could recapitulate mentioned primary layer of human skin, dermis and epidermis. The upper thin layer (N1: 1.4 wt% CNF, 0.35 wt% PVA) with lower porosity, preventing the lower layer from microorganisms acting as a protective barrier and the lower thick layer (N2: 0.4 wt% CNF, 0.1 wt% PVA) with high porosity preventing dehydration and providing for the transfer of nutrients essential for cell proliferation (Fig. 7). Lower concentration of polymers often leads to higher porosity and increased water uptake and as a result, poor mechanical properties. The intra−/inter-molecular hydrogen bond formation between the hydroxyl groups of both CNFs and PVA covered up for the weak mechanical properties of scaffolds.

2.3. Medical implants

In medical implants, CNCs are used with different matrices which help in the development of vascular prostheses. CNC with hydrophilic polyurethane is used to make new nanocomposites that can be used for vascular grafts. Nanocomposites made with hydrophilic PVA and CNFs were found to be useful in cardiovascular soft tissue replacement as it has similar mechanical properties of human tissues. PVA-CNF composite formulated by Millon et al. [65], which showed mechanical behavior analogous to that of the cardiovascular tissues such as heart valve leaflets and aorta, is one of the earliest works in this field. The mechanical properties of PVA-NCF nanocomposites were found to be similar to cardiovascular tissues. Nanocomposites based on CNCs derived from tunicates and PVA have been used in intracortical implants for delivery of curcumin [66]. Bhat et al. [28] prepared two different biocomposites hydrogels from two different polymers namely polyurethane and PVA. Vascular grafts were prepared from polyurethane for vascular prostheses (Fig. 8(a)). At 5 wt% of CNC, and film thickness of

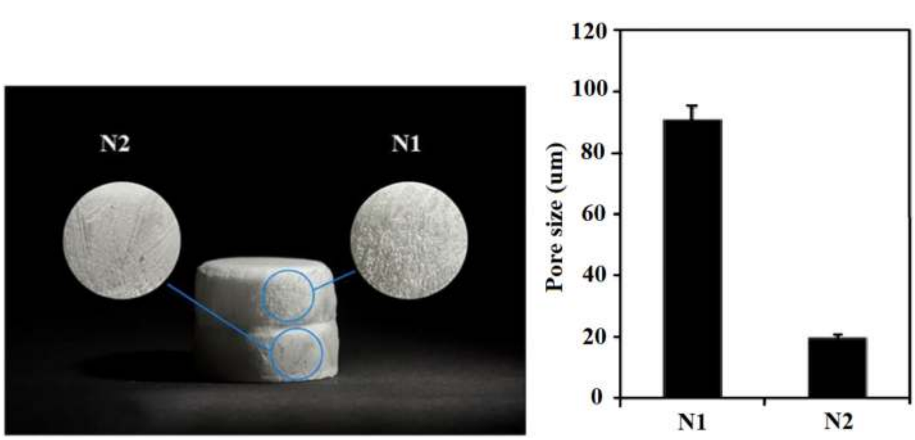

Fig. 7. CNF/PVA scaffolds for skin tissue engineering with low and high porosity layers, N1 and N2 respectively. The graph shows the thickness of each layer.
(Reproduced with permission) [64].

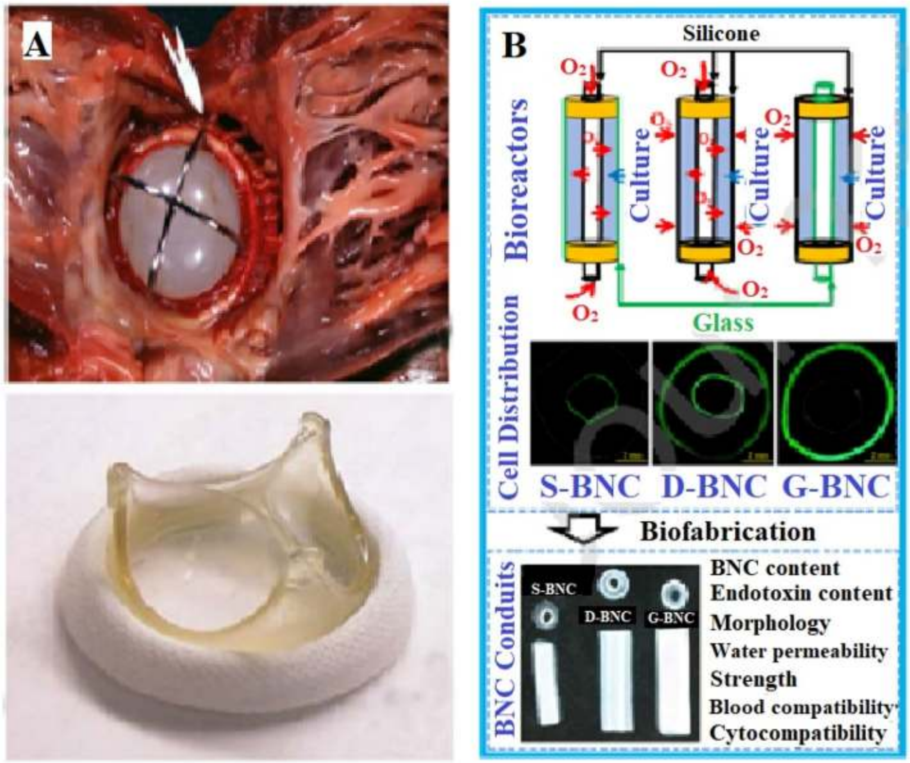

Fig. 8. A) Cardiovascular prosthesis based on Polyurethane/CNC [28]; B) three different conduits prepared in bioreactors based on BNC. (Reproduced with permission) [68].

0.7–1.0 mm, the grafts resisted an exceptional 300 kPa of hydraulic pressure with the elongation at break of about 800–1200%. The composite prepared from PVA and NC was observed to be an optimum material for soft cardiovascular tissue replacement. The composite exhibited faster relaxation rates and lower residual stresses compared to the replaceable tissues, which is indispensable for cardiovascular applications. Not only thin films but the addition of NC adds to the mechanical properties of aerogels and foams as well [67]. The combination of low

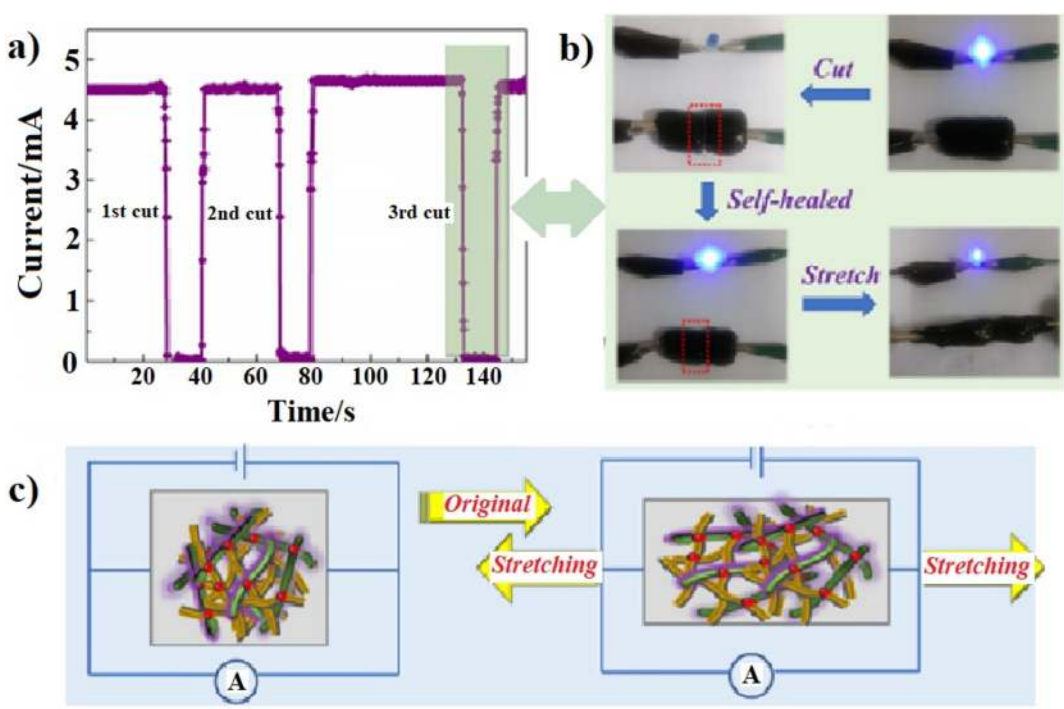

Fig. 9. a) Variation of current across the PANI@CNF-PVA composite under repeated damaging and healing process b) change in luminance of the light bulb with cutting, stretching and healing of the composite; c) depiction of change in resistance in conductive 3D hydrogel on stretching. (Reproduced with permission) [75].

density, and brilliant mechanical properties and their porous structure makes aerogels and foams suitable for biomedical applications such as scaffolds and many more.

Bao et al. [68] attempted in making an artificial blood vessel by preparing three different bacterial cellulose based conduits (tube like structure through which fluids can pass) in bioreactors, testing them in vitro and characterising them based on their mechanical properties, luminal surface etc. The inner walls of human blood vessels have a thin, smooth layer of endothelial cells, known as the luminal surface. This thin layer of cells forms an interface of circulating blood and tubular walls of the vessel. Use of BNC in the composite conduits was found to provide a scaffold for growth of these endothelial cells. The authors prepared three different conduits, S-BNC, D-BNC, and G-BNC, and tested them in vitro (Fig. 8(b)). The conduits referred to as S-BNC and D-BNC, were prepared based on previous studies [69,70]. The third conduit (G-BNC), was fabricated by the authors, using a small caliber BNC tube on the inside and silicone tube on the outside, with the help of a glass rod serving as a template. The artificial blood vessels were placed in bioreactors to study cell culture, determine cell distribution, water permeability, blood compatibility, cytocompatibility, etc. These three conduits were ranked as: S-BNC < D-BNC < G-BNC considering the smoothness of the luminal surface. Among these three conduits, G-BNC had the smoothest luminal surface and high hemocompatibility but a loose network structure. Considering the mechanical properties of the as-prepared conduits, S-BNC conduits were the ones with weakest mechanical properties. D-BNC conduit had dense network structure and highest BNC content, thus showing strong mechanical properties. Concluding the study, both D-BNC and G-BNC were accepted as small vascular grafts based on in vitro. Even though different biomaterials have been proposed in the past [71], no artificial grafts have yet been prepared, to serve as artificial blood vessels. However, implantation and study of these grafts using animal mode are needed in the future, in order to verify current results.

Self-healing composite materials can also be applied or used in biomedical implants [72]. These materials have the ability to regain/retain their shape after the application of any external force or deformation. Molecular interaction in hydrogels are non-covalent reversible bonds, which break on the application of external forces and mend again on lifting of the force. A Hydrogen bond is one such non-covalent bond strategized for fabricating self-healing hydrogels [73]. Hydrogen bonds can be formed and broken depending on the conditions such as temperature and medium, thus forming the basis of preparing self-healing hydrogels [74]. In situ polymerized PANi on CNFs were intermixed with PVA crosslinked borax hydrogel to fabricate electroconductive hydrogel [75]. CNFs served as a platform for polymerization of polyaniline forming an integrated structure with good electrochemical as well as mechanical properties. The purpose of the study was to investigate the capability of hydrogel, to be used as an electrode for tissue-like implantable bioelectronic device. With a self-healing ability of within 15 s, the composite had appealing mouldability, thermo-reversibility, and pH sensitivity (Fig. 9). Compared to pure PVA gels, the composite hydrogel had 400- and 3.5-times better storage modulus (31.5 kPa) and maximum compressive stress (48.8 kPa). As another strategy to make self-healing hydrogel, Liu et al. [76] prepared a hydrogel constituting of CNC, quaternary ammonium xylan (QAX) and high concentration of PVA (20 wt%). The electrostatic interaction between QAX and CNC, hydrogen bond formation between CNC and PVA formed the basis of self-healing properties of the so prepared hydrogel. Impressive 1.56 MPa of compressive strength and 771% elongation at break was obtained which can be attributed to the crosslinking between the constituents resulting from strong bond formation (Fig. 10a)). Without any external stimuli, the hydrogel also exhibited self-healing behavior at room temperature, healing efficiency of 37.03% within 48 h (Fig. 10(b)).

All the application mentioned in this section of medical implants, are based on soft tissues present in human body (cardiovascular tissues, blood vessels, etc.). Considering the use of NC based implants involving hard tissues, such as tooth enamel, the mechanical stability needs to be significantly better. It has been inferred by Marrelli et al. [77], that the mechanical properties of the implants deeply depend on their processing methods or preparation procedure. They employed three-point bending test procedure to study the effect of the preparation procedure on the mechanical properties of dental restorations composed of zirconia ceramics. A similar procedure can be adopted in order to test the mechanical stability of NC-based hard tissue implants, in the future.

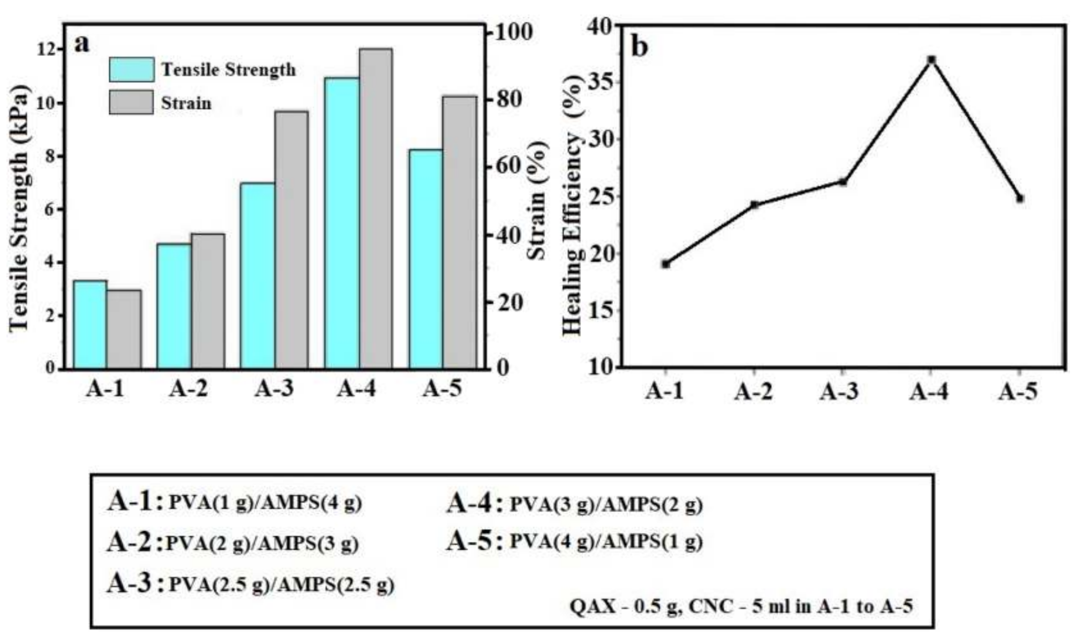

Fig. 10. The self-healing behavior of nanocomposite hydrogels (a) Tensile stress and strain values of healed nanocomposite hydrogels, A-1 to A-5 are hydrogels having different concentration PVA and AMPS; (b) healing efficiency curves of A-1 to A-5 hydrogels.
(Reproduced with permission) [76].

3. Biosensing applications

Biosensing is often used for detection of biomolecules with the help of a biosensor (analytical device). These biosensors combine biological components such as sweat, saliva, or other vital bodily fluids with a physicochemical detector. As discussed earlier, these detectors can be heavy machinery or even small, portable, or wearable devices. The latter has recently attracted more attention resulting from the ease of testing, portability of sensors, very little time for diagnostics, reduced need for sample preparation etc. This section discusses some of the recently produced biosensors for health monitoring, testing and diagnostics, spectrometry and so on.

3.1. Electrochemical sensing

Electrochemical biosensors transform biochemical information into analytical signals such as current or voltage. These sensors are often used to detect analyte concentrations from the measurable electrical response, which is proportional to analyte concentration observed in the electrode. Detection of adenine and guanine provides valuable information in DNA sequencing, oxidative damage, and protein metabolism in cells [78]. These bases moreover determine immune deficiency and help in detection of diseases such as Alzheimer, HIV infection, cancer, etc. [79–81]. A thin film comprising of NC and single-walled carbon nano horns (SWCNHs) were fabricated for the sensitive and selective detection of adenine and guanine bases in RNA and DNA [82]. It was anticipated that the so-formed film would demonstrate antifouling property and high catalytic activity. The detection of these bases was carried out using linear and cyclic sweep voltammetry. The limit of detection was confined to a maximum value of 1.4×10^{-6} mol L^{-1} for adenine and 1.7×10^{-7} mol L^{-1}. The electrode was also utilized in determining the concentration of these bases in fish sperm and synthetic human serum.

Blood test mainly rely on spectroscopy for diagnostics [83]. Human skin secretes sweat from eccrine and apocrine glands which is a carrier for sugars, electrolytes, proteins, metabolites, acids, and hormones [84,85]. These are termed as biomarkers and carry valuable information which is useful in analyzing genetic disorders or infections [86–88]. monitoring athletic performance, etc. [89]. Enzymatic sensors have gained credible attention in electrochemical sensing, owing to their ease of operation, portability, little requirements for sample preparation, etc. Wearable devices such as rings [90], patches [85], wristbands [91], and tattoos [92], are suitable substrates to fabricate electrochemical platform for biosensing applications. Use of conducting polymers in biosensors allow faster electron transfer and provides good platform for immobilizing biomolecules. A recent study targeted to achieve the goal for the development of a sensitive enzymatic cholesterol biosensor using the PANi/CNC nanocomposite followed by coating a thin layer of ionic liquid (IL) in order to put the synergistic effects of the CNCs to host enzymes and bio molecules [93]. On the basis of the immobilization cholesterol oxidase (ChOx), a sensitive electrochemical cholesterol biosensor was produced on the polyaniline/CNC/IL modified Screen-Printed Electrode (PANi/CNC/IL/SPE) (Fig. 11). The PANi/CNC/IL/glutaraldehyde/ChOx-modified electrode was able to monitor cholesterol in the range of 1 μM to 12 mM with limit of detection of 0.48 mM. The biosensor also exhibited very high sensitivity value, 35.19 μA mM cm^{-2} under optimized conditions and little to no effects of existing electrically active compounds (glucose, uric acid or ascorbic acid) on the biosensor were observed. The fascination of electron transferring between enzyme and surface was done by the nanocomposite to ensure permeable determination of cholesterol with the least measured interference. The fabricated biosensor presented good reproducibility, stability and operational repeatability, suggesting that it could be used in food industries and clinical diagnostics for determination of cholesterol levels. It has been found that the synergistic properties of CNC structures and electrochemical properties of IL/conducting polymers in relation to the electrocatalytic properties of nanocomposites of high porosity can be integrated to make this material a good candidate for hosting enzymes and bio molecules.

Recently, a breakthrough was obtained in health monitoring via a smart wearable sensing device [94]. The wearable sensor used BNC as a substrate for detecting lactate in artificial sweat. This was achieved by immobilization of lactate oxidase on the BNC substrate directly. Fabrication of the mentioned electrochemical platform constitutes of

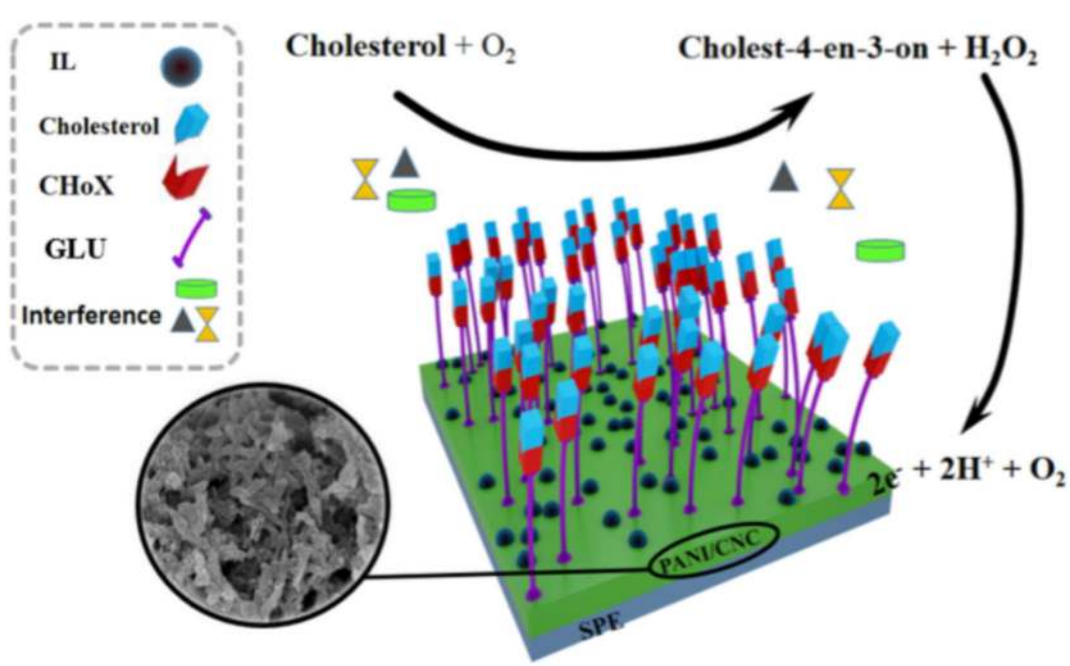

Fig. 11. Essential redox reaction between Cholesterol and Cholesterol Oxidase immobilized on Glutaraldehyde modified PANi/CNC electrode coated over with ionic liquid, for electrochemical detection of cholesterol.
(Reproduced with permission) [93].

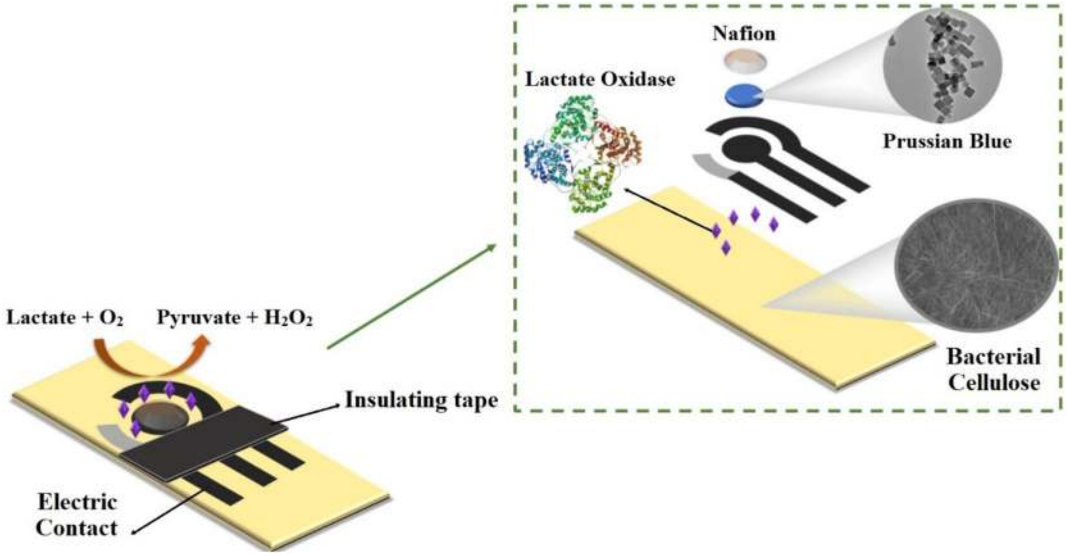

Fig. 12. Smart wearable lactate biosensor.
(Reproduced with permission) [94].

Prussian blue nanocubes modified carbon based-electrode and lactate oxidase (LOx) immobilized directly onto the surface of BNC (Fig. 12). Prussian blue nano cubes efficiently serve as electron mediators for H_2O_2. The biosensor successfully responded to lactate in artificial sweat with concentration ranging from 1.0–24.0 mmol L^{-1}.

Another electrochemical biosensing platform was fabricated by Silva et al. [95] with BNC as substrate and screen-printed carbon electrodes (SPCE) as sensing units on BNC. The porous structure of BNC makes a commendable skin substitute, often used in wound dressing applications. The sensors were designed with a detection limit of 1.01 μM for cadmium ion (Cd^{+2}) and 0.43 μM for lead ion (Pb^{+2}), sufficient enough for detection of these ions in human sweat or urine. These SPCEs were also functionalized through anodic pre-treatments, designed to detect 17β-estradiol and uric acid with detection limits 0.58 μM and 1.8 μM respectively. This green skin-adherent wearable sensing device (Fig. 13) combined the benefits of improved hydrophilicity, wettability and semipermeable, renewable and non-allergenic features of BNCs.

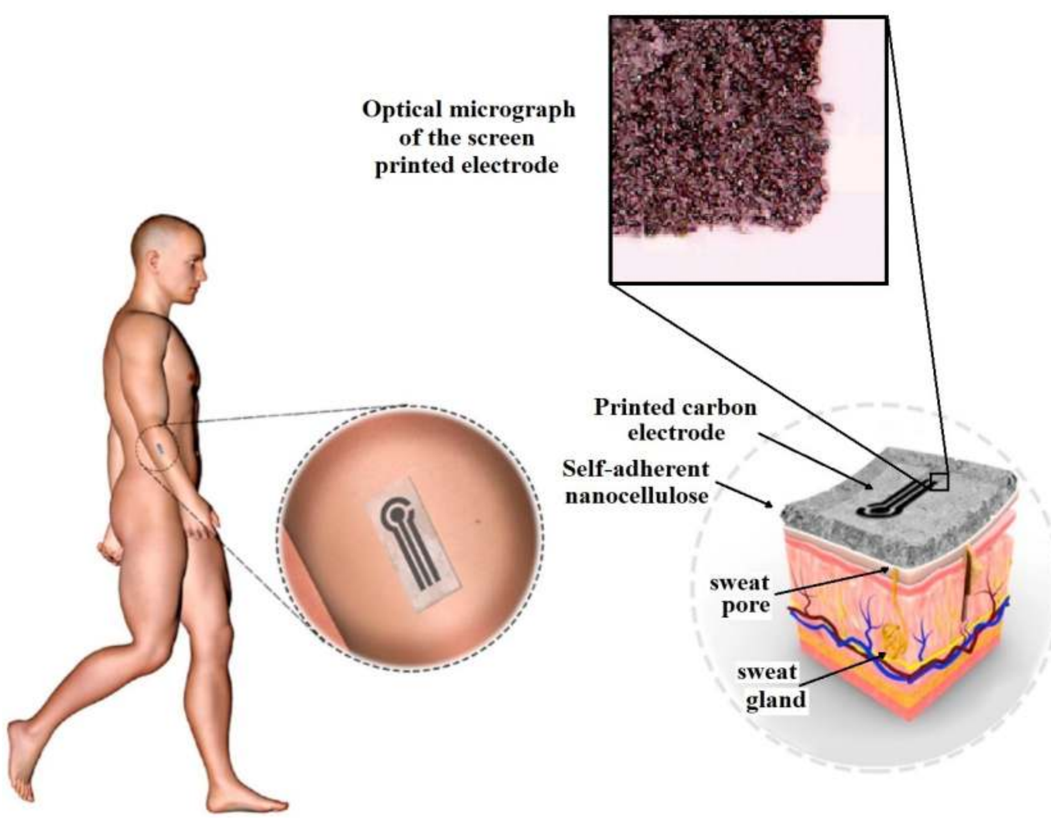

Fig. 13. Pictorial representation and schematic layered diagram of screen-printed carbon electrode on BNC substrate for electrochemical biosensing application.
(Reproduced with permission) [95].

Fig. 14. Linear increase in color concentration with increase in concentration of Cu^{2+} ions. (Reproduced with permission) [100].

3.2. Colorimetric biosensing

Heavy metals or metal ions such as Cu^{2+} ions, Ag^{+} ions, etc. are highly abundant and are potentially toxic for the human body. The severity of problems resulting from uptake of such ions ranges from vomiting, spams to neurodegenerative diseases, organ damage (liver, kidney) or even death [96–99]. Milindanuth et al. [100] prepared test strips by immersing Rh-2, a Rhodamine B derivative, in BNC. BNC acting as a template, shows remarkable biocompatibility with Rh-2. These strips allowed detection of color change through the naked eye, which is a suitable method of spectroscopy. They observed linear relation between the Cu^{2+} concentration and color strength (Fig. 14). The color changed from colorless to pink on increase in concentration of copper ions.

In recent research, colorimetric sensor based on NC grafted with 2,5-dithiourea (DTu) for sensing of Cu^{2+} and Ag^{+} ions [101]. The use of NC makes the sensor cheap, and portable, disposable analytical equipment and an ideal substrate for fabricating sensors for heavy metal ions [102,103]. The fabricated sensor showed a notable color change from white to yellow-red against the addition of Ag^{+} ions, up to 10^{-3} mol L^{-1} (limit for naked-eye color detection) and from white to light-grey against the addition of Cu^{2+} up to 10^{-4} mol L^{-1}. The response was as quick as 5 s from addition of ions. Apart from the test carried out, the sensor proved to be equally efficient in terms of rapidity, simplicity in in-situ identification of these ions in real water.

Incorporation of fluorescent material into the composites along with other components for making sensing platform allows the detection of color change via naked eyes. This is another potential method for making spectroscopy easier. Materials such as carbon quantum dots (CQDs) [104], fluorescent elastase peptide [105], have been worked on to successfully prepare NC based colorimetric sensors. Palomero et al. [104] fixated quantum dots of graphene doped with nitrogen and sulphur onto TEMPO-oxidized NC hydrogels, to prepare a fluorescent sensing platform for laccase enzyme detection. The composite was reported to have a detection limit of 0.048 U-mL^{-1}. These composite hydrogels were also found to be sensitive against carcinogenic 2,4,5-trichlorophenol (TCP) found in herbicides and fungicides. High intensity fluorescence was observed testing the hydrogel against TCP in specific water and red wine samples [106]. In order to detect Human Neutrophil Elastase (HNE), an inflammatory disease marker, Fontenot et al. [105] manufactured an NC-elastase peptide biosensor. Fluorescent groups released as products of reaction between peptide and the biomarker. Numerous fluorescent bioimaging platforms have also been made using NC. In a recent study, a sensor containing elastase tripeptide, was placed in a layered wound dressing for detection of HNE, with the bottom layer providing essential fluid flow, and the top layer protecting the wound (Fig. 15(a)) [107]. The method of detection required the removal of the dressing and place it under a UV lamp source. If the sensor emits high intensity fluorescent light (Fig. 15(b)), it would indicate saturation in relation with the detection limit. This proved effective healing of wound.

To mention one such application, NC can be used as fluorescent material for cell bioimaging. Cui et al. [108] utilized aggregation-induced emission effect, for producing images. The CNC-PhE-poly(ethylene glycol) methyl ether methacrylate (PEGMA) composite was tested by evaluating

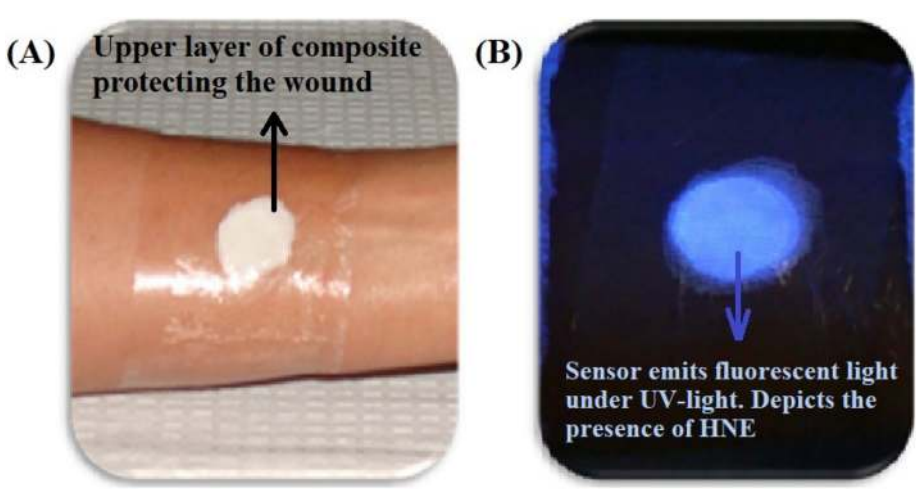

Fig. 15. a) Layered dressing housing the fluorescent biosensor between the layers; b) dressing placed under UV light source for examination of HNE content. (Reproduced with permission) [107].

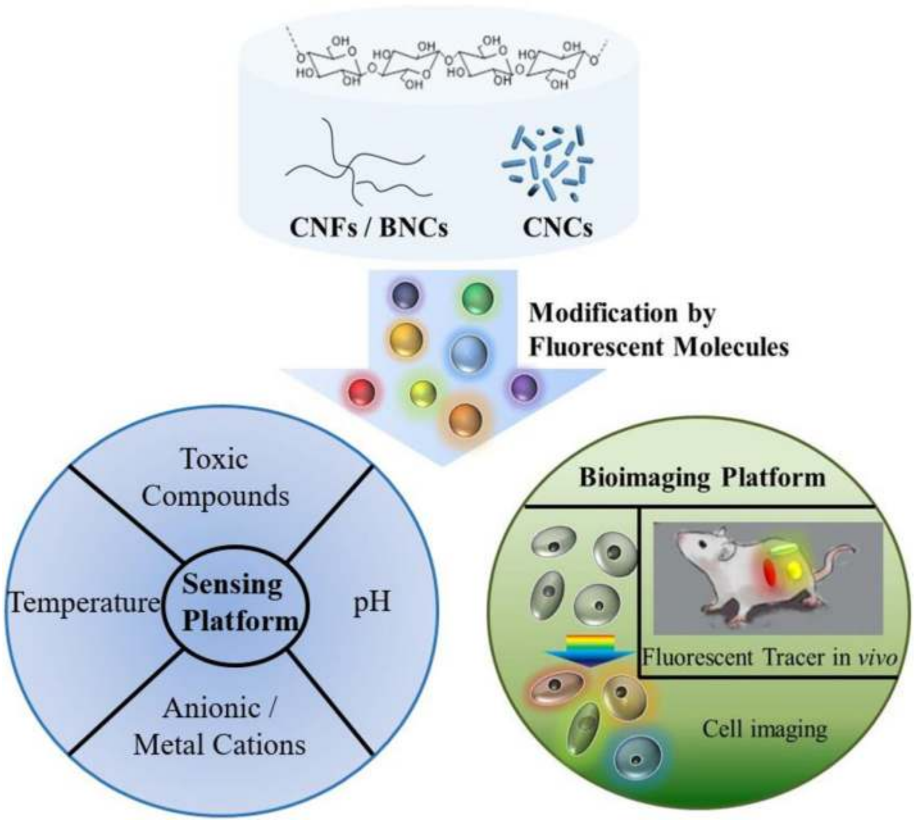

Fig. 16. Different biosensing applications involving use of fluorescent materials for spectroscopy and bioimaging. (Reproduced with permission) [109].

the growth of L929 cells. During a cultivation period of 24 h, the images showed significant intracellular fluorescence with 50 μg mL^{-1} of the composite, and measured cell survival rate of 95% or above. Fig. 16 summarises different prospects of fluorescent biosensing [109].

4. Conclusion

NC is biodegradable, exhibits good biocompatibility and cytocompatibility, and is available in abundance in nature. In biocomposites, for biomedical applications, NC is capable of forming an interconnected network with biopolymers with the help of crosslinkers. This 3-D network provides mechanical stability to the composite while maintaining adequate porosity, which is crucial for supply of essential nutrients, proliferation of cells, etc. In this review, we highlighted numerous biomedical applications from the recent past in wound dressing, drug delivery, tissue engineering and scaffolds, medical implants, and biosensing. It is quite often observed that NC-based composites progressively decompose post tissue regeneration, attributed to the biodegradability of NC. However, in most of these researches, tests were carried out in vitro. The lack of relevant enzymes in the human body limits the in vivo degradability of NC. This challenge stands as a major concern in the interaction between host tissues and those inside composite scaffolds. The interaction of fabricated nanocomposites with the micro-organisms present in the local environment is yet to be explored. Certain microbials have been observed to develop resistance against antiseptics. Side effects of regular and long-term use of Chlorhexidine as an oral antiseptic is a good example [110]. Studies evaluating the long-term effect of NC-based composites on the behavior microbials present in the local environment, need to be carried out. Some of the highlighted studies in this review involve the use of biopolymers. It can be noted here that, only a few of these studies involve the use of conducting biopolymers. Research in the future will be dedicated to combining the electrochemical properties of conducting polymers and structural stability of NC hydrogels for making self-healing, implantable electronic devices. These might be capable of replacing the existing medical devices such as retinal implants, cochlear implants, electronic pacemakers, smart contact lenses, and so on [111].

Biosensors based on NC have good flexibility, mechanical and thermal stability, stability in water, exhibit optical transparency, etc. In terms of structural stability, BNC substrates show superior mechanical stability compared to those based on other forms of NC (CNCs or CNFs), even when hydrated. This shows that BNC is a better option for making substrates for SPE devices. Besides, the porous structure of BNC-, or even that of CNC- or CNF-hydrogels, would eliminate the possibility of skin irritation resulting from prolonged use of modern wearable devices. Use of IL along with BNC can also be explored further, as the former enhances the sensitivity of these devices. These SPE devices can further be employed and tested as immunosensors or can be combined with drug delivery therapies in therapeutics and diagnostics as well. The biosensors highlighted in this review, require a considerable amount of electrolyte fluid for generating signals useful for analysis. Fabricating skin mountable nanocellulose based membranes with embedded systems for sensing, and capable of providing real-time analysis, may serve as a solution for the same. There are a lot of combinations and facets that are yet to be explored in biomedical research. In the long term, NC-based biocomposites are capable of revolutionising the state-of-the-art of biosensing and biomedical technology.

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