



Bacterial nanocellulose as a versatile scaffold for biomedical applications: Synthesis, functionalization, and future prospects

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ABSTRACT

Bacterial nanocellulose (BNC) consists of nanofibres of cellulose randomly entangled during their synthesis by particular bacterium cells during fermentation. Thus, it may be regarded as a unique three-dimensional of pure cellulose that is consolidated during the generation of the fibres by their microbiological factories. The metabolic synthesis and purification processes are approached, underscoring its potential as an ideal template for the production of biomedical composite solutions, based on its inherent nano-driven assets. Also, BNC properties can be greatly enhanced by the incorporation of bioactive substances, which may be inorganic or organic, leading to the formulation of superior wound dressings and implants. In wound dressings, the most relevant agents encompass silver and copper nanoparticles, curcumin, and hyaluronic acid. BNC implants cover several organs such as blood vessels, heart valves, nerve scaffolds, cornea, tympanic membranes, vocal cords, bone, cartilage, dental structures, contrast and drug delivery agents. The functionalization of BNC with iron oxide nanoparticles, graphene, alginate, chitosan, silk fibroin, or bone morphogenetic protein 2 human in implants allows the enhancement or mimicking of mechanical properties, cell migration, proliferation, differentiation, and biocompatibility. This highlights the potential of using BNC as a scaffold for biomedical applications, offering a compelling alternative to petrochemical-based biomaterials.

1. Introduction

Bacterial nanocellulose (BNC) is a versatile polysaccharide excreted by bacteria that has gained interest in the biomedical field due to its intrinsic physicochemical properties. This biopolymer presents high purity and flexibility, high water retention (> 90 %), high degree of polymerization (2,000–6,000) and crystallinity (70–90 %). Its entangled nanofibrous structure results in a very porous material similar to the extracellular matrix (ECM). In addition, the BNC hydrogel possesses excellent mechanical properties, namely high tensile strength and elastic modulus [1–3]. Such properties endow BNC with a broad spectrum of possible applications in the biomedical field. Hydrogels are some of the most promising biomaterials because they are prone to chemical modifications with bioactive chemicals, genes, and cells, thus opening an

array for a variety of applications in dental care, wound dressing, and controlled drug delivery. Tissue engineering has considerably invested in the regeneration and reconstruction of damaged tissues. Three-dimensional (3D) structural scaffolds have led to the development of functional engineered tissues for mechanical support during *in vivo* implantation, such as cartilage, bone, skin, heart valves, nerves, and tendons [4].

A variety of inorganic nanoparticles (NPs) are used in healthcare applications: silver (Ag), gold (Au), copper (Cu), platinum (Pt), titanium (Ti), and their oxide derivatives. Their size ranges from about 1 to 100 nm and vary in shape, e.g., nanodisks, nanowires (Nws), nanotubes, and nanorods (NRs). These intrinsic physicochemical properties remarkably revolutionize healthcare, including therapeutics, optoelectronics, drug discovery, diagnostic biological probes, catalysis, display instruments,

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biological sensors, and the detection of environmental toxic metals or reagents and medicines. Metal or bare NPs are known to potentially induce toxicity by directly interacting with cells, or through the release of toxic metal ions, hindering their use in biomedical applications. Thus, biological processes are taking over the production of NPs, due to its safer and eco-friendlier approach. These processes include the use of distinct biological resources such as plants, bacteria, actinomycetes, fungi and yeasts, viruses, and a wide variety of marine and freshwater algae [5]. Another aspect to consider regarding metal NPs is their high surface energy and *van der Waals* forces that facilitate aggregation, hindering their effectiveness. Aggregation may be mitigated through the use of a substrate able to stabilize the NPs [6].

Hyaluronic acid, bone morphogenic protein 2, gelatine, chitosan, lignin, and polylactic acid (PLA), are some examples of biocompatible organic fillers used in biomedical applications. These fillers are less likely to cause an immune response or other adverse reactions. However, similar to inorganic NPs, their synthesis can be both laborious and hazardous. Added features include improved strength, flexibility, promotion of cell growth, and antimicrobial properties [7–11].

This review provides a detailed description of the synthesis of BNC and its mechanical properties according to the physical state of the biopolymer. In addition, it focuses on the various applications of BNC when functionalized or in combination with inorganic or organic compounds, and its significance in wound dressings, implants, and cancer therapy.

2. Bacterial nanocellulose

2.1. Synthesis

Different genera of fermentation bacteria are recognized as possessing the required genes for BNC production such as *Rhizobium*, *Azotobacter*, *Agrobacterium*, *Aerobacter*, *Salmonella*, *Escherichia*, among others. Nevertheless, the most prominent belong to the *Acetobacteraceae* family, namely: *Novacetimonas* and *Komagataeibacter* [12]. The biosynthesis of BNC corresponds to an extremely complex and highly regulated metabolic process that undergoes several steps and involves a series of genes responsible for the encoding of both enzymes and different

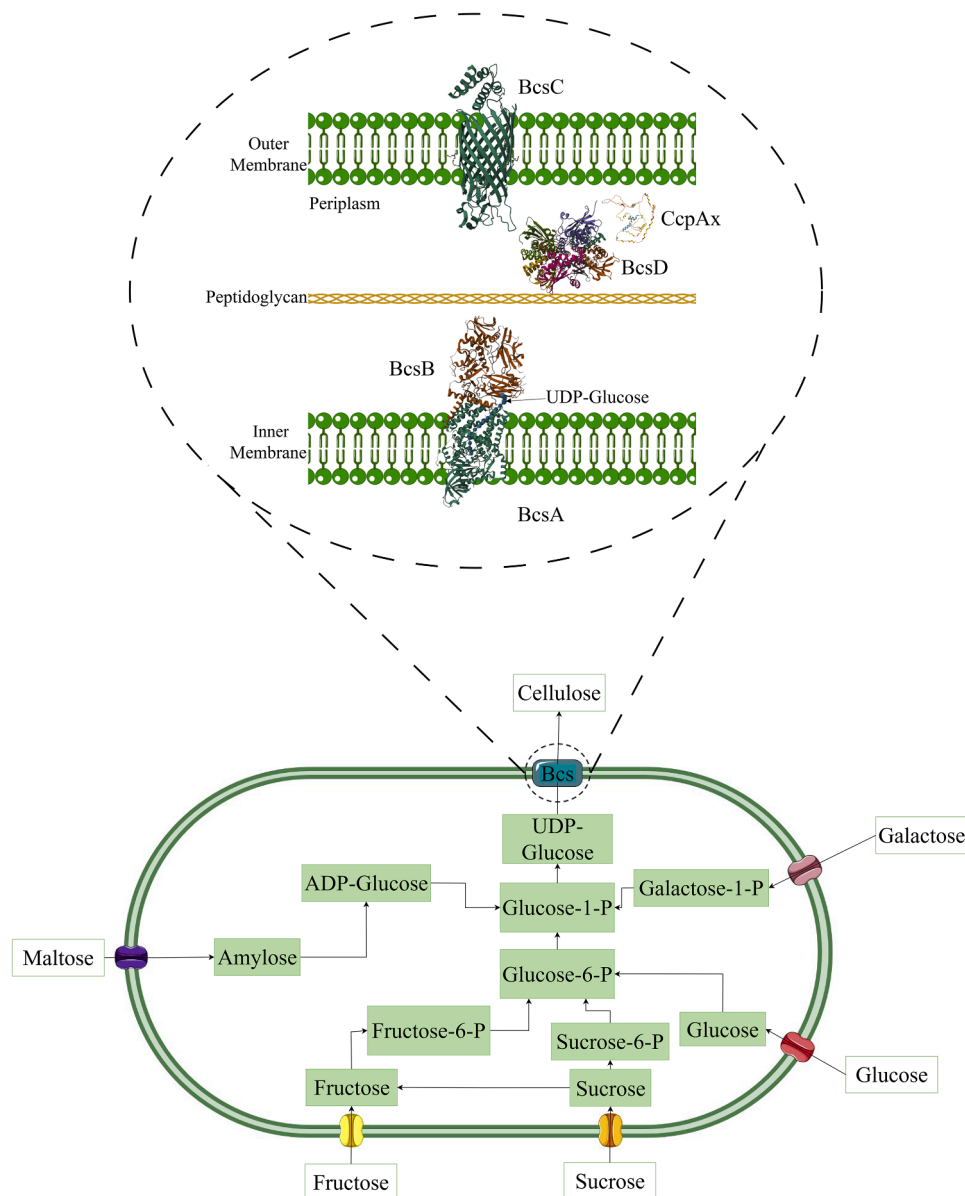


Fig. 1. Schematic representation of the biosynthetic pathway for BNC and organization of the various subunits in the bacterial cellulose synthase complex. Adapted from Ref. [14], under the Licence CC BY 4.0, 2021, Nanjing Medical University.

catalytic complexes [13,14]. BNC is produced as fibres that intertwine with each other. As their concentration increases, a 3D network is developed thus creating a floating macroscopic porous membrane. This structure is formed by the bacterial cells entrapped between the produced cellulosic ribbons, which allows for these non-motile, strictly aerobic bacteria to grow at the interface of the culture medium [12,15]. This process can use multiple carbon sources for the BNC building blocks such as glucose, fructose, and galactose. Therefore, it is important to note that BNC will vary depending not only on the carbon source used, but also on the bacterial strain. Different carbon sources and different metabolic profiles will govern BNC properties such as the mechanical properties, crystallinity, and thickness of the produced BNC [15,16].

BNC biosynthesis (Fig. 1) using glucose as the main carbon source is the most common and straightforward metabolic pathway. It may be broken down into four main enzymatic steps: (i) phosphorylation of glucose into glucose-6-phosphate via glucokinase; which is followed by the (ii) preparation of linear glucan chains, meaning the isomerization of glucose-6-phosphate by phosphoglucosyltransferase into glucose-1-phosphate; (iii) uridine diphosphate glucose (UDP-Glc), a direct precursor of cellulose, is synthesized by UDPG-pyrophosphorylase, which translates into the assembly and crystallization of glucan chains into hierarchically structured cellulose ribbons; lastly, (iv) UDP-Glc polymerizes into cellulose by a membrane protein complex called cellulose synthase, and it is secreted to the extracellular environment [13,14,17–20]. It should be underscored that for BNC synthesis using disaccharides, such as sucrose, lactose, and maltose, the initial metabolic process is different. The disaccharides must first be converted into their respective monosaccharides through an additional hydrolysis step and only then undergo the cycle referred above [16,17]. As for the genes involved in this process, it is important to highlight those belonging to the BNC synthase operon (*bcs*), namely *bcsA*, *bcsB*, *bcsC*, and *bcsD* [12,13,17]. *BcsA* has a catalytic role in cellulose production and its activity can be regulated through the cyclic di-GMP (di-guanosine monophosphate) messenger. It is embedded in the inner membrane as eight transmembrane helices and consists of two cytoplasmic domains: a catalytic β -1,4-glycosyltransferase domain conserved between transmembrane helices four and five and a C-terminus containing a PilZ domain. The binding of cyclic di-GMP to *BcsA* occurs in its PilZ domain and induces a conformational change that allows UDP-Glc access to the catalytic site. Once activated, this catalytic domain promotes the polymerization of UDP-Glc monomers into β -1,4-glucan chains [14,17]. *BcsB* is a large periplasmic protein anchored to the inner membrane through a single transmembrane helix. This protein interacts with *BcsA*, being responsible for the *BcsA* stabilization and enabling its catalytic activity. It is assumed that *BcsB* also has an important function in the transportation of the synthesized glucan chain through the periplasm into the outer membrane [14,17].

BcsC is a β -barrel type outer membrane porin protein. It is suggested that *BcsC* leads to the formation of membrane pores that allow cellulose secretion into the extracellular environment. In periplasmic domain of *BcsC* there is a tetratricopeptide repetition [13,14,17]. *BcsD* is a periplasmic protein that assists in the association and crystallization processes of the glucan chain. Moreover, the hierarchical cellulose assembly process is also largely controlled by the *BcsD*-driven arrangement of linear terminal complex arrays and their orientation longitudinal to the axis of the cell. It also prepares the glucan chain for secretion through *BcsC* protein; however, the mechanism of its function is not fully described yet. Nevertheless, it is evident that this protein is essential for the optimal production of BNC [13,14,16,17]. The up regulation of carboxymethyl cellulase has been suggested as an additional factor for BNC enhanced production. This enzyme catalyses the hydrolysis of endo- β -1,4-glycosidic bonds of cellulose chains. However, its activity has been correlated with higher production of BNC due to its potential as cellulose error correction enzyme. This enzyme is expressed by the *cmcAx* gene [14]. Other proteins with a hypothesized relevant role in BNC production are *ccpAx* protein and β -glucosidase. *CcpAx* protein possibly assists BNC production during the crystallization phase. It

interacts with the *BcsD* subunit and is also able to facilitate protein-protein interactions during the assembly of the cellulose synthase complex [12,14]. Finally, β -glucosidase (expressed by *bglxAX* gene), is envisioned to modify and affect the degree of polymerization of the newly synthesized glucan chains. However, the details of its actual role have not been unveiled yet [14,17]. Initially, sub-fibrils are formed, then they crystallize and organize themselves to form fibrils, which in turn combine to form cellulose nano- or microfibrils, consisting of about 1,000 individual glucan chains [13,14,20].

Thus, BNC consists of an ultra-thin 3D network developed and stabilized through inter- and intramolecular hydrogen bonds. The high amount of hydrogen bonds endow the material with its remarkable water-holding capacity (about 200 times its dry weight) [14] and also the formation of its hierarchical structure. The nanofibrous structure of BNC is able to mimic the fibrous architecture of the ECM (e.g. collagen, elastin and fibrin fibres that are components of connective tissues), and its resistance, flexibility, and porosity are important in the support of tissues that undergo constant stress and deformation like skin, muscle and cartilage, and the porosity and permeability that allows diffusion of gases or nutrients [13,14,16–18] [16,19].

The morphology of BNC can vary depending on the culture methods used, ranging from uniform membranes to spherical or irregular pellet-shaped hydrogels. There are two main types of bacterial culture used to obtain BNC: static and agitated culture. Fig. 2 displays some examples of BNC produced from different strains, obtained from static and agitated cultures. This distinction influences not only the supramolecular structure of the obtained product but also its mechanical characteristics and physical properties [14]. Due to its simplicity, static culture represents the most commonly used technique for the production of BNC as a flat membrane with a homogeneous supramolecular structure [13,14,18,20,21]. This approach allows the use of containers of any shape, which in turn, will dictate the shape of the membrane formed [17,18]. In this technique, BNC producing bacterium are incubated for 2 to 20 days at temperatures between 25 and 30 °C [13,14]. The most commonly used culture medium is the Hestrin-Schramm medium, which uses glucose as the main carbon source, yeast extract as a source of nitrogen and growth factors, peptone as a nitrogen source, and citrate-phosphate buffer to prevent pH reduction caused by gluconic acid during the bacteria aerobic growth [16,21]. It should be noted that the variation of any of these parameters affects the final product, making the incubation time the most critical factor. It is relevant to mention that the membrane is generated at the air-liquid interface, where there is more oxygen available. As the membrane produced on the medium grows downwards and entangles the bacteria, it limits the oxygen supply, resulting in reduced oxygenation and overall lower productivity. Incubation time is the main factor that governs the thickness of the BNC membrane produced [13,14,16–18,20,22]. However, historically, static culture is characterized as incompatible with industry. The most common arguments are the high costs associated with culture medium and the assurance of aseptic conditions in a large area. An established strategy to reduce costs is the use of alternative culture media based on agricultural and industrial wastes particularly rich in carbon sources, such as sugar cane processing industries and fruit juice production plants [14]. The agitated or dynamic culture comprises agitation or mixing of the culture medium to enhance the dissolved oxygen and improve the availability of nutrients concentration, consequently increasing the rate of BNC biosynthesis [14,16–18,21]. The shape and size of BNC is governed by the applied rotational speed. It usually presents itself with a spherical, cocoon-shaped, pellet-shaped, or even irregular clump-shaped morphology, therefore its applications are generally different from the membranes obtained through static culture [13,17,18,22]. Furthermore, BNC from dynamic culture possesses higher water absorptivity and higher suspension viscosity. The dynamic culture increases the amount of dissolved oxygen [21,22]. Furthermore, in dynamic culture, BNC usually possesses lower crystallinity and inferior mechanical properties such as lower Young's modulus, due to the decreased degree of

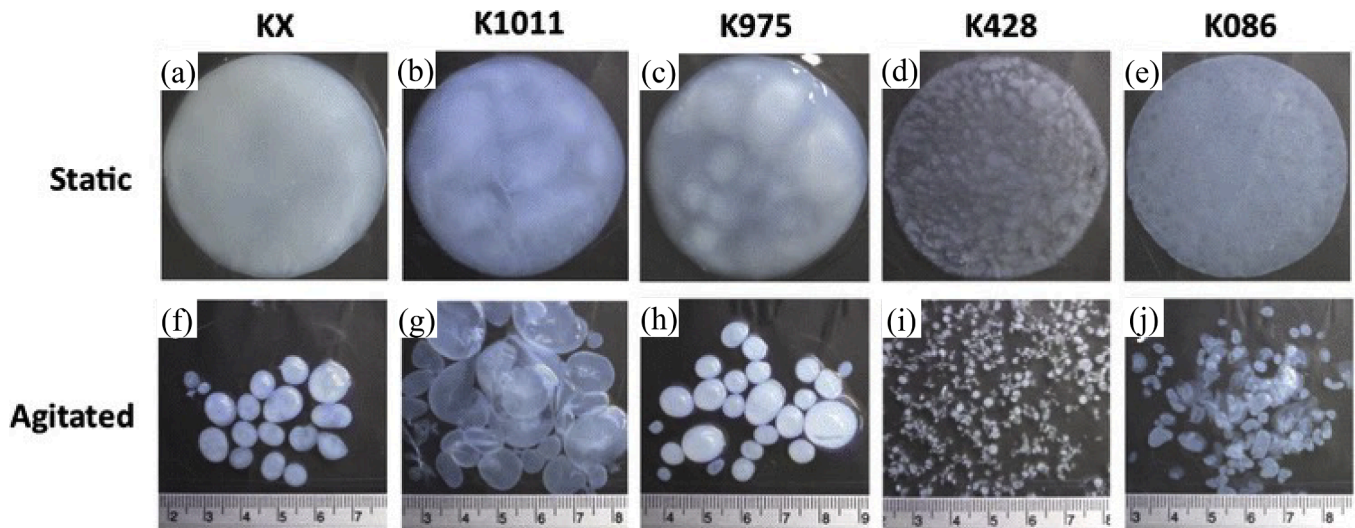


Fig. 2. Optical images of BNC produced by five strains of *K. xylinus*, (a-e) in static condition, and (f-j) in agitated condition. Reproduced from Ref. [23] with permission, Copyright 2018, Springer Nature.

polymerization and organization of the BNC nanofibres [13,14,16,21].

Bioreactors are able to ensure a suitable control of media flow and aeration which aids in the proper growth of microorganisms and animal cells. It is thus expected that they should be able to overcome those previously identified disadvantages. However, despite several types of bioreactors being tested for the scale-up production of BNC, the ideal automated process for industrial-scale production of this material has not yet been achieved [13,19,20]. There are a series of bioreactors that have been studied in this process, including the stirred, airlift, membrane, and rotating disc reactors, as illustrated in Fig. 3. The most appealing property of stirred-type bioreactors for the industrial production of BNC is their high volumetric mass transfer coefficient for the

transfer of oxygen. However, the agitation associated with this type of bioreactor can harm the productivity of BNC by the same principles of the agitated culture. In addition, this process is energy-intensive when compared to other bioreactors, especially airlift bioreactors [17,24]. Airlift bioreactors are particularly interesting due to their simple design and facile maintenance. Besides, they have proved to be an energy-efficient method that involves less shear stress on the membranes than the one felt in typical agitated methods. Normally, airlift produced BNC bioreactor is characterized by either a fibrous or pellet shape, with higher water-holding capacity. Its mechanical properties can be altered by changing the number of separating plates [14,17,18]. Membrane reactors, on the other hand, often use oxygen-permeable silicone

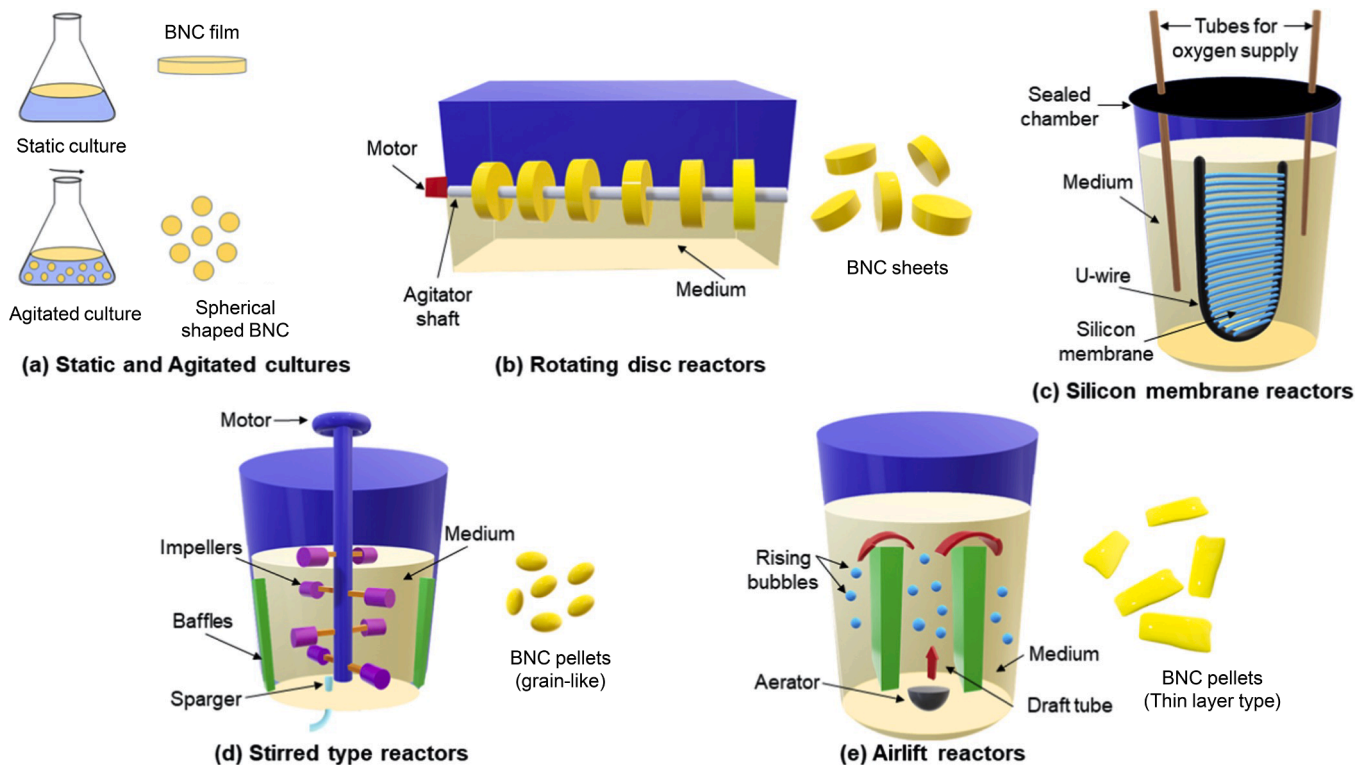


Fig. 3. Schematic representation of bacterial cellulose production in (a) static and agitated reactors (b) rotating disc reactors (c) silicon membrane reactors (d) stirred type reactors (e) airlift reactors, their components and shape of BNC produced. Reproduced from Ref. [17] with permission, copyright 2022, IPO Science.

membranes so that a BNC membrane can be formed on its surface. The degree of roughness of this membrane directly correlates with the rate of BNC that can be produced. These bioreactors are characterized by their high oxygen permeability and large surface area but they are associated with high operating costs and difficulties in the harvesting of the BNC [17,20]. A different option is the rotating disc reactors, which are designed so that half of the disc's surface is in contact with the air, while the other half is submerged in the culture medium. This particular configuration allows for better access of the bacterium attached to the disc to oxygen and nutrients present in the reactor, which increases the yield of the process at low rotation speeds. The production rate can be altered by changing either the rotational speed of the discs or the distance between them. Despite this, it has been shown that the yield of BNC is not that higher than the one obtained from static culture [17,20,24]. Although all these bioreactors offer slightly improved yields compared to conventional static and agitated methods [20]. One possible explanation for the absence of higher BNC yields through dynamic culture may be correlated to the shear stress of dynamic cultures which is reflected by the presence of a higher concentration of negative cellulose producing mutants [13,14,17,20–22].

2.2. Purification

Ubiquitously BNC is embedded with bacterium cells and culture medium containing secondary metabolites [25]. Living bacterium cells and other impurities represent potential safety hazards and will impact the quality and characteristics of the BNC fibres. Thus, BNC must be submitted to different purification steps. Typical purification procedures are described in Table 1 [26]. Pure BNC displays improved mechanical strength, biocompatibility, and enhanced functional characteristics, such as water-holding capacity. In addition, if BNC contains a low thickness, it becomes more transparent, becoming more adequate for optics and electronics applications [27]. Commonly, the purification protocols displayed in Table 1 are sufficient to meet biomedical applications requirements, such as physicochemical, cytotoxicity, hemocompatibility, and biocompatibility [28]. Numerous industries are exploring the potentialities of BNC, and as this field of study develops, so do novel methods for purification and functionalization.

As observable in Table 1, all purification methodologies comprise the use of an alkali solution (NaOH or KOH). Thus, all purification processes involve the mercerization of BNC. Mercerization will simultaneously degrade amorphous regions of cellulose and will convert cellulose I polymorphs to cellulose II. Cellulose II polymorph contains one additional hydrogen bond per cellulose monomer; thus, it is a more thermodynamically stable polymorph. Therefore, purified BNC exhibits enhanced properties in comparison to unpurified BNC [38].

2.3. Properties for biomedical applications

Considering the various applications in biosensing, immunotherapy, drug delivery, tissue engineering and regeneration, implants, and medical devices, it is of utmost significance that the materials potentiate a favourable environment for the growth and differentiation of cells. Non-toxicity, biocompatibility, mechanical, and physical requirements are also mandatory when classifying a biomaterial as ideal. Naturally occurring polymers, such as: chitosan, alginate, gelatine, starch, cellulose, and silk fibroin (SF), present enhanced biocompatibility. It is hypothesized that their similarity with ECM prevents immunological reactions and inhibits chronic inflammatory response. Their degradation within the human body may or may not be desirable, depending on the proposed application [39,40].

BNC is highly biocompatible and has been tested in animals and humans as long-term implants for up to one year. Furthermore, the literature supports this statement through tests performed with BNC-based implants that showed no severe signs of inflammation, no formation of fibrotic capsule, nor did it present toxicity according to

Table 1

Purification methods applied to different bacteria and the carbon source used throughout their fermentation.

Bacterium	Carbon Source	Purification Method	Ref
<i>Bacillus</i> sp. strain SEE-3	Glucose	• Distilled water (wash and boil) • 0.1 M NaOH (3 h, 80 °C)	[25]
<i>Komagataeibacter europaeus</i> SGP37	Glucose	• Distilled water (until neutral pH) • Tap water (wash) • 0.5 M NaOH (1 h, boil) • Distilled water (30 min, boil) • Distilled water (until neutral pH) • 0.5 % aqueous KOH solution	[29]
<i>Starkeya</i> sp. strain N1B	Naphthalene	• Water (until neutral pH) • 1 or 4 M NaOH (depending on the thickness, 10 to 30 times)	[30]
<i>Komagataeibacter</i> sp.	High fructose corn syrup (Hungrasweet F50)	• Distilled water (until neutral pH) • Tap water (24 h, wash) • 0.1 % solution of NaOH (15 min, 70 °C)	[31]
<i>Komagataeibacter pasteurianus</i> ATCC 23760	Glucose	• Distilled water (until neutral pH) • Sterilized by Gamma irradiation (20 kGy) • 1 M KOH solution (48 h)	[32]
<i>Komagataeibacter hansenii</i>	Glucose, mannitol, fructose, sucrose, and glycerol	• 0.5 M HCl bath (1 h) • Distilled water (until neutral pH) • 0.1 M NaOH solution (2 h, 80 °C)	[33]
<i>Komagataeibacter sucrofermentans</i> DSM15973	Glucose, pure glycerol, and crude glycerol (20 %)	• Distilled water (until neutral pH) • 0.5 % (w/v) aqueous NaOH solution (6 h, 80 °C)	[34]
<i>Komagataeibacter xylinus</i> ATCC 23770	Glucose	• Deionized water (until neutral pH) • 0.5 M NaOH solution (3 days, solution replaced twice a day)	[28]
<i>Komagataeibacter sucrofermentans</i> ATCC 700178	Fructose	• Deionized water (until neutral pH) • Distilled water (12 h, water changed hourly)	[35]
<i>Taonella mepensis</i>	Glucose	• 1 % NaOH solution (1 h, 80 °C) • Distilled water (until neutral pH) • 2 % sodium dodecyl sulphate (SDS) solution (magnetic stirrer, overnight)	[36]
<i>Komagataeibacter hansenii</i> ATCC 53582	Glucose	• Distilled water (until SDS removal) • 1 M NaOH solution (50 rpm, 90 min, 60 °C) • Distilled water (until neutral pH)	[37]

genetic and cellular assays [2]. This proves that BNC can be an excellent material for wound dressing, hard and soft tissue engineering (e.g. skin regeneration, artificial dura mater membrane, facial nerve regeneration, prosthetic hernioplasty, cancer diagnosis, drug delivery, tissue-engineered cornea stroma, and neuroendovascular applications) [40–42].

Relating to its *in vivo* degradation, that is, degradation under

physiological conditions, BNC is known to be principally degraded by cellulase, an enzyme that is not produced by the human body. So, in principle, this is beneficial for long-term implants. But it also limits its applications, for instance, to achieve a drug delivery system, BNC should be degraded within a short period [2,40]. Factors affecting biodegradation are the mechanical stress of bodily movements or inflammatory cellular response, that contribute to changes in crystallinity and molecular weight. In addition, several other mechanisms that contribute to BNC degradation have been identified: hydrolysis, enzymatic degradation, oxidation, and physical degradation. The chemical structure of BNC is composed of linear glucose units, each with one primary hydroxyl (OH) group and two secondary OH groups. These acting sites form glycosides that can be chemically attacked by body fluids constituents forming oligomers, and consequently monomers. During hydrolysis, the biomaterial absorbs the body fluids, leading to swelling and degradation. Enzymatic degradation occurs due to the presence of endogenous hydrolase enzymes present in plasma and interstitium that accelerate the hydrolysis of the glycosidic linkages. Oxidative degradation occurs as a result of hydrogen removal from the polymer chains by oxidants produced by tissues. Physical and chemical scission of bonds in the polymer chain can be due to swelling and wearing, which accelerate the damaging of the amorphous region by environmental stress [40].

If desired, a biomaterial can be tailored to become biodegradable through modification with metal NPs or bioactive molecules [40]. Considering the fibrous nanosized 3D network, high OH content, and water retention capacity, it is expected that BNC can retain considerable concentrations of drugs, either small (e.g., diclofenac, octenidine, and lidocaine) or large molecules (polymeric drugs, peptides, or proteins) relevant for drug delivery. Specific applications include dermal/transdermal, oral, and dental administrations [2]. A drug-loaded BNC for dental therapies was reported by Weyell and co-workers [43] and demonstrated slow partial degradability under physiological conditions in the mouth, showing potential as an innovative wound dressing and drug delivery systems. A hydrolytic degradable BNC-based hydrogel scaffold incorporating calcium phosphate for bone tissue regeneration was developed by Basu *et al.* [44].

The mechanical strength of BNC is equivalent to synthetic polymers, resulting from the complex interlaced nanofibres that develop into a

porous matrix. The arrangement of BNC microfilaments within the 3D nanofibrillar network gives rise to a thin, extremely fluid, and hydrophilic structure. In addition, the crucial mechanical strength of BNC is attributable to its linear cellulose chains and high cohesion between macromolecules [45].

The Young's modulus and stiffness are significantly higher in dry samples compared to hydrated BNC [46]. Drying at atmospheric pressure and temperature leads to a higher density of cellulose fibrils due to its smaller volume (thickness), reflected in less porosity, more homogeneous structure, higher quantity and stronger hydrogen bonds and higher crystallinity, ultimately improving strength and Young's modulus. The freeze-drying method maintains the cavities between the layered structure leading to lower crystallinity and less quantity and weaker hydrogen bonds between the cellulose molecules, impacting Young's modulus [47]. If on the one hand, the typical behaviour of brittle materials was evidenced by oven-dried BNC, freeze-dried BNC on the other hand, exhibited low Young's modulus and higher elongation before fracture [48]. A summary of the different values obtained in the literature is presented in Table 2.

3. Bioactive substances and antimicrobial agents and their applications

Bioactive substances promote tissue growth by interacting with and integrating into damaged tissue, thus exhibiting biological activity. The incorporation of bioactive molecules within BNC, such as natural polymers (e.g., proteins: collagen, silk, gelatine, and fibrin; polysaccharides: starch, chitosan, cellulose, and alginate; polynucleotides: DNA, RNA) and inorganic compounds (e.g., metal NPs: Cu, Ag, Ti, and Au; minerals: hydroxyapatite, calcium phosphate, and silica (Si)) instil very desirable properties to the polymeric scaffold. These may include structural, regenerative, and/or antimicrobial properties, or act as cancer treatment agents. Some of these properties can be achieved through the application of external stimuli like radiation and ultrasound. Internal stimuli include pH, enzymes, and redox [54,55]. Hyaluronic acid is often used in ligament tissue engineering, osteochondral defects, and vascular grafts. Chitosan applicability can be found in bone formation and cartilage tissue engineering. Proteins like collagen or gelatine are largely used in drug delivery and tissue engineering scaffolds, and silk can be

Table 2
Mechanical properties of BNC produced under static mode, according to the physical state of the sample.

Sample	Size (cm)	Thickness (μm)	Load (kN)	Strain rate (mm min ⁻¹)	Young's modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)	Ref
Vacuum dry	n.d.	n.d.	n.d.	n.d.	5.13	n.d.	n.d.	[47]
Dry	n.d.	n.d.	n.d.	n.d.	13.02	n.d.	n.d.	[47]
Oven dried	2.0 × 6.0	22.7	1.0	5.0	5.6±0.76	37.2±3.3	2.20±0.06	[48]
Dry (25 °C)	1.5 × 10	300–500	n.d.	10	n.d.	17.38±7.23	6.63±1.55	[49]
Dry (25 °C) - rehydrated	1.5 × 10	300–500	n.d.	10	n.d.	17.40±3.68	11.04±3.68	[49]
Dry (40 °C)	6.5 × 2	2670±670	10	2–4	0.139±0.022	10.34±3.69	7.94±3.39	[46]
Dry (80 °C)	1.5 × 10	300–500	n.d.	10	n.d.	12.94±1.80	6.40±1.61	[49]
Dry (80 °C) - rehydrated	1.5 × 10	300–500	n.d.	10	n.d.	8.49±5.47	11.08±2.75	[49]
Hydrated	0.6 × 3.5	800	0.005	10	0.0056±0.0030	n.d.	n.d.	[50]
Hydrated	0.6 × 3.5	800	0.005	10	0.0011±0.0004	n.d.	n.d.	[50]
Hydrated	1.0 × 10	300–500	n.d.	n.d.	9.14	112.4	0.6	[51]
Hydrated	1.5 × 10	300–500	n.d.	10	n.d.	1.18±1.01	12.80±2.17	[49]
Moist (1.82 %)	1.5 × 10	280	n.d.	4.8	1.044	20.76	2.28	[52]
Moist	6.5 × 2.0	2670±670	10	2–4	0.02638±15.22	4.64±0.32	21.74±6.25	[46]
Freeze dried	2.0 × 6.0	34.9	1.0	5.0	0.18±0.06	7.2±1.1	4.92±1.2	[48]
Freeze dried	6.0 × 1.0	n.d.	n.d.	n.d.	2.68±0.73	n.d.	n.d.	[53]

n.d. – not defined

found in drug delivery applications. Biopolymers, for example PLA or poly-(γ -glutamic acid) (PGA), are found in implant and suture [39]. A more thorough description and examples of the applicability of such bioactive molecules within the BNC matrix are described below.

3.1. Wound healing and wound dressing

Skin disorders, including wounds, can profoundly affect the well-being, resulting in a noticeable decrease in overall quality of life [56]. Despite the availability of several therapeutic strategies, the ongoing demand for developing and enhancing new wound healing methods remains crucial [57,58]. As part of the treatment for skin wounds, the application of wound dressings plays a pivotal role. These wound dressings are designed to foster an optimal healing environment, enhance patient comfort, and offer mechanical properties that simplify their application for enhanced efficacy [59]. To establish an optimal environment for wound healing, it is required to uphold moisture retention, improve exudate absorption, facilitate oxygen exchange, and promote re-epithelialization, minimizing wound exposure duration and preventing infections. These essential elements contribute to an effective and accelerated wound healing process [57]. Being a biopolymer endowed with remarkable characteristics, BNC fulfils the fundamental requirements for promoting an exceptional and accelerated wound healing process, either through its incorporation as a skin substitute or direct application as a wound dressing (see Fig. 4) [60,61].

Extensive evidence indicates that BNC exhibits superior properties to traditional gauze-based wound dressings or synthetic products, making it an outstanding choice for wound healing applications [26]. The biocompatibility of BNC is attributed to its structural similarity to collagen, which facilitates both epithelialization and angiogenesis. This remarkable property enables its effective application in treating diverse wound types, including ulcers, burns, skin grafts, lacerations, and skin substitutes. Its wide-ranging efficacy in managing diverse types of wounds further accentuates its promising prospects for applications in wound healing [20,62]. Furthermore, high water retention enhances moisture retention and contributes to minimizing the adherence of the wound dressing to the affected area, particularly in burn cases [63]. Consequently, removing the wound dressing considerably reduces pain and discomfort, ensuring patients a more comfortable and less distressing wound healing experience [64]. The remarkable flexibility of BNC wound dressings allows for full adaptation and coverage of the affected area, making them a highly versatile and optimal choice for treating various wound types, including chronic, acute, and burns. This adaptability provides consistent wound support throughout the healing

process, leading to better patient outcomes and enhanced healing potential [61]. Due to its exceptional intrinsic properties, this biopolymer has led to the commercialization of various wound dressings. These wound dressings show exceptional effectiveness, leading to positive and notable outcomes [24]. Table 3 provides examples of commercialized BNC-based products specifically developed for wound dressing and wound healing.

Modification of BNC with active compounds (e.g., antibiotics, anti-inflammatory drugs, peptides, proteins, and inorganic NPs) facilitates the healing process by quickly enhancing it, thus minimizing exposure to the inflammatory phase [57,71]. The BNC structure's abundance of OH groups provide exceptional capacity for modifications. This intrinsic versatility empowers the production of BNC as a versatile material with potential application in several biomedical fields, including wound healing, drug delivery, and tissue engineering [13,72–74].

3.1.1. Inorganic compounds in wound dressings

The large surface-to-volume ratio of inorganic NPs, together with their heat resistance and long-term stability, are interesting and effective characteristics that demonstrate therapeutic potential against infections. This is especially important considering the substantial increase in antimicrobial resistance. In fact, multidrug-resistant pathogens are responsible for the death of 700,000 people yearly, according to the World Health Organization (WHO) [75]. Metal NPs can improve the antibacterial properties of wound dressing materials by killing the bacteria, thereby controlling the infections and promoting wound healing processes. They can stimulate the cellular and molecular process to maintain the wound microenvironment such that the healing process is facilitated by inducing antibacterial, antimicrobial, anti-inflammatory, and angiogenic effects [76]. Several attempts were made to improve the antibacterial properties of wound dressing materials and enhance wound healing by incorporating metal NPs such as AgNPs, CuNPs, zinc oxide (ZnONPs), and magnesium oxide NPs (MgONPs) [77–81].

Effective against bacteria, fungi, and viruses, the mechanism of action of AgNPs can be based on Ag^+ ion release that interacts with the cell membrane of bacteria leading to cell death. In addition, these ions generate reactive oxygen species (ROS), thus targeting the bacterial cell envelope causing DNA damage [75]. The shape and size of AgNPs are other factors that influence their antibacterial effectiveness. This can be controlled with the synthesis methodology, and the application fields vary [82]. The use of AgNPs in wound dressings is known to promote wound healing due to decreased inflammatory response. However, there is evidence of significant transdermal penetration of AgNPs into

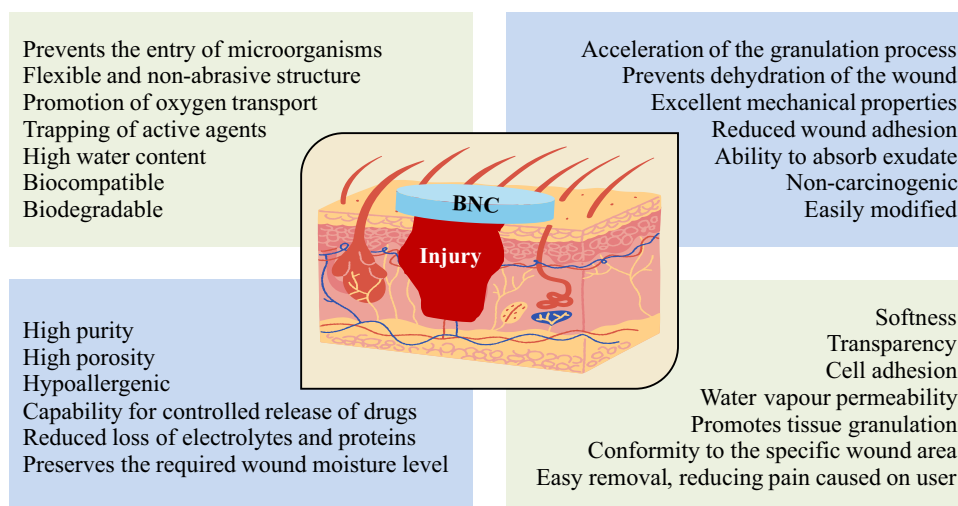


Fig. 4. Properties that classify BNC as an ideal scaffold for wound healing.

Table 3

Examples of commercialized BNC-based products specifically developed for wound dressing and wound healing.

Commercial name (producing bacteria)	Company/distributor	Active compound	Properties	Wound type	Ref
Biofill® (<i>Acetobacter xylinum</i>)	BioFill Produtos Bioetecnologicos, Curitiba, Brazil	None	Reduced risk of infection Facilitates the healing process Pain relief Water vapor permeability Durable	Burns Abrasions Ulcers	[24, 65]
Bioprocess® (n.d.)	BioFill Produtos Bioetecnologicos, Curitiba, Brazil	None	Acceleration of the healing process	Burns Abrasions Ulcers	[66]
Dermafill™ (<i>Acetobacter xylinum</i>)	Cellulose Solutions Ltd, Georgia, USA	None	Pain relief Acceleration of the healing process	Burns Abrasions Lacerations Ulcers	[65]
Nanoskin® (<i>Komagataeibacter xylinum</i>)	Innovatec	None	Non-toxic Biocompatible Hypoallergenic Maintenance of a humid environment Facilitates oxygen exchange Promotes granulation and epitelisation	Ulcers Skin cancers Amputations	[57, 67]
Membracel® (<i>Komagataeibacter xylinum</i>)	Vuelo Pharma, Curitiba, Brazil	None	Rapid skin regeneration Maintenance of wound moisture Pain relief Facilitates oxygen exchange Hypoallergenic	Ulcers Burns Lacerations	[57]
CelMat® (n.d.)	Bowil Biotech Sp., Wladyslawowo, Poland	None	Non-toxic Promotes cell regeneration Protects against entry of micro-organisms	Burn wounds	[57]
EpiProtect®2117 (n.d.)	S2Medical AB, Linköping, Sweden	None	Permeable to water vapour and air Impermeable to liquids Bacterial barrier Facilitates evaporation of exudate	Burn wounds	[68]
Bionext® (<i>Komagataeibacter xylinum</i>)	Bionext Produtos Biotecnológicos, Curitiba, PR Brazil	None	Pain relief Reduced risk of infection Facilitates the healing process	Ulcers Burns Lacerations	[69]
Prima Cell™ (n.d.)	Xylos Corporation, US	None	Pain relief Facilitates the healing process	Ulcers	[65]
Nanoderm™ (n.d.)	Axcelon Biopolymers Corporation, Ontario, Canada	None	Prevents infections Pain relief Reduces bacterial proliferation Minimises hydroelectrolytic loss	Infected wounds	[57]
Nanoderm™ Ag (n.d.)	Axcelon Biopolymers Corporation, Montreal, Canada	Ag	Controlled release of Ag Sustained antimicrobial activity Flexible Economical	Infected wounds	[26, 57]
Suprasorb X® (n.d.)	Lohmann & Rauscher International, Neuwied, Germany	Polyhexamethylene biguanide (PHMB)	Maintenance of wound moisture High exudate absorption Comfortable Reduced risk of infection Antimicrobial activity	Ulcers Burns Post-surgical wounds Skin grafts Abrasions Lacerations	[26]
Xcell® (n.d.)	Xylos Corporation, US	PHMB	Pain relief Accelerates the granulation process Antimicrobial activity Maintenance of wound moisture	Ulcers	[70]

n.d. – not defined

capillaries during the use of surgical dressings. In fact, dose-dependent histopathological abnormalities in the skin have been reported [83]. Despite some negative views and facts over the use of AgNPs, films of AgNws with BNC prepared by Wan *et al.* (2020) were able to provide biophysical cues that facilitated cell proliferation and progressed the wound-repairing process, along with the expected antibacterial effect and excellent stretchability and flexibility, as illustrated in Fig. 5 [84]. Ag, in the form of nanoprisms (AgNPrs), were prepared using a light-induced transformation reaction with light-emitting diodes (LED) to reduce their inherent toxicity to mammalian cells. Cell viability of the

films using human dermal fibroblasts (HDFa) was in the range of 68.86–124.19 % for direct cytotoxicity test and 89–118 % for indirect cytotoxicity test, indicating that the films can be considered non-toxic. The composite also demonstrated good antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus* [85]. Xie and co-workers (2019) used polydopamine (PDA) as a reducing agent to produce AgNPs to reduce their toxicity and enhance the biological compatibility of the BNC composites. The chelation between PDA and Ag and the 3D network of BNC plays a crucial role in the stable release of Ag⁺ ions. The samples showed good

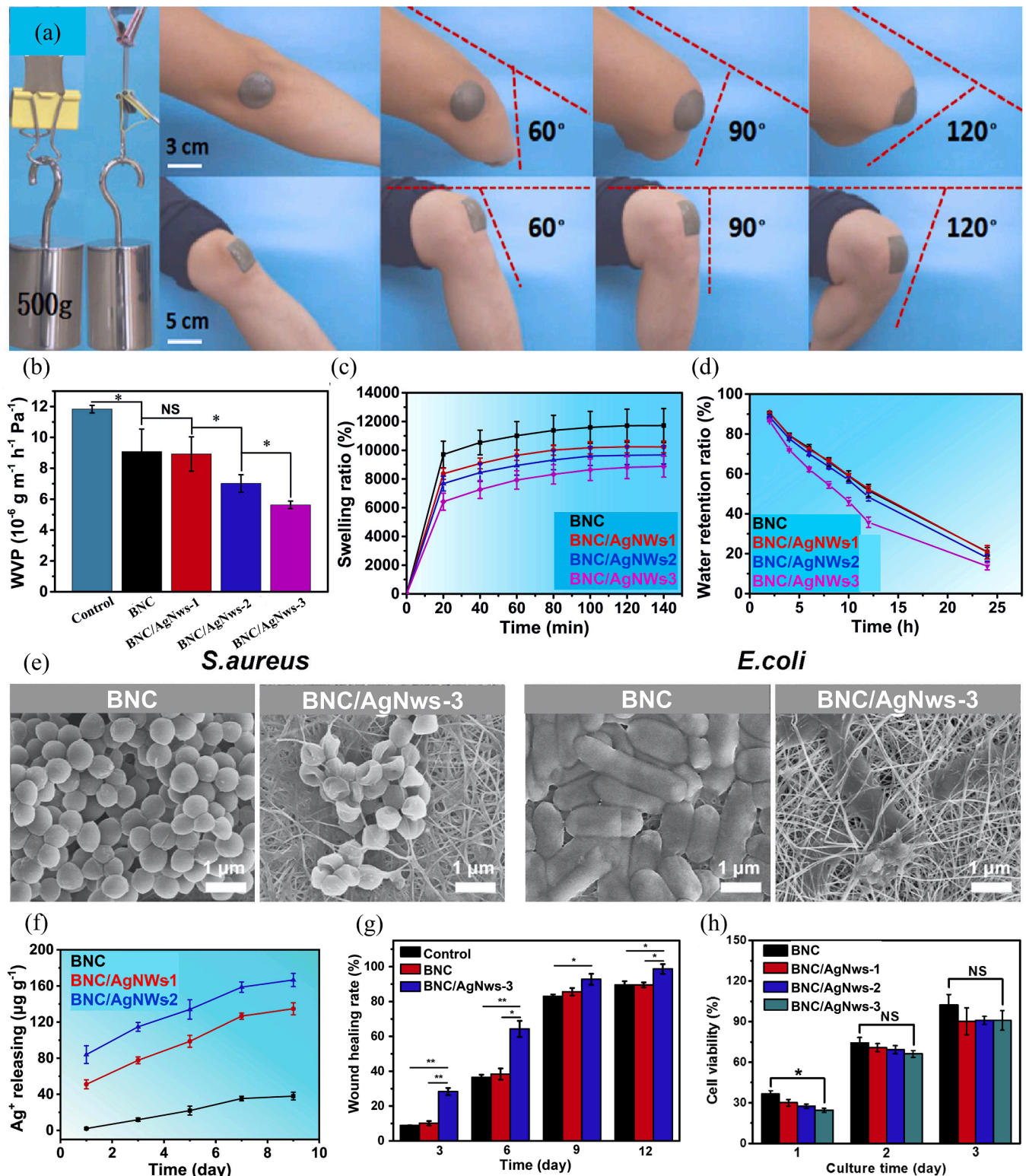


Fig. 5. (a) Digital images showing the robustness and excellent stretchability and flexibility of the BNC/AgNws dressings, (b) water vapour permeability (WVP), (c) water uptake, (d) water retention rate, of BNC/AgNws dressings, (e) Scanning Electron Microscopy (SEM) images of bacteria on BNC/Ag dressings (scale bars: 1 μm), (f) release profiles of Ag^+ from BNC/AgNws dressings (* $p < 0.05$, $n = 4$, NS means insignificant, $p > 0.05$), (g) wound healing rate (WHR) after *in vivo* animal experiment (h) viability of NIH/3T3 cells cultured on BNC/AgNws dressings. Adapted from Ref. [84] with permission, Copyright 2020, Elsevier.

cytocompatibility against normal human dermal fibroblasts, demonstrating that PDA supported cell growth and eased the stable release of Ag^+ [86]. The reducing properties of the catechol groups of PDA were similarly explored by Jiji *et al.* (2020). Cell viability was tested using NIH/3T3 cells and found that the BNC/PDA/AgNPs composite showed

cell viability, a non-toxic nature, and improved cell proliferation. A synergistic effect of AgNPs and PDA has assisted in promoting fibroblast proliferation, granulation tissue formation, angiogenesis, and re-epithelialization. AgNPs eliminate the infection from pathogens and lead to an effective healing process. The antimicrobial activity

mechanism of the AgNPs is justified by encountering the sulphur in membrane proteins and phosphorus contents in the cells that activate ROS to create oxidative stress and eventually death of cells [87].

Clays have attracted interest in the medical field due to their crystalline structure, colloidal particle size, cation exchange, and swelling capacity. Besides being abundant in nature and thus of low-cost, the amount of clay required for incorporation into a polymer matrix is very low, while still conveying the desired characteristics [88]. In line with this, montmorillonite (MMT) is being explored in the production of wound dressing applications. Horue *et al.* (2020) engineered a low-cost film based on BNC and Ag-modified MMT. The developed films revealed inhibition in the growth of both *S. aureus* and *P. aeruginosa* bacteria. Cytotoxicity was tested by using mouse skin fibroblast L929 cells and the results suggested that the samples are non-toxic to this cell line, however, toxicity may be dose-dependent, particularly for AgNPs [89]. Cerium (Ce), a metal that is used in the production of ceramics as stabilizers, has antimicrobial activity in the form of cerium nitrate ($\text{Ce}(\text{NO}_3)_3$). In addition, it is believed to exert a protective effect against post-burn immunosuppression. Taking this into consideration, Teixeira and co-workers (2019) idealized BNC membranes functionalized with $\text{Ce}(\text{NO}_3)_3$ and AgNPs. Despite the cell viability of mouse fibroblasts L929 maintaining in the range of 70 %, these results show that higher toxicity levels are verified for $\text{Ce}(\text{NO}_3)_3$ compared to AgNPs [90].

Copper oxide (CuO) is one of the most extensively explored metal oxides due to its excellent antibacterial properties and electrochemical properties. Several works showed the enhancement of the antibacterial properties of BNC membranes in the presence of Cu or CuO NPs [79,91]. Xie *et al.* (2019) reported a work where BNC was blended with graphene oxide (GO) to promote the growth of CuO nanosheets. The composite membranes showed good antibacterial activity against *E. coli* and *P. aeruginosa* and was particularly significant in Gram positive *S. aureus* and *Bacillus subtilis*. Good cell viability of NIH/3T3 cells was verified due to the slow release of Cu^{2+} [92].

ZnONPs are widely used due to their antimicrobial activity against a large spectrum of bacterial microorganisms [93], and their applicability ranges from drug delivery, cosmetics, food industry, medical field, and agriculture [94]. They are also listed by the Food Drug and Administration (FDA) as a generally regarded as safe (GRAS) material to use in the food industry [95]. ZnONPs have particular interest in the treatment of skin diseases as they are biogenic and exhibit immunomodulatory properties, therefore good for wound healing [96].

The introduction of carbon-based materials to BNC, endows additional functionalities such as conductivity, thereby producing heat with the application of a low voltage. The heat provided can promote wound healing by accelerating wound cell migration. The combination of BNC, GO, dopamine, and AgNPs proved compatible with the mouse fibroblast cell line NIH/3T3. The addition of AgNPs further stimulated migration and proliferation of cells (keratinocytes) and largely facilitated wound closure [97]. The addition of GO makes the film conductive, and the heat produced can assist in the stimulation of deep or surface skin cells at the wound, promoting the healing process [98]. The physical stability of the BNC hydrogel can be improved with the addition of polyacrylic acid (PAA). Nonetheless, Chen *et al.* [50] verified that the addition of GO led to a decrease in the mechanical properties of hydrogels and this behaviour can be explained by the reduction of functional groups due to the electron beam irradiation, used in the samples' preparation. In this case, biocompatibility of the hydrogels was improved with GO content indicating good cell growth and favourable cell proliferation site due to the improved water swelling properties [99]. Hydrogels with good bactericidal effect on strains like methicillin-resistant *S. aureus* (MRSA) are essential to reduce nosocomial infections. In this regard, Zmejkoski *et al.* (2021) proposed the use of graphite in the form of quantum dots along with BNC. The graphene quantum dots (GCD) composite hydrogel showed bactericidal effect against MRSA, *E. coli*, and *P. aeruginosa*. The results from scratch assay have shown that BNC/GCD hydrogel had the desired effects on human gingival fibroblast (HGF), assisting in

angiogenesis and regeneration process and resulting in the wound closure. The percentage of fluid absorption and water retention was higher in the BNC/GCD hydrogels in comparison with the control BNC samples. The bactericidal activity against MRSA is crucial as it appears in hospitals and can cause surgical wound infections and so on. Hence BNC/GCD hydrogels can be a good candidate not only for wound dressing but also as wound healing and disinfectant [100]. Delayed healing of diabetic wounds is causing a great burden on the healthcare system. This is triggered by the long inflammatory stage, reduced angiogenesis, and interference in cellular proliferation. The results have suggested that multiwall carbon nanotubes (MWCNT) have a positive effect on cellular adhesion and proliferation due to their nanofibrous structure. Hence, Khalid *et al.* (2022) incorporated MWCNT into the BNC matrix via *ex situ* method to favour cell adhesion, proliferation, reepithelization, and facilitate a faster wound healing process. The samples were tested for antibacterial and wound healing properties. They have used bacterial strains such as *S. aureus*, MRSA, *E. coli*, *P. aeruginosa*, *Salmonella Typhi*, and *Klebsiella pneumonia*. The assays indicated that the introduction of MWCNTs showed an antibacterial effect. The inhibition zones of samples with MWCNT were higher than that of control BNC and a standard drug (neomycin sulphate+bacitracin zinc). The wound healing suggested that the samples with MWCNTs had the highest wound healing ability (99 %), compared to the positive control group (95 %) and negative control group (77 %) at the 21st day. All samples showed moderate epithelization [101].

Properties such as biocompatibility, good antibacterial activity, and strong oxidation have made titanium dioxide NPs (TiO_2 NPs) a promising material for wound dressing applications. However, to improve the response to light and separation of electron-hole pairs, researchers suggested doping with other NPs. In this regard, carbon quantum dots (CQD) are considered noteworthy because of their properties such as hydrophilicity, high aqueous stability, and low toxicity. On the other hand, CQD could also participate in the process of angiogenesis by enhancing the expression of the anti-angiogenic factors and enhancing the interleukin-6 expression in the inflammation phase [102]. Hence, Abolghasemzade *et al.* (2021) used CQD in conjunction with SiNPs and SF to promote wound healing. The produced BNC/CQD/SiNPs/SF nanocomposite showed an increase in tensile strength of 60 % and a decrease in Young's modulus by 50 %. Improved antibacterial activity in comparison with other samples against *E. coli* and *S. aureus*, was also verified. The average wound area was less than the control samples which suggests that it helps the proliferation and migration of NIH/3T3 cells, helping in the healing process [103].

BNC membranes were functionalized with gold nanoclusters (AuNCs) modified by Wang *et al.* (2021) with aminobenzenboronic acid (ABA). The tensile strength of the membranes improved with the addition of modified AuNCs and showed good antibacterial activity against Gram negative bacteria. The healing ratios of the composite were higher than the BNC membranes, with cell viability of more than 80 % [104]. Table 4 lists the aforementioned works developed using inorganic compounds for BNC-based wound dressings and wound healing applications.

Although inorganic compounds offer potential advantages in wound healing, it remains imperative to confront distinct drawbacks and hurdles to minimize risks and guarantee the users' well-being and safety [105]. Indeed, the cytotoxic effect remains the primary disadvantage of inorganic NPs. Nevertheless, it is essential to acknowledge that several factors influence this property, including the type of NPs, concentration, size, agglomeration, and surface charge. These factors influence their application's safety and potential risks [106]. Moreover, incorporating these NPs may affect biocompatibility, increasing the toxicity of the final composite. These can potentially trigger an inflammatory response, interfering with the normal wound healing mechanism [107,108]. Despite the increasing number of studies focusing on incorporating inorganic NPs, more clinical evidence is still warranted.

Table 4

Examples of BNC functionalized with inorganic compounds for wound dressings and wound healing applications.

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Wound dressing	Commercial AgNWs (n.d.)	n.d.	<i>In situ</i> – spraying of AgNWs onto the BNC substrate in culture media containing bacteria	Water uptake: >6000 % Water retention: 13.7 % after 24 h Porosity: <92 % Water contact angle: from ~28 to 68.7° Thermal stability improved Complete wound healing Good antibacterial activity against <i>E. coli</i> and <i>S. aureus</i> Tensile strength: 1.56 MPa Young's modulus: 2.88 GPa Strain: 44 % Good antimicrobial activity against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>E. faecalis</i> , and <i>S. aureus</i> Human dermal fibroblasts Non-cytotoxic	[84]
Wound dressing	AgNPs (Decahedral, 31.62 ±0.60 nm, pH=11) (Triangular, 75.16±6.06 nm, pH=10) (Truncated octahedron, 117.4±18.09 nm, pH=9)	n.d.	<i>Ex situ</i> – Immersion	Porosity: 28.75 % Swelling properties: reduction to 103 % Tensile strength: 1.72 MPa Young's Modulus: 232.34 MPa Elongation-at-break: 179.65 % Antibacterial activity: 80 % inhibition against <i>E. coli</i> after 72 h Cell growth inhibition by about 7.5 % compared to the blank and BNC Ag ⁺ release rate less than 0.35 mg L ⁻¹ within 48 h Good thermal stability below 100 °C Ag ⁺ release <0.005 mg L ⁻¹ Thermally stable below 100 °C Swelling ratio decreased Tensile strength improved Antibacterial activity: <i>E. coli</i> (7.8 mm after 12 h) <i>E. coli</i> (6.6 mm after 72 h) Good bactericidal fastness Non-cytotoxic BNC/Cu ₂₀ Improved thermal stability Release of Cu ²⁺ : Burst release during the first days. Sustained release from 60–90 days	[85]
Wound dressing	AgNPs (n.d., ~50 nm)	n.d.	<i>In situ</i> – immersion of BNC/chitosan in PDA followed by another immersion in a AgNO ₃ solution and reduced under UV lamp	Antibacterial activity: 80 % inhibition against <i>E. coli</i> after 72 h Cell growth inhibition by about 7.5 % compared to the blank and BNC Ag ⁺ release rate less than 0.35 mg L ⁻¹ within 48 h Good thermal stability below 100 °C Ag ⁺ release <0.005 mg L ⁻¹ Thermally stable below 100 °C Swelling ratio decreased Tensile strength improved Antibacterial activity: <i>E. coli</i> (7.8 mm after 12 h) <i>E. coli</i> (6.6 mm after 72 h) Good bactericidal fastness Non-cytotoxic BNC/Cu ₂₀ Improved thermal stability Release of Cu ²⁺ : Burst release during the first days. Sustained release from 60–90 days	[109]
Wound dressing	AgNPs (n.d., >50 nm)	AgNO ₃ reduced using an UV lamp (80 W, λ =254 nm, distance of 45 cm, 60 min.)	<i>In situ</i> – immersion of BNC/GO in a PDA solution followed by another immersion in a AgNO ₃ solution, and reduced under a UV lamp	Antibacterial activity: <i>E. coli</i> (7.8 mm after 12 h) <i>E. coli</i> (6.6 mm after 72 h) Good bactericidal fastness Non-cytotoxic BNC/Cu ₂₀ Improved thermal stability Release of Cu ²⁺ : Burst release during the first days. Sustained release from 60–90 days	[98]
Wound dressing	CuNPs (quasi-spherical, n.d.)	CuCl ₂ ·2H ₂ O reduced with NaBH ₄	<i>In situ</i> – immersion of BNC in different solutions of CuCl ₂ . After rinsing, immersion in NaBH ₄ followed	Antibacterial properties: <i>S. aureus</i> (14.7±1.2 mm) <i>E. coli</i> (14.0±1.0 mm) Non-cytotoxic: Cell viability: >70 % BNC/SPG/ZnONPs Swelling ratio: 725±19 % Tensile strength: ~70 MPa Antibacterial properties: <i>E. coli</i> (90 %) <i>S. aureus</i> (70 %) Non-cytotoxic: Cell viability (normal human fibroblast cells) >95 % BNC/ZnONPs/BDP Release of Zn ²⁺ after 14 days: 1.56 ppm Non-cytotoxic: Cell viability (L929) 99.3±4.5 % Wound Area (cm ²): 12.825±0.311 Fe/Alginate/Casein/BNC ₅₀ Compressive moduli: 94.0±3.6 %	[91]
Wound dressing	Commercial ZnONPs (n.d., 30 nm)	n.d.	<i>Ex situ</i> – immersion of BNC in an aqueous SPG solution with ZnONPs followed by crosslinking with glutaraldehyde	Non-cytotoxic: Cell viability (normal human fibroblast cells) >95 % BNC/ZnONPs/BDP Release of Zn ²⁺ after 14 days: 1.56 ppm Non-cytotoxic: Cell viability (L929) 99.3±4.5 % Wound Area (cm ²): 12.825±0.311 Fe/Alginate/Casein/BNC ₅₀ Compressive moduli: 94.0±3.6 %	[110]
Wound dressing	ZnONPs (hexagonal, 11.5–17.5 nm)	n.d.	<i>Ex situ</i> –spraying of ZnONPs onto BNC <i>In situ</i> – immersion of BNC in a solution of zinc nitrate tetrahydrate, followed by oxidation with NaOH	Antibacterial activity: <i>E. coli</i> (17.2±1.4 mm) <i>S. aureus</i> (30.7±2.6 mm) Non-cytotoxic: Cell viability >80 % BNC/PAA/GO (60/20/20 %v/v, 50 kGy) Swelling ratio: 2074.98±92.46 %	[96]
Wound dressing	Fe ₃ O ₄ (spherical, 47.2 ±13.7 nm)	n.d.	<i>Ex situ</i> – immersion in culture medium containing <i>K. xylinus</i> 3611 T	Antibacterial activity: <i>E. coli</i> (17.2±1.4 mm) <i>S. aureus</i> (30.7±2.6 mm) Non-cytotoxic: Cell viability >80 % BNC/PAA/GO (60/20/20 %v/v, 50 kGy) Swelling ratio: 2074.98±92.46 %	[111]
Wound dressing	Commercial GO (n.d.)	n.d.	<i>Ex situ</i> – mixing	Swelling ratio: 2074.98±92.46 %	[99]

(continued on next page)

Table 4 (continued)

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Wound dressing	GQD (disc-like, 2–20 nm)	n.d.	<i>Ex situ</i> – immersion	Water vapour transmission rate: 484.83±16.32 % Necessary mechanical properties required for wound dressing Cell availability 90.23±4.01 % Bactericidal effect against MRSA and <i>E. coli</i> Biocompatible Wound fluid absorption: 117.7 % BNC/CQD/SiNPs /SF Tensile stress: 17.95 MPa Elongation: 10.60 % Young's Modulus: 320 MPa Water uptake: 73.5 %	[100]
Wound dressing	CQD (21.8±0.6 nm) CQD/SiNPs (43.9±2.4 nm) > CQD/SiNPs/SF (91.1±3 nm)	n.d.	<i>Ex situ</i> – spraying	Antibacterial activity (MIC): <i>E. coli</i> (2±0.1 mg mL ⁻¹) <i>S. aureus</i> : (2±0.3 mg mL ⁻¹) Non-cytotoxic: Cell viability (NIH/3T3) 108±5.82 % Antibacterial activity: <i>B. subtilis</i> (9 mm) <i>K. pneumoniae</i> (12 mm) <i>S. aeromonas</i> (6 mm) <i>E. coli</i> (11 mm) <i>Kluyvera spp</i> (10 mm)	[103]
Wound dressing	TiO ₂ (170±35 nm) AgNPs (n.d.)	n.d.	<i>In situ</i> – TiO ₂ sol-gel coating of BNC/SiO ₂ followed by immersion in AgNO ₃ and reticulated in glycerol	Antibacterial property: bacteriostatic against <i>S. aureus</i> and <i>E. coli</i> was 85 % Ag ⁺ release rate: ~0.1–0.2 µg mL ⁻¹ Water absorption: 274 % No water penetration occurred during the vertical wicking test Increased alkaline resistance	[112]
Wound dressing	AgNPs (n.d., 100 nm)	n.d.	<i>In situ</i> – immersion of BNC/PDA in Tollens' reagent	Antibacterial activity: <i>S. aureus</i> (26±0.35 mm) <i>E. coli</i> (28±0.16 mm) PVA/BNC/Ag (4 mM) Swelling Property: 1604±58.2 % Water retention: 12.38 %	[86]
Wound dressing and biomedical applications	AgNPs (n.d., 30–50 nm)	n.d.	<i>In situ</i> – initial reduction of AgNO ₃ followed by addition in bacteria culture	Antibacterial activity: <i>S. aureus</i> (99.38±0.48 %) <i>E. coli</i> (99.72±0.14 %) Wound closure rate after 15 days: 97.89±0.97 % Non-cytotoxic: Cell viability (L929) 134 % Tensile strength: 5.21±0.03 MPa Ag ⁺ release after 24 h: ~24 µg mL ⁻¹	[113]
Wound healing	AgNPs (n.d.)	n.d.	<i>In situ</i> - reduction of AgNO ₃ by mixing with PVA in a solution of BNC containing sodium hydroxide, urea	Antibacterial activity: <i>E. coli</i> (15±1.0 mm) <i>P. aeruginosa</i> (11±0.5 mm) <i>S. aureus</i> (13±0.05 mm) <i>L. fusiformis</i> (14±0.75 mm) Non-cytotoxic Improved wound healing 5 wt.% BNC/ZnO Increased water vapour transmission: 2506.56 g ⁻¹ m ⁻² day ⁻¹ to 2856.60 g ⁻¹ m ⁻² day ⁻¹ Tensile strength (29.68 MPa) Young's modulus (2387.41 MPa) Elongation at break (1.30 %)	[114]
Wound healing	AgNPs (spherical, 15 nm)	n.d.	<i>In situ</i> – immersion of reduced GO/BNC/DOPA in AgNO ₃	Antibacterial activity: <i>E. coli</i> (15±1.0 mm) <i>P. aeruginosa</i> (11±0.5 mm) <i>S. aureus</i> (13±0.05 mm) <i>L. fusiformis</i> (14±0.75 mm) Non-cytotoxic Improved wound healing 5 wt.% BNC/ZnO Increased water vapour transmission: 2506.56 g ⁻¹ m ⁻² day ⁻¹ to 2856.60 g ⁻¹ m ⁻² day ⁻¹ Tensile strength (29.68 MPa) Young's modulus (2387.41 MPa) Elongation at break (1.30 %)	[97]
Wound healing	ZnONPs (spherical, n.d.)	n.d.	<i>In situ</i> – modified BNC membranes were immersed in different zinc acetate solutions and oxidized with NaOH	Non-cytotoxic Bacteriostatic rate: <i>S. aureus</i> (78.64 %) <i>E. coli</i> (37.67 %) Area of infected wound after 14 days: from 37.5 % to 7.5 % Decrease in inflammatory cells in the wound at day 7: from 483.1 to 142.4 BNC/PP/Fe/HCMS/GOx Water uptake capability: ~900 % Good antibacterial activity against <i>S. aureus</i> and <i>E. coli</i>	[115]
Wound healing	Fe@HCMS (hollow spherical shape, n.d.)	n.d.	<i>Ex situ</i> – immersion	Water uptake capability: ~900 % Good antibacterial activity against <i>S. aureus</i> and <i>E. coli</i>	[116]

(continued on next page)

Table 4 (continued)

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Wound healing, biofilms	MgO NPs (1) Wet chemistry (nanorod, 55–70 nm; nanodisc, 7000–400 nm) (2) Sonochemistry (sphere, 45–70 nm) (3) Commercial (leaf-shaped nano-sheet, diameter 40–45 nm, width 55–90 nm, length 135–240 nm)	n.d.	<i>In situ</i> – (1) Immersion and calcination (2) Immersion, sonication, and calcination (3) <i>Ex situ</i> – immersion and calcination	Hemocompatible (hemolysis ratio <1.5 %) Non-cytotoxic: Cell viability (NIH/3T3) 81 % Improved thermal stability Water contact angle: MgO NPs/sono 93.4° MgO NPs/ <i>ex situ</i> 109.8° Release 24 h: MgO NPs/sono 17.76 % MgO NPs/wet 14.21 % MgO NPs/ <i>ex situ</i> 28.21 % Mechanical properties: Sonochemical – Tensile strength (31.19±0.43 MPa), Young's modulus (163.87±2.43 MPa), Strain to break (6.19±0.54 %) <i>Ex situ</i> – Tensile strength (24.00±0.26 MPa), Young's modulus (82.93±0.97 MPa), Strain to break (6.11±0.39 %) Antibacterial activity: Wet chemistry – <i>E. coli</i> (11.99±2.13 mm), <i>S. aureus</i> (18.21±1.43 mm) Sonochemistry – <i>E. coli</i> (14.87±1.00 mm), <i>S. aureus</i> (15.65±0.98 mm) Commercial – <i>E. coli</i> (18.32±1.19 mm), <i>S. aureus</i> (25.11±3.02 mm) Improved thermal stability Non-cytotoxic up to 25 % MMT/Ag Antibacterial properties: <i>E. coli</i> (24.3±0.7 mm) <i>P. aeruginosa</i> (23.6±0 mm) <i>S. aureus</i> (19±0 mm) MRSA (18.8±0.3 mm) <i>S. typhi</i> (26.6±0.7 mm) <i>Klebsiella sp</i> (25.0±0 mm) Wound healing percentage: 99 % Antibacterial activity: <i>S. aureus</i> (17 mm) <i>P. aeruginosa</i> (13 mm) Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[81]
Chronic wounds	MMT/Ag (n.d.)	n.d.	<i>Ex situ</i> – immersion	Improved thermal stability Non-cytotoxic up to 25 % MMT/Ag Antibacterial properties: <i>E. coli</i> (24.3±0.7 mm) <i>P. aeruginosa</i> (23.6±0 mm) <i>S. aureus</i> (19±0 mm) MRSA (18.8±0.3 mm) <i>S. typhi</i> (26.6±0.7 mm) <i>Klebsiella sp</i> (25.0±0 mm) Wound healing percentage: 99 % Antibacterial activity: <i>S. aureus</i> (17 mm) <i>P. aeruginosa</i> (13 mm) Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[89]
Diabetic wounds	Commercial MWCNT (n.d.)	n.d.	<i>Ex situ</i> – immersion	Improved thermal stability Non-cytotoxic up to 25 % MMT/Ag Antibacterial properties: <i>E. coli</i> (24.3±0.7 mm) <i>P. aeruginosa</i> (23.6±0 mm) <i>S. aureus</i> (19±0 mm) MRSA (18.8±0.3 mm) <i>S. typhi</i> (26.6±0.7 mm) <i>Klebsiella sp</i> (25.0±0 mm) Wound healing percentage: 99 % Antibacterial activity: <i>S. aureus</i> (17 mm) <i>P. aeruginosa</i> (13 mm) Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[101]
Skin burns	Commercial AgNPs (n.d., 20 nm)	n.d.	<i>Ex situ</i> – immersion	Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[90]
Catalytic, magnetic, conductive, and biomedical applications.	BNC-ox-10Ag (n.d., 41.4 ±2.4 nm)	n.d.	<i>In situ</i> – mixing of AgNO ₃ in an oxidized BNC suspension followed by thermal treatment	Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[117]
Antibacterial materials	CuONPs (nanosheets, length: 50–200 nm, width: 20–50 nm)	Copper chloride dihydrate (CuCl ₂ ·2H ₂ O) reduced in the presence of GO and NaOH at 100 °C	<i>In situ</i> – mixing of CuCl ₂ ·2H ₂ O in a dispersion of GO nanosheets and NaOH. Followed by mixing of the obtained powder with BNC slurry	Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[92]
Clinical applications	AuNPs (lattice structure, 2 nm)	n.d.	<i>Ex situ</i> – immersion	Improved thermal stability with the addition of AgNPs and Ce(NO ₃) ₃ Wet to dry strength (W/D): 22.3 % Antibacterial property: compelling activity against <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and moderate effect against <i>C. albicans</i> BNC/GO/CuO _{10%} Antibacterial Properties: <i>S. aureus</i> (17.8±0.2 mm) <i>E. coli</i> (14.0±0.4 mm) <i>B. subtilis</i> (28.1±0.2 mm) <i>P. aeruginosa</i> (12.2±0.2 mm) Non-cytotoxic BNC/ABA/AuNCs 12 µg mL ⁻¹ Antibacterial activity: <i>P. aeruginosa</i> (16.2±0.18) multi-drug-resistant (MDR) <i>P. aeruginosa</i> (17.4±0.11) <i>E. coli</i> (15.6±0.18) MDR <i>E. coli</i> (13.8±0.11) Non-cytotoxic: Cell viability (human umbilical vein endothelial cells and human aortic fibroblasts) > 80 %	[104]

n.d. – not defined.

3.1.2. Organic compounds in wound dressings

Organic NPs represent a relevant strategy to minimize risks and ensure users' well-being and safety [13,118]. This is highly relevant for improving the wound healing practices. In response to this perspective, there has been a considerable upsurge in the optimization of research studies, explicitly targeting applying organic to replace inorganic NPs. This approach remains crucial, even if addressing biodegradability challenges is required [59].

The wound healing process involves three stages: inflammatory/hemostasis, proliferative, and remodelling [119]. The first phase, potentially the most critical, involves the presence of anti-inflammatory agents responsible for decreasing cyclooxygenase (COX) formation and inhibiting the production of post-inflammatory mediators, leading to pain relief. These agents include non-steroidal anti-inflammatory drugs (AINEs). Hoff *et al.* (2021) proposed a natural and organic alternative to

AINEs by incorporating α -tacophenol derivatives (α -13'-COOH) into BNC. A potential active wound dressing for advanced skin lesion therapy was developed. The biocompatible BNC composite, combined with this anti-inflammatory agent and polyethylene glycol (PEG) 400, exhibited advantageous effects in promoting wound healing process under both healthy and diabetic conditions, as depicted in Fig. 6, significantly decreasing wound closure time and the number of bacteria in burns after 3 weeks. A synergistic effect was established by combining α -13'-COOH and PEG400 [120]. Furthermore, integrating chitosan and collagen in the BNC matrix positively impacted multiple phases of the healing process. If on the one hand the incorporation of collagen *in situ* promoted the granulation process by encouraging the growth of skin cells, on the other hand, the *ex situ* application of chitosan increased antibacterial activity while enhancing macrophage activation and establishing binding interactions with erythrocytes. Notably, these effects did not

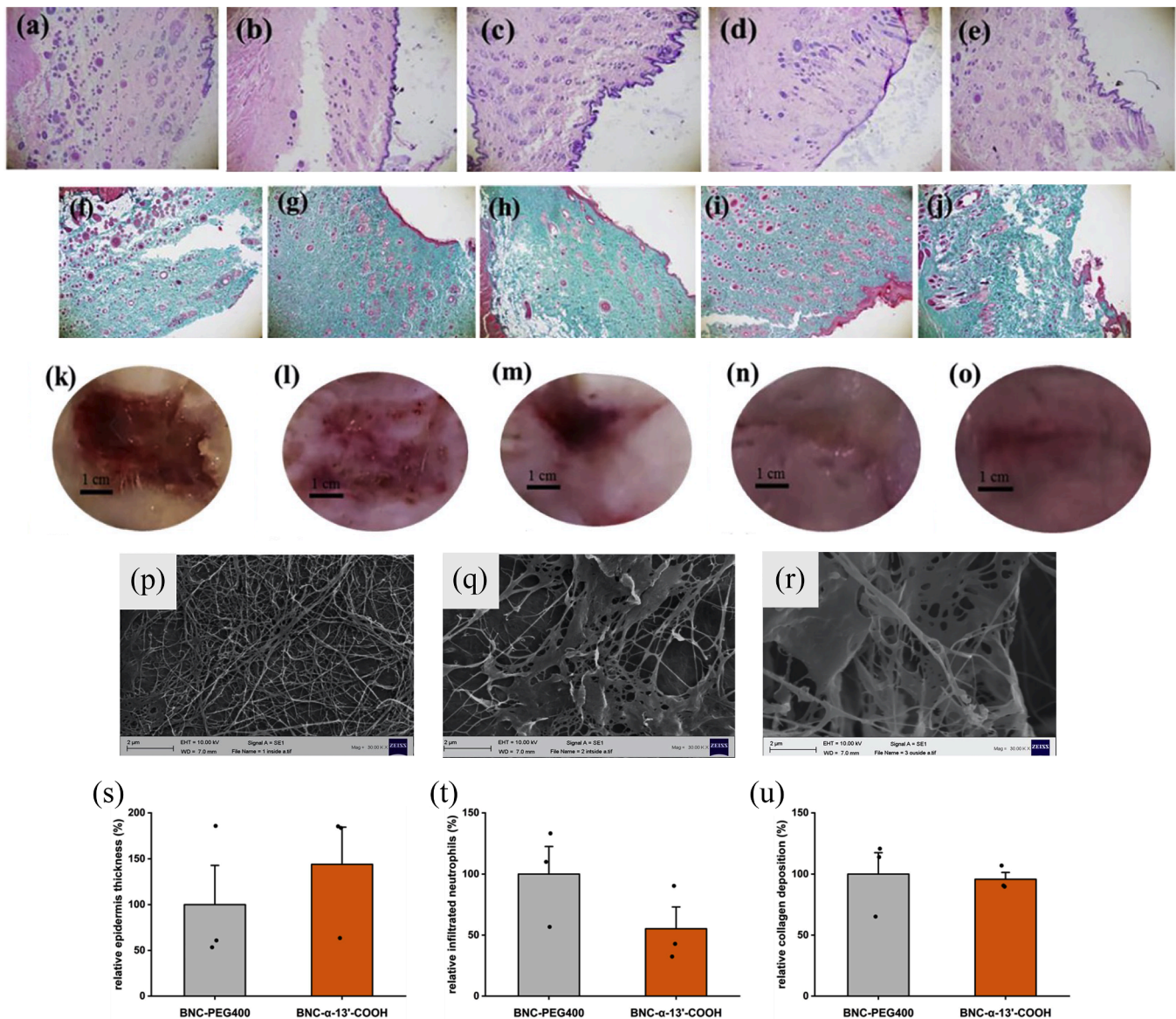


Fig. 6. Histopathological observations of skin tissue on day 21 with various treatments (a) control (–), (b) control (+), (c) BNC, (d) BNC/collagen, and (e) BNC/collagen/chitosan. Masson trichrome staining of skin tissue on day 21 with various treatments (f) control (–), (g) control (+), (h) BNC, (i) BNC/collagen and (j) BNC/collagen/chitosan. Photo of granulation of burned skin tissue on day 21 (k) control (–), (l) control (+), (m) BNC, (n) BNC/collagen, and (o) BNC/collagen/chitosan. SEM morphology of (p) BNC, (q) BNC/collagen, and (r) BNC/collagen/chitosan wound dressings. (s–u) Characterization of the wounds by (s) epidermal thickness, (t) neutrophil infiltration, and (u) collagen deposition after 10 days. Data are represented as mean bar plots with S.E. and individual data points. * Unpaired t-test compared to BNC-PEG400 control, $p < 0.05$. Adapted from Ref. [120,121], under the Licence CC BY 4.0, 2021, MDPI and with permission, Copyright 2023, Elsevier, respectively.

compromise the composite's hemocompatibility for burn treatment. Fig. 6t shows that BNC benefited from the loading with PEG400 that enabled constant moisture of the BNC over 24 h, essential during the wound healing process. Histological analysis of the wounds presented in Fig. 6v-x demonstrate a larger percentage of thicker epidermis, lower infiltrated neutrophils into wound tissue, and no pathological collagen deposition. The mechanical stability, represented by the compression strength of the loaded BNC, was increased, which is essential during handling and fixation by second dressing materials [121]. Yuan *et al.* (2019) also engineered an oxidized BNC nanocomposite, incorporating the functional properties of collagen and chitosan. Once again, this synergy of natural compounds supported hemostasis. The modification of BNC through TEMPO-oxidation heightened the composite's degradability. This amplified the hemostatic effect, resulting in an accelerated healing process that surpassed the performance of the commercial wound dressing Surgicel® [122].

Alongside chitosan and collagen, several organic compounds/NPs can be incorporated into the BNC structure to support the overall healing process. These include natural biopolymers such as chitosan, curcumin, lignin, alginate, hyaluronic acid, gelatine, collagen, and keratin. Furthermore, bioactive substances, including aminoacids, such as lysine, tyrosine, and arginine, promote cell proliferation and collagen synthesis. Synthetic compounds, namely PHMB, PVA, and PHEMA, can also be integrated into the structure, further enhancing its potential [59,123].

Curcumin is recognized as a wound healing agent, in addition to antimicrobial, antioxidant, and anti-inflammatory [59,124]. Consequently, curcumin has been incorporated into nanocomposites for wound dressings, including burns [125]. The hydrophobic nature of this macromolecule represents its main disadvantage. It reduced the water vapour permeability of the BNC composite, combined with the decrease in available spaces and the compaction of the structure. Notwithstanding, the permeability value remains above 150 %, indicating the potential to establish an environment promoting healing [126]. Encapsulating this compound offers a viable strategy to counteract its hydrophobic nature. Gupta (2019) encapsulated curcumin in hydroxypropyl- β -cyclodextrin. The resulting BNC composite demonstrated improved solubility while upholding essential criteria for wound dressing application, including hemocompatibility, water vapor permeability, as well as antioxidant and antimicrobial properties against *S. aureus*. Nevertheless, the highest dose of curcumin (2 % w/v) impacted cell viability, suggesting a potentially toxic effect on vital organs that warrants a reduction in dosage [127]. Lignin is the second most abundant class of lignocellulosic materials. It has an amorphous structure consisting of three basic phenylpropanolic monomers: (i) p-coumaryl, (ii) coniferyl, and (iii) sinapyl alcohols [128]. These monomers, characterized by their antimicrobial attributes, represent a favourable option for its incorporation in wound dressing formulations [57]. Incorporating a dehydrogenative polymer derived from coniferyl alcohol into DMSO-pre-treated BNC resulted in an accelerated healing process, pain relief, and notable antimicrobial efficacy [129]. Moreover, incorporating hyaluronic acid, another natural biopolymer, enhanced surface density and improved the smoothness of the BNC. The composite's observed enhanced biocompatibility (L0202) can be attributed to the presence of hyaluronic acid [130]. The BNC-hyaluronic acid composite exhibited non-cytotoxicity and improved water retention capacity after drying, surpassing that of traditional wound dressings (with polyacrylate fibre superabsorbent commercial dressing at 1400 % and Hydrofibre superabsorbent commercial dressing at 2600 %, in contrast to 3300 % for the developed wound dressing) [131].

Proteins present a viable agent for wound healing. An example of this is keratin, possessing an intrinsic capacity to expedite hemostasis and facilitate cell proliferation while retaining biocompatibility [132]. Although a limited number of studies in the literature assess the formulation of BNC-keratin composites, Keskin *et al.* (2017) evaluated the incorporation of this protein by *in situ* and *ex situ* processes, being subsequently freeze-dried. The non-cytotoxicity requirement on

hypersensitive sites 2 (HS2) keratinocytes was achieved regardless of the incorporation method adopted. The *in situ* process demonstrated an absence of cytotoxic properties, whereas the *ex situ* approach exhibited approximately 60 % cell viability in nanocomposites containing a 1:1 ratio of BNC-keratin. However, both nanocomposites resulted in enhanced dermal fibroblast cell adhesion. This improvement can be attributed to keratin facilitating cellular interactions between keratinocytes and fibroblasts, enhancing it as an ideal candidate for optimizing the wound healing process [133]. The combination of keratin and ionizing radiation yielded promising outcomes for wound treatment, indicating a potentially viable option to synergize these two approaches in the context of wound healing [134]. Additionally, a synergistic effect was observed between crosslinked BNC and gelatine, which contributed to the acceleration of the wound healing process by applying an electric field stimulation. This combined approach demonstrated a therapeutic effect superior to the commercial wound dressing – Tegaderm™ [135].

The high porosity of BNC is one of its main characteristics (~75–82 %) [20]. Nevertheless, not all particles can penetrate the BNC structure. This process is influenced by BNC's pore size and the specific particle dimensions [58]. The average pore size can exhibit variability, influenced by bacterial type and production techniques, ranging from 10 to 350 μm [20]. Due to its molecular weight and viscosity, chitosan exemplifies significant constraints in integrating within the BNC matrix. Several strategies were analysed for the functionalization of BNC with chitosan. The dip crosslinking method using tripolyphosphate is the most prevalent, straightforward, and cost-effective approach. However, this technique often alters the morphological attributes of the composite, resulting in increased smoothness and modifications in the alignment of the biopolymeric chains' structure [136]. Wei *et al.* (2021) incorporated chitosan NPs into the BNC matrix to address this issue. NPs were then generated through a pressurized injection process, introducing elongated polymer chains into the BNC through ionic gelation. Submerging this mixture in an aqueous sodium sulphate solution facilitated gelation by adjusting pH. The method employed for applying chitosan NPs ensured the preservation and potential enhancement of the mechanical properties of the composite. This method was made more accessible by omitting pre-treatment procedures and not relying on commonly employed dispersants and crosslinking agents [137].

Mohamad *et al.* (2018) produced a BNC hydrogel modified with acrylic acid. Moreover, the incorporation of keratinocyte (4×10^4 cells mL^{-1}) and fibroblasts (6×10^4 cells mL^{-1}) yielded a synergistic outcome, effectively expediting the healing process (77.34 ± 6.21 %, 13 days) when compared to the BNC/acrylic acid hydrogel (71.51 ± 2.35 %, 13 days) [138]. In combination with the inherent challenges of burn recovery, the proliferation of pathogens within the affected area increases the overall process duration. The most prevalent pathogens found in burn wounds: *Candida*, *Klebsiella* and *Staphylococcus* genera, contribute to excessive exudate and compromised healing processes. Incorporating thymol, an essential oil, into BNC led to the formulation of a composite for burn treatment. This composite exhibited antimicrobial effectiveness against these strains, outperforming gentamicin (a standard antibiotic). The resulting non-cytotoxic nanocomposite demonstrated remarkable antimicrobial activity and an ability to accelerate the healing process (90.7 %, 20 days). Particularly facilitated granulated tissue production (epithelialization) without fibrosis due to its inherent anti-inflammatory action and deposition of ECM. These attributes fulfil key criteria for applying wound dressings [139]. To overcome the limitations of available drugs to treat such infections with e.g. *Candida* spp., that can potentially result in fatalities (1–33 %) [140], Vilela *et al.* (2019) developed an antifungal nanocomposite through the crosslinking of BNC with poly ([2-(methacryloyloxy) ethyl] trimethylammonium chloride) (10 and 40 wt.%) using *N,N'*-methylenebis(acrylamide). This composite exhibited a weak to strong disinfectant activity against *C. albicans*. Despite increased crosslinker agent concentration, cell viability was reduced at 40 wt.%. This concentration did not reveal a cytotoxic effect

(72.4±3.1 %) on human keratinocytes (HaCaT). Thus, this composite presents promising attributes for potential application in diabetic or burn wound dressings due to its ultraviolet (UV) protection, non-cytotoxicity, transparency, and antifungal activity [141]. Table 5 summarizes examples of BNC functionalized with organic compounds for application in dressings and wound healing.

3.1.3. Combination of organic and inorganic compounds in wound dressings

Combining organic and inorganic compounds holds important potential for producing nanocomposites for wound healing. Third-degree burns can be treated by applying a bioadhesive nanocomposite composed of BNC, PDA, and AgNPs. BNC serves as an exceptional ECM and a scaffold for *in situ* incorporation of AgNPs. Applying PDA coating facilitates the reduction of silver nitrate, leading to the formation of AgNPs. Moreover, the distinctive adhesive properties of PDA and its surface functionalization capabilities contribute to its intriguing and versatile role in wound dressing materials [87]. Chronic wounds are difficult to treat due to the elevated number of microorganisms, and to the significant increase in antibiotic resistance. Ag is a broad-spectrum natural antimicrobial that has attracted attention in wound management. The incorporation of AgNPs onto BNC showed considerable efficacy against the wound-infecting pathogenic microbes like *S. aureus*, *P. aeruginosa*, and *Candida auris*, according to a work presented by Gupta and co-workers (2020). In a development of their previous work [127], the complex curcumin:hydroxypropyl- β -cyclodextrin was in this case used as reducing agent of AgNO₃ to prepare AgNPs. The loading of both AgNPs and curcumin proved to be cytocompatible demonstrating potential to be used in wound dressing applications [142].

Similarly, Zhao *et al.* (2022) have produced films using BNC, AgNPs, PDA, and chitosan to improve the antibacterial action and protect wounds from infections caused by bacteria. A synergistic effect was observed with improved antibacterial action against *S. aureus* and *P. aeruginosa*. The tensile strength of the film increased due to increase of hydrogen bonds by the presence of chitosan. The films that were tested exhibited the absence of cytotoxicity using NIH/3T3 cells. However, the presence of AgNPs showed a slight inhibition of NIH/3T3 cell growth, below 5 % [109].

The significant increase in antibiotic resistance poses a major global health challenge [143]. A multifunctional nanocomposite based on BNC, gelatine (1 wt.%), and selenium NPs (SeNPs) (30 mmol L⁻¹) has also been developed to address the antibiotic resistance issue. SeNPs can recognize and distinguish between bacteria and healthy mammalian cells in addition to being non-toxic. In a work by Mao and co-workers (2021), SeNPs are used along with gelatine to functionalize BNC hydrogel. The incorporation of SeNPs has shown antibacterial effect. The presence of SeNPs has facilitated ROS generation and induced oxidative stress to destroy the bacteria. The coating with gelatine reduced the porosity of the hydrogel which helps in the control release of SeNPs. Cytotoxicity was tested using NIH/3T3 cells, and the samples prepared using concentrations of H₂SeO₃ below 0.06 mol L⁻¹ were found to be non-toxic. These accelerated the wound healing process by promoting granulation tissue formation, collagen deposition, and angiogenesis, by reducing the inflammatory response. The mechanical results suggested that the tensile strength and Young's modulus improved with the addition of gel due to the formation of hydrogen bonds and even more with the addition of an optimized amount of SeNPs. This composite meets a wide range of requirements for wound dressing applications. Biocompatibility, biodegradability, hemocompatibility, excellent mechanical properties, high swelling capacity, flexibility, remarkable wound healing, as well as antioxidant, anti-inflammatory, and antimicrobial activities, are among the achieved features. A synergistic effect between gelatine and SeNPs has been established [144].

Schizophyllan (SPG) is an extracellular polysaccharide obtained from fungus *Schizophyllum commune* with good antimicrobial, antioxidant, anti-wrinkle, and moisturizing effects and can also promote cell

proliferation and regeneration. Hence, aminoalkylsilane groups and SPG were simultaneously used by Hamed *et al.* (2021) to modify the BNC hydrogel. Thereafter, ZnONPs were loaded on functionalized BNC. The obtained samples were tested against *E. coli* and *S. aureus* and showed inhibition effect. Suppression of the respiratory chain enzymes by the released Zn²⁺ ions and additionally generation of a huge amount of ROS can be the factors leading to the death of bacteria. Considering the cell viability of more than 70 % from cytotoxic tests, it can be understood that the samples are non-toxic to fibroblasts and the presence of SPG can lead to cell proliferation and thus promote a smooth wound healing process. The swelling behaviour of the samples with SPG improved in comparison with BNC due to the hydrophilic nature of SPG and the formation of hydrogen bonds with water molecules. However, the addition of ZnONPs reduced the swelling ratio due to the chelation of ZnONPs with the hydrophilic sites of the polymer. Nevertheless, the swelling ratio remained higher than that of the pristine BNC. The hydrogen bonding between the OH groups in the polymer and oxygen bonds in the NPs resulted in improved tensile strength of the hydrogel [110].

Nanozymes are able to regulate ROS levels to provide good antibacterial effect. Hence, a study investigating the effects of enzyme-based wound dressing was conducted by Zhang *et al.* (2022). In this study, a nanocomposite was developed using polypropylene (PP) enveloped BNC and hollow mesoporous nanocatalyst iron (Fe@HCMS) together with glucose oxidase (GluO_x). The antibacterial results indicated that BNC/PP/Fe@HCMS/GluO_x has good antibacterial activity against *S. aureus* and *E. coli*. Noticeable hemocompatibility with rabbit red blood cells with the hemolysis ratio below 1.5 %. The composite was able to reduce the bleeding. The cell viability was 81 %. The formation of new epithelial layers in combination with a good antibacterial effect due to the conversion of glucose to a hydroxyl radical (•OH) at the wound sites has promoted wound healing of BNC/PP/Fe@HCMS/GluO_x [116]. Hydrogels consisting of alginate, casein, BNC and Fe₃O₄NPs synthesized by co-precipitation were prepared by Patwa *et al.* (2020). The Fe₃O₄NPs promoted a rough surface necessary for cell adhesion. The swelling properties of the hydrogel improved with the addition of both BNC and Fe₃O₄NPs at large concentrations. This can be attributed to the increase of irregular porous size that facilitated more water take-up. The antibacterial activity was observed in all the hydrogels and the inhibition zone is higher against *S. aureus* in comparison with *E. coli*. Cell viability over 80 % was found for all samples when tested against murine embryonic fibroblasts [111].

In a different study, Luo and co-workers (2020) modified BNC with maleic anhydride. Thereafter it was used to synthesize spherical-shaped NPs, since maleic anhydride enhances the bonding between the BNC and the ZnNPs. The antibacterial properties of the sample were greater against *S. aureus* in comparison with *E. coli*. The incorporation of ZnO has maintained adequate pore size and porosity of the sample and enhanced the water vapor permeability. The water vapor transmission rate was observed to be 2856.60 g m⁻² and this value is well within the limit of a desired value (2500–3000 g m⁻²) for a wound dressing material. The cytotoxicity results have suggested that at lower concentrations (5 wt.%) of ZnO, it was non-toxic to the mouse fibroblast cells. No irritation on the skin for 5 wt.% ZnONPs but at 20 wt.% ZnONPs exhibited irritation and crust formation. The sample also showed a good wound healing process due to the improved re-epithelialization and wound contraction. The tensile strength and Young's modulus increased with the addition of ZnONPs and the properties indicate that the membranes were flexible, comfortable, and resilient [115]. Any potential cytotoxic effect of ZnONPs seems to have been overcome by functionalizing BNC with botulin diposphate (BDP). An antioxidant, anti-burn, and antitumor compound that, according to Melnikova *et al.* (2021), when in combination with ZnO demonstrated good values of cell availability (L929 mouse fibroblasts) and no significant difference compared to pristine BNC. Furthermore, it presented good wound healing properties due regulation of oxygenation and microcirculation,

Table 5

Examples of BNC functionalized with organic compounds for wound dressings and wound healing applications.

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Wound healing	Commercial α -13'-COOH (n.d.)	n.d.	<i>Ex situ</i> – impregnation	Controlled release of α -13'-COOH (24 h) Preservation of a moist environment Enhancement of compression strength Collagen deposition (10 days) Facilitates dermal thickness (~150 %) High swelling capacity: 166 %, 15 min Tensile strength: 128.6±4.7 kPa Young's modulus: 382.3±6.7 kPa Elongation rates: 4.8±1.1 % Density: 5.9±0.3 mg cm ⁻³ Antimicrobial activity: <i>E. coli</i> (97.6 %) <i>S. aureus</i> (98.3 %) <i>K. xylinus</i> (97.4 %) Non-cytotoxic Hemostatic Degradable: 86 %, 30 days Promotes fibroblast production and reduces inflammatory response (<i>in vivo</i>) Tensile strength: ~0.54–0.9 MPa Young's modulus: ~1.25–2 MPa Strain at break: ~30–45 % High water retention capacity Non-cytotoxic: Cell viability (L929) >80 %	[120]
Wound healing	Commercial Collagen/chitosan (n.d.)	n.d.	<i>Ex situ</i> – immersion	Antimicrobial activity: <i>E. coli</i> (97.6 %) <i>S. aureus</i> (98.3 %) <i>K. xylinus</i> (97.4 %) Non-cytotoxic Hemostatic Degradable: 86 %, 30 days Promotes fibroblast production and reduces inflammatory response (<i>in vivo</i>) Tensile strength: ~0.54–0.9 MPa Young's modulus: ~1.25–2 MPa Strain at break: ~30–45 % High water retention capacity Non-cytotoxic: Cell viability (L929) >80 %	[122]
Wound healing	Commercial Hyaluronic acid (n.d.)	n.d.	<i>Ex situ</i> – impregnation	Strain at break: ~30–45 % High water retention capacity Non-cytotoxic: Cell viability (L929) >80 %	[130]
Wound healing	Keratin (n.d., 83.73 nm)	n.d.	<i>In situ</i> : 3 % w/v keratin under static fermentation <i>Ex situ</i> –impregnation	<i>In situ</i> : non-cytotoxic (L929 fibroblasts and HS2 keratinocytes) <i>Ex situ</i> : non-cytotoxic. Cell viability (L929, except for the 1:1 ratio BNC: keratin; HS2 keratinocytes) Stress-strain: ~200–800 MPa Tensile strength: ~110–785 MPa Young's modulus: ~20–35 GPa) Transparent: >75 % transmittance Moderate wettability: <90° Non-cytotoxic: Cell viability (NIH/3T3) >87 %, 5 days Hemocompatible: ~0.3–1.1 % Good wound healing ability Inhibits scar formation Promotes angiogenesis Biocompatible (TNF- α , IL-6, and IL-1 β) Moderate water absorption capacity: ~190–300 %	[133]
Wound healing	Commercial Gelatine (n.d.)	n.d.	<i>Ex situ</i> – immersion	Non-cytotoxic: Cell viability (NIH/3T3) >87 %, 5 days Hemocompatible: ~0.3–1.1 % Good wound healing ability Inhibits scar formation Promotes angiogenesis Biocompatible (TNF- α , IL-6, and IL-1 β) Moderate water absorption capacity: ~190–300 %	[135]
Wound dressing	Curcumin (n.d.)	n.d.	<i>Ex situ</i> – immersion	Sustained release (initial burst in the first 8 h, maximum of 64 %) Antimicrobial activity (Log reduction): <i>S. aureus</i> (1.42–2.63) <i>E. coli</i> (0.36–0.91) Humidity absorption: 97.63±0.057 % Transparency: 66.13±2.36 % transmittance Water vapour permeability: 2258.53–2460.63 g m ⁻² /24 h Biocompatible Hemocompatible: <0.20 % (<i>in vitro</i>) Non-cytotoxic: Cell viability (A549) 60 %	[126]
Wound dressing	Curcumin (n.d.)	hydroxypropyl- β -cyclodextrin as encapsulating agent	<i>Ex situ</i> – immersion	Controlled release of curcumin: ~77 % at 6 h and 82 % at 48 h Antimicrobial activity: <i>S. aureus</i> (11.08±0.90 mm) Antioxidant activity: IC50=1087.49 ±6.47 μ g mL ⁻¹ Water vapor permeable: 12.34±0.10 g m ⁻² h	[127]
Wound dressing	Chitosan/ ciprofloxacin (n.d.)	n.d.	<i>Ex situ</i> – immersion	Sustained antibiotic release: ~74 %, 6 h Antimicrobial activity: <i>P. aeruginosa</i> (4.3 mm) <i>S. aureus</i> (5.5 mm)	[135]

(continued on next page)

Table 5 (continued)

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Wound dressing	Chitosan NPs (n.d., 339–700 nm)	n.d.	<i>Ex situ</i> – immersion	Non-cytotoxic: Cell viability (GM07492) 84.2 % BNC/chitosan 2 %: Young's modulus: 2.06 MPa Tensile strength: 0.95 MPa Elongation at break: 68 % Antimicrobial activity: <99 % reduction against <i>S. aureus</i> and <i>E. coli</i> Non-cytotoxic Promotes cell adhesion and proliferation Biocompatible Stimulates collagen production Acceptable inflammatory response UV-A and UV-B resistance High water absorption capacity: 225–873 %, 48 h Storage modulus: >1.7 GPa	[137]
Wound dressing	Commercial Poly ([2-(methacryloyloxy) ethyl] trimethylammonium chloride) (10 and 40 wt.%) (n.d.)	n.d.	<i>In situ</i> – mixing and polymerization	Young's modulus: ≥ 2.4 GPa Elongation at break: >2.4 % Non-cytotoxic: Cell viability (HaCaT) 72.4–81.4 % Antifungal activity (Log reduction): <i>C. albicans</i> (3.4–5.5) Moisture content: 95.5 $\pm$ 0.10 % Hemocompatible: 1.51 $\pm$ 0.01 % Facilitates oxygen exchange: 76.8 $\pm$ 0.07 % porosity	[141]
Burn wound dressing	Commercial Collagen/chitosan (n.d.)	n.d.	<i>In situ</i> – mixing of collagen in culture medium <i>Ex situ</i> – immersion in chitosan	Flexible: tensile strength of ~ 0.8 MPa Microbial reduction Non-cytotoxic: Cell viability (mammalian cells) 90 % Reduced healing process time (<i>in vivo</i>) Non-cytotoxic Enhances cellular adhesion Antimicrobial activity: <i>E. coli</i> (15 $\pm$ 0 mm) <i>P. aeruginosa</i> (16.3 $\pm$ 0.4 mm) <i>S. typhimutium</i> (16 $\pm$ 0 mm) <i>S. aureus</i> (15.5 $\pm$ 0.4 mm) Improves wound healing process: 64.25 %, 15 days Reduces scar formation Increases cell proliferation and collagen production Enhances wound healing: 77.34 $\pm$ 6.21 %, 13 days Increases collagen deposition Facilitates reepithelialization process High water absorption capacity: >400 g m ⁻² d ⁻¹ Antimicrobial activity: <i>S. aureus</i> (40.33 $\pm$ 1.15 mm) <i>E. coli</i> (18.33 $\pm$ 1.15 mm) <i>P. aeruginosa</i> (20.67 $\pm$ 0.57 mm) <i>K. pneumoniae</i> (41.33 $\pm$ 1.15 mm)	[121]
Burn wound dressing	Curcumin (n.d.)	n.d.	<i>Ex situ</i> – immersion	Non-cytotoxic: Cell viability (NIH/3T3) 88.81 % Facilitates cell proliferation Enhances wound healing process: 90.7 %, 20 days Reduction in scar formation High swelling: ~ 74 –97 % Controlled and sustained release for 72 h (initial burst of 34 % in 1 h) Antimicrobial activity High swelling capacity: 3334.21 $\pm$ 353.54 %, 24 h Non-cytotoxic	[122,125]
Burn wound dressing	Acrylic acid/ keratinocytes/ fibroblasts (n.d.)	n.d.	<i>Ex situ</i> – mixing		[125,138]
Burn wound dressing (third degree)	Commercial Timol (n.d.)	n.d.	<i>Ex situ</i> – immersion		[129,139]
Chronic wound healing	Dehydrogenative polymer of coniferyl alcohol (n.d.)	n.d.	<i>Ex situ</i> – immersion		[129,131]
Chronic wound dressing	Commercial Hyaluronic acid (n.d.)	n.d.	<i>Ex situ</i> – impregnation		[131,133]

n.d. – not defined.

reduction of oxidative stress, and hypoxia in a burn wound [96].

The enhancement of the mechanical properties with the incorporation of AgNPs was also verified by Song *et al.* (2021), who produced a hydrogel based on PVA/BNC/AgNPs. Both the stress at break and elongation at break improved, but only at an optimized concentration of AgNPs. This was due to AgNPs agglomeration at higher concentrations

that disturb the formation of the hydrogel network, contributing to the decline in properties. These hydrogels were tested for antibacterial activity against *E. coli* and *S. aureus* showing significant antibacterial effects. Good cytocompatibility using L929 cells was verified. The addition of AgNPs increased the re-epithelialization due to the anti-inflammatory activities, improved angiogenesis and formation of a hair follicle [114].

Table 6

Examples of BNC functionalized with organic and inorganic compounds for wound dressings and wound healing applications.

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Burn wound healing (third degree)	AgNPs (n.d.)	PDA	<i>In situ</i> – polymerization	Antimicrobial activity: <i>S. aureus</i> (22.33±0.57 mm) <i>E. coli</i> (12.00±1.00 mm) <i>P. aeruginosa</i> (15.67±0.57 mm) <i>K. pneumoniae</i> (15.00±0.00 mm) Non-cytotoxic: Cell viability (NIH/3T3) >70 % Water vapor permeability: ~400 g m ⁻² d ⁻¹ Promotes cell proliferation, reepithelialization, and collagen deposition (IL-1α, IL-6, IL-10, VEGF-A, VEGF-B, bFGF, TGF-β1, TGF-β3, and SMAD-3) Effective in wound healing: 94.35 % healing rate at 20 days and 100 % after 25 days No scar formation Non-cytotoxic: Cell viability (U251, MSTO and Panc 1) >80 %	[87]
Wound healing	AgNPs (n.d., 42.71±17.97 nm) /Curcumin-Cyclodextrins	n.d.	<i>In situ</i> – immersion	Hemolysis: 6.85±1.12 % Antimicrobial activity: significant for <i>P. aeruginosa</i> , <i>S. aureus</i> , and <i>C. auris</i> Antioxidant activity Tensile strength: ~0.74–0.85 MPa Young's modulus: ~2.77–3.08 MPa Failure strain: ~33.4–40 % High swelling: >2000 % Antioxidant activity: ~25–80 % 24 h Degradable: 100 %, 180 min Controlled release of SeNPs: ~20.4–25.2 %, 3 days	[142]
Wound dressing	Gelatine/SeNPs (n.d., 75 nm)	n.d.	<i>In situ</i> – immersion and reduction	Antimicrobial activity against <i>E. coli</i> , MDR <i>E. coli</i> , <i>S. aureus</i> , and MDR <i>S. aureus</i> Non-cytotoxic at concentrations below 60 mmol L ⁻¹ Hemocompatible: hemolysis rate <2 % Facilitates wound healing (96 %, 14 days) Anti-inflammatory activity (TNF-α and IL-6) Promotes fibroblast and collagen production Promotes angiogenesis Biocompatible (<i>in vivo</i> , TNF-α, IL-6, and IL-1) Release profile: – BC/Ag/gCN/EE after 30 h: 61% of Ag and 67% of EE – PVA/BC/Ag/gCN/EE after 12 h: 62% of Ag and 74% of EE Biodegradability: – PVA/BC/Ag/gCN/EE ~44 % – uncrosslinked PVA/BC/Ag/gCN/EE 79 % – crosslinked PVA/BC/Ag/gCN/EE improved dissolvability significantly Mechanical properties: – BC/Ag/gCN/EE tensile strength 6.99 MPa Young Modulus 121.37 MPa – uncrosslinked PVA/BC/Ag/gCN/EE tensile strength 6.56 MPa Young Modulus 412.88 MPa – PVA/BC/Ag/gCN/EE tensile strength 8.44 MPa Young Modulus 564.84 MPa Antibacterial: strong antibacterial activity against <i>E. coli</i> and <i>S. aureus</i> Viability: – BC/Ag/gCN/EE 89 ± 2.31 % – PVA/BC/Ag/gCN/EE 96 ± 3.28 % Scratch area reduction: – BC/Ag/gCN/EE 18.69% – PVA/BC/Ag/gCN/EE 23.97 %	[144]
Wound dressing	Ag/graphitic carbon nitride(gCN)/eucalyptus extract(EE) Electrospinning (with PVA)	PVA	Immersion (without PVA)	– BC/Ag/gCN/EE tensile strength 6.99 MPa Young Modulus 121.37 MPa – uncrosslinked PVA/BC/Ag/gCN/EE tensile strength 6.56 MPa Young Modulus 412.88 MPa – PVA/BC/Ag/gCN/EE tensile strength 8.44 MPa Young Modulus 564.84 MPa Antibacterial: strong antibacterial activity against <i>E. coli</i> and <i>S. aureus</i> Viability: – BC/Ag/gCN/EE 89 ± 2.31 % – PVA/BC/Ag/gCN/EE 96 ± 3.28 % Scratch area reduction: – BC/Ag/gCN/EE 18.69% – PVA/BC/Ag/gCN/EE 23.97 %	[145]

n.d. – not defined.

Table 6 summarizes examples of BNC functionalized with organic and inorganic compounds for application in wound dressings and wound healing.

3.2. Implants

Implants assist as support, or as replacement of damaged body parts, and can also function as delivery agents or for monitoring of physiological activity. Once introduced into the body, medical implants can remain permanently or serve their purpose for a temporary term. Examples of implants are artificial joints, surgical meshes, breast implants, cochlear implants, intraocular lenses, pacemakers, or other cardiac implants, and intrauterine contraceptive devices. Medical body implants can be made of metal, plastic, ceramic, skin, bone, or other tissues [146]. Biocompatibility and lack of toxicity are two essential features for 3D scaffolds, in association with the ability to promote cell adhesion, proliferation, and differentiation. Another essential property is its porosity, which should enable cell infiltration, vascularization, nutrient exchange, and possess adequate mechanical properties. It has been found that the implant morphology should resemble that of the ECM [147].

BNC is a biomaterial applied in several biomedical applications (see Table 7), attributed to its outstanding mechanical, physical, and chemical properties. Furthermore, different sizes can be achieved by regulating its biosynthesis [148]. Nevertheless, the lack of antimicrobial, anti-inflammatory, and antioxidant properties limits its application. BNC modification adaptability is advantageous to mitigate this limitation, facilitated by its abundance of OH groups and a neutral electrostatic charge [65]. Therefore, the risk of infection or rejection after implantation can be easily mitigated or even overcome if the right antimicrobial agents are incorporated within the implant matrix, avoiding the need for a second surgery. An extensive array of positively/negatively charged organic/inorganic compounds can be introduced using various methods (*in situ* and *ex situ*), resulting in composites that fulfil the requisites of the biomedical field [149,150]. BNC-based functional composites have been applied in the development of wound dressings, cancer therapy, drug delivery, biosensors, and implants [40, 151]. Some examples of implants are displayed in Fig. 7. Their application has been prioritized over synthetic products due to their hemocompatibility, biocompatibility, ease of sterilization, and lack of cytotoxicity [13]. Another notable property is the degradability of BNC [152]. In specific contexts, a sought-after attribute involves the degradation and absorption of materials within the body upon implantation, particularly within tissue engineering. This property enhances cellular production, growth, and adhesion, facilitating the gradual replacement of the implant with newly regenerated tissue [153]. Nonetheless, it can be easily modified to increase degradability (e.g., oxidation processes

[122], cellulase treatment, and radiation [154]).

3.2.1. Soft tissue implants

BNC can be applied in the replacement of synthetic soft implants, namely, cardiovascular systems (e.g., artificial blood vessels and heart valve replacement), nervous system (e.g., nerve scaffold and dura mater replacement), ophthalmic applications (e.g., artificial cornea and contact lenses), urinary conduits, and skeletal system (e.g., cartilage regeneration, meniscus implant, artificial ligament/tendon, tympanic membranes, and vocal cords) [150,157,158].

3.2.1.1. Inorganic compounds in soft tissue implants. According to several studies, the treatment for neurological disorders like Parkinson's and Alzheimer's disease, or cervical spinal cord injury, can potentially rely on cell-based therapy based on neural stem cells (NSC) to protect and restore damaged neurons. However, there is an urgent need to regulate the growth and differentiation of these cells and to ensure the successful integration of implanted tissues essentially, transitioning from the laboratory to clinical application. Neurogenesis, as with any cell culture, is very particular regarding the surrounding physiological conditions. The surface in which they differentiate and grow is of particular importance, therefore significant efforts have been made to develop a scaffold able to provide the microenvironment to develop NSC. An example is a work developed by Guo *et al.* (2021), who developed an electrically conductive scaffold by making use of the conductivity of 3D-graphene (3D-G). Despite the biocompatibility of this inorganic foam, the large pores of 100–300 μm signify that cells tendentially attach, proliferate and differentiate along the walls and not on the inside. To simulate a more realistic environment, BNC was added to the 3D-G by culturing *K. xylinum* on its surface. The resulting superior surface area and reduced pore size provided a broad array of oxygen groups that increased biocompatibility, proliferation, and differentiation. In fact, primary cortical neurons cultured on this composite formed an intense neuronal network with greater network activity than the one formed on the graphene foam alone. RNA-Seq analysis suggest that the composite offers a more promising 3D conductive substrate for neural tissue engineering. Furthermore, the incorporation of BNC onto the 3D-G surface lowers the Young's modulus of the biopolymer to levels similar to soft-tissue membranes, since the inorganic matrix presents approximately 1,000–2,000 MPa [159].

Endothelial cells are important constituents of the blood vessels and the lymphatic system, operating as a selective barrier for the transport of molecules between blood/lymph and tissues. The blood vessel endothelium layer is extremely important in vascular integrity therefore, an intact endothelium ensures the normal working of blood vessels. It regulates blood flow, vascular tone, angiogenesis, monocyte/leukocyte

Table 7

Examples of commercialized BNC-based products specifically developed for medical implants.

Commercial name (producing bacteria)	Company/distributor	Active compound	Properties	Wound type	Ref
SYnthesized Cellulose (BASYC®) (n.d.)	University Jena and Polymer Jena, Germany	None	High mechanical strength	Blood vessel repair	[24]
Xylos® Porous Surgical Mesh (n.d.)	Xylos Corporation®	None	High water retention Low roughness of inner tube surface	Abdominal and thoracic defects	[155]
Xylos® Vessel Guard (n.d.)	Xylos Corporation®	None	Reinforcement	Cover for vessels during anterior vertebral surgery	[155]
Gore-Tex® membranes	Gore-Tex®	n.d.	Protection	Dental implant	[156]
Securian	n.d.	n.d.	Periodontal tissue improvement	Tendon repair	[156]
Gelfoam™	Pharmacia & Upjohn Company LLC	n.d.	Tissue Reinforcement matrix Tympanic membrane perforations	Tissue repair	[156]

n.d. – not defined.

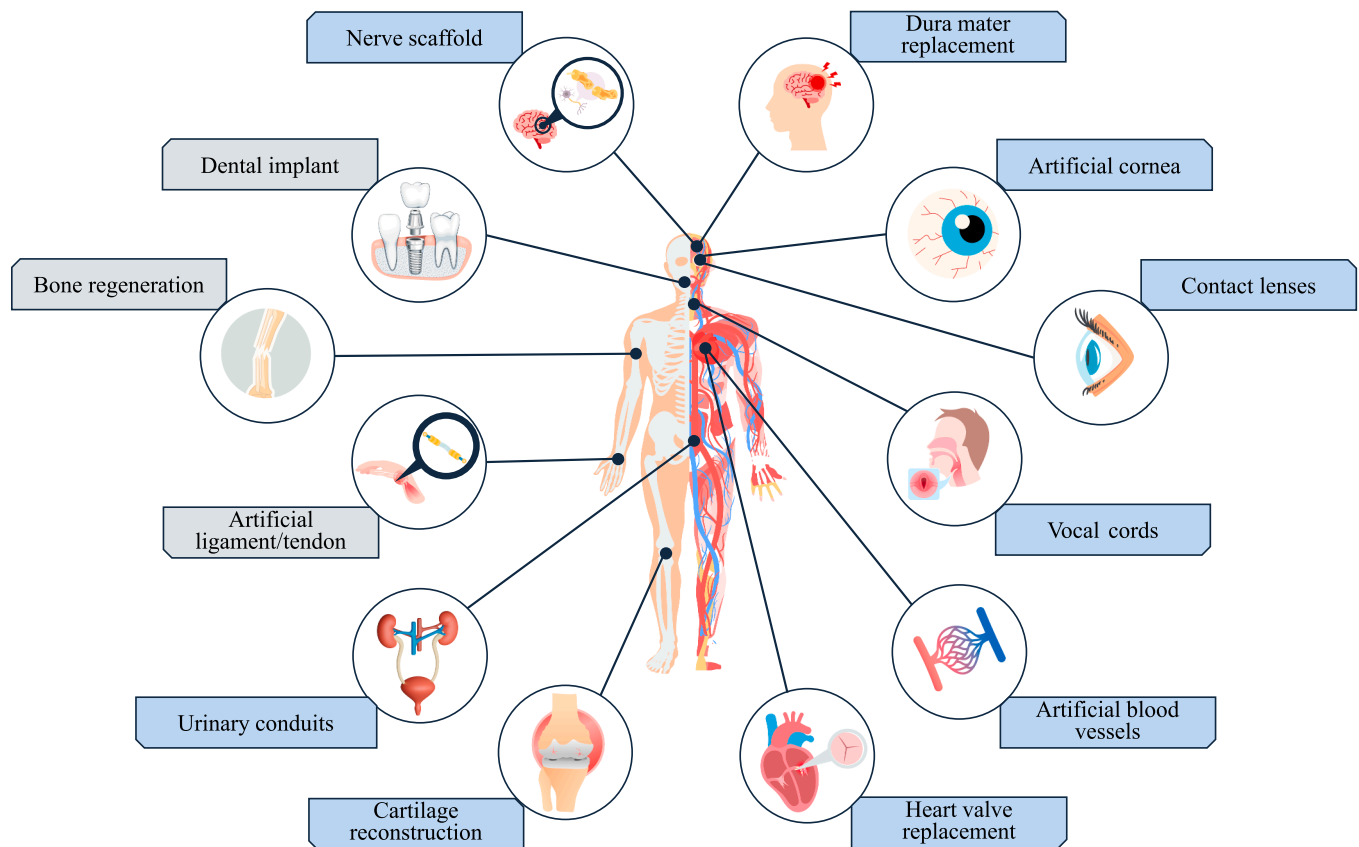


Fig. 7. Examples of soft (blue boxes) and hard (grey boxes) BNC-based implants. Created with BioRender.com.

adhesion, platelet aggregation, and facilitates normal functions of tissues or organs. Malfunction may result in different pathologies such as aneurysms, stroke and heart disease, diabetes, among others. In tissue engineered vascular grafts (TEVG), autologous saphenous vein and synthetic vascular grafts are used to circumvent these issues [160,161]. Modified synthetic nondegradable polymers, biodegradable synthetic or natural polymers have been used for constructing vascular grafts, among them polytetrafluoroethylene (e-PTEF) or polyethylene terephthalate (PET). Nonetheless, problems in seeding endothelial cells onto prosthetic grafts via physical forces (gravitational or hydrostatic seeding) could not fulfil the preclinical requirements. In addition, surface hydrophobicity or hydrophilicity are another major issue. Hydrophobic surfaces tendentially adsorb non-specific proteins, leading to platelet adhesion and thrombus formation, whilst highly hydrophilic materials can hinder adhesion thus full coverage of endothelial cells, disabling long-term applications of vascular grafts [161]. Biologically active cells are able to achieve tissue remodelling and regrowth of injured blood vessels, but it is a lengthy process since cellular denudation occurs due to the high dynamic shear stress at the damaged site, impairing tissue growth. A magnetic field gradient can potentially target magnetic NPs-loaded cells locally to the BNC surface and be a retention platform to increase cell homing at damaged vasculature. The incorporation of magnetite (Fe_3O_4) within BNC was possible through *in situ* precipitation of Fe^{3+} and Fe^{2+} . To avoid oxidation, dextran was used to protect the iron oxide NPs and improve cytocompatibility. The characterization of this magnetic hydrogel showed a superparamagnetic behaviour since no hysteresis loop was formed. The Young's modulus of the composite for all the experimental samples (200–380 KPa) was higher than that reported for healthy carotid walls during the cardiac cycle (130 ± 15 KPa in systole and 80 ± 10 KPa in diastole) and with no significant differences to the untreated BNC membrane (320.51 ± 149.58 KPa). This shows the mechanical suitability of the hydrogel as vascular graft. The release of

weakly bonded metal NPs was verified for 100 mM and 50 mM Fe^{3+} containing BNC, whilst the 25 mM sample remained almost invariant throughout the 8 days of experiment. Cell viability via live/dead and MTT assays presented 90.56 % live cells and 9.43 % dead cells of a total of 339 cells for the 25 mM Fe^{3+} BNC, similar to pristine BNC (98 % live cells of a total of 227 cells). The other concentrations of Fe^{3+} , 50 mM and 100 mM showed poor results of viability. Cultured human aortic smooth muscle cells (HASMC) showed an extended morphology and were well adhered to all the substrates, seemingly proliferating faster in pristine BNC and 25 mM magnetic BNC. Cell targeting was achieved at low iron oxide NPs concentration (25 mM), but the cell coverage in the magnetic BNC was lower than the systems based on magnetized steel stents. The reason can be due to the coating of the NPs with dextran, which is known to resist cell adhesion [160]. Zhang *et al.* (2020) demonstrated a non-invasive and convenient method to remotely regulate the adhesion of endothelial cells based on an oscillating magnetic field. BNC membranes were modified using PEG-coated iron oxide NPs with grafted Arg-Gly-Asp peptide used as cell anchors. Seeded murine endothelial cells were subjected to a magnetic field at different frequencies (0 Hz, 0.1 Hz, 2 Hz), and adhesion and growth of endothelial cells showed to be significantly oscillation frequency dependent, with cell growth promotion under lower frequency, leading to successful endothelialization on the modified BNC membranes. The magnetic BNC composite demonstrated high mechanical strength with Young's modulus of 1.75 ± 0.3 MPa, only slightly lower than pristine BNC (2.21 ± 0.4 MPa). Similar tensile strength and elongation at break to BNC were also verified. Cell viability, illustrated in Fig. 8, was analysed by the CCK-8 assay using murine endothelial C166 cells. The results showed no reduction of the composite (1 mg mL^{-1} for 24 h) viability by more than 20 %. Increased incubation for up to 7 days (at concentrations higher than $125 \mu\text{g mL}^{-1}$) caused some toxic effects, however, not enough to label it as non-biocompatible. Release of iron oxide NPs was less than 0.1 % after

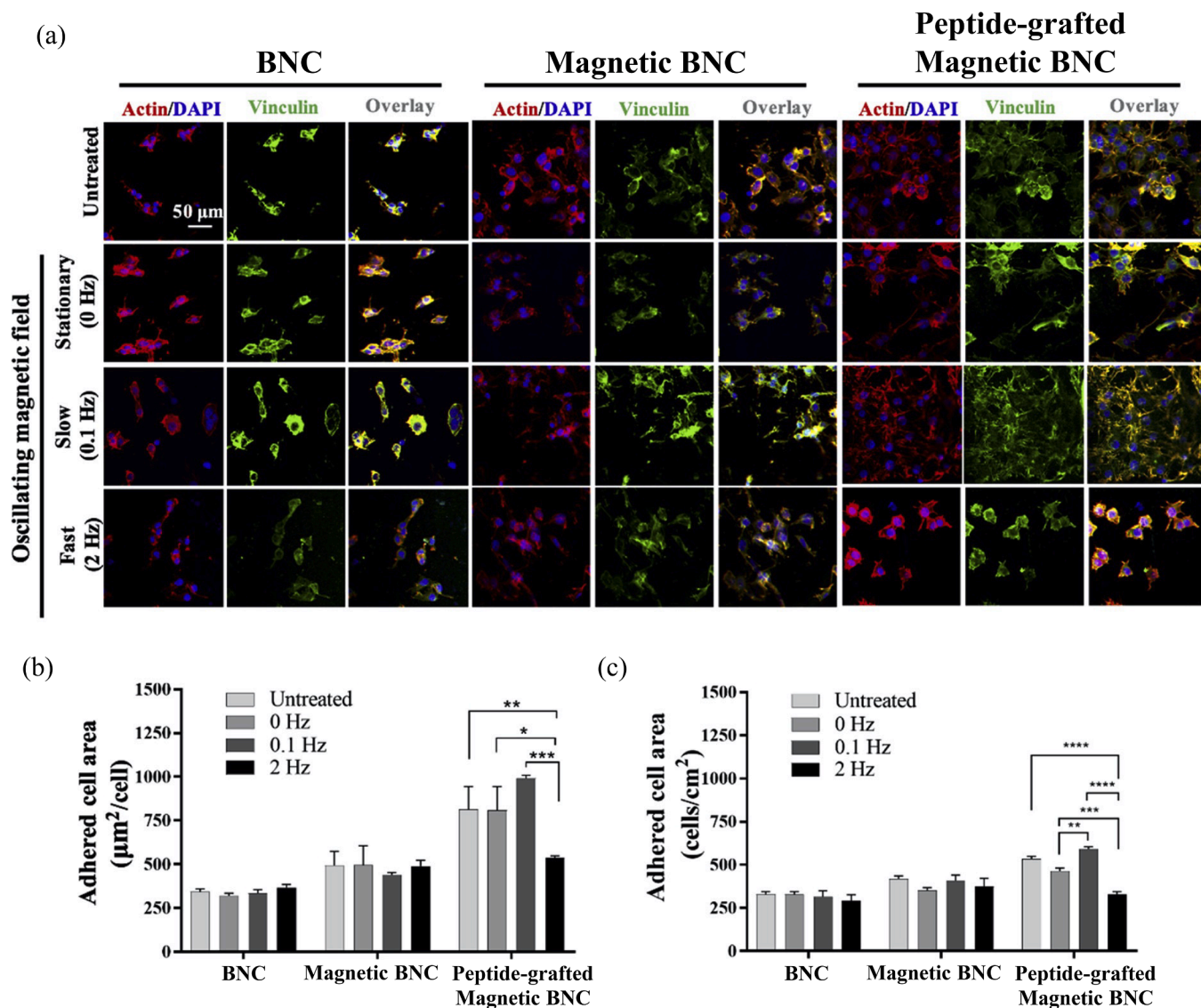


Fig. 8. (a) Immunofluorescence staining micrographs of vinculin (green), F-actin (red), and nuclei (blue) after incubation of C166 cells on BNC/magnetic BNC/peptide-grafted magnetic BNC for 24 h under “stationary”, “slow”, and “fast” conditions by applying various oscillation frequencies (0, 0.1, and 2 Hz) of a magnetic field. The untreated cells on each type of membrane are control groups. Scale bars represent 50 μm . (b,c) Corresponding quantifications of adhered cell density and area. Data are shown as mean $\pm$ standard errors ($n = 30$). The statistical significance of the results was determined using one-way ANOVA, *, **, ***, and **** represent the significance at $p < 0.05$, $p < 0.01$, $p < 0.005$, and $p < 0.001$. Reproduced from Ref. [161] with permission, Copyright 2020, American Chemical Society.

incubation for 14 days. C166 cells show higher coverage on peptide-modified membranes than pristine BNC, irrespective of the applied magnetic field. The former showed higher cell seeding density and more extended cellular morphology. The reason could be due to the peptide's ability to interact with the integrin receptors on the cell membrane and enhance cell adhesion and affinity [161]. Different applications of magnetic NPs include actuators. These materials produce mechanical motion or force in response to an external stimulus. These can be applied into various medical devices or implantable systems for specific purposes. Superparamagnetic iron oxide NPs (SPIONs) are a good example since their applicability in biomedicine includes: drug delivery, making use of their targeting through an external magnetic field [162], contrast agents for magnetic resonance imaging to enhance the visibility of specific tissues [163], and hyperthermia therapy due to the ability of SPIONs to generate heat when exposed to an alternating magnetic field [164]. The incorporation of these NPs within BNC to be potentially used as actuators was reported by Roig-Sanchez and co-workers (2021), who tested a static and agitated culture of BNC in the presence of SPIONs. Magnetic actuation is conferred by SPIONs. The

combination of platinum NPs (PtNPs) or AuNPs with SPIONs allows the controlled motion of the hydrogels by a magnetic field and an H_2O_2 oxidation reaction. These Janus Pt/SPIONs structures increased by five times the directionality and movement [165]. Table 8 summarizes examples of BNC functionalized with inorganic compounds for soft tissue implant applications.

3.2.1.2. Organic compounds in soft tissue implants. Loss of function of articulation and muscles due to cartilage damage constitutes a problem. Such injuries are frequent in the aging population and athletes. The human body's capacity for self-regeneration faces limitations due to the restricted proliferation of chondrocytes, the absence of blood vessels and nerves, and the relatively low metabolic rate [158,166]. The predominant treatment method involves autografting chondrocytes, specialized cells that stimulate the generation of new cartilage tissue. Nonetheless, the demand for dual surgeries and an extended recovery period has prompted exploring a biomaterial-based approach to cartilage transplantation [158]. Implants require high porosity to support chondrocyte migration and ECM production. However, BNC presents different sizes

Table 8
Examples of BNC functionalized with inorganic compounds for soft tissue implants applications.

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Neural tissue engineering	3D-G (n.d.)	n.d.	<i>In situ</i> – culturing of <i>K. xylinum</i> on the 3D-G surface	Non-toxic: Cell viability > 99 % Kept physiological levels of stemness Young's modulus similar to soft-tissue membranes Superparamagnetic behaviour Young's modulus: 200 – 380 KPa	[159]
Vascular grafts	Fe ₃ O ₄ (n.d., 49.81 ± 20.77 nm)	n.d.	<i>In situ</i> – mixing and reduction	Non-toxic: Cell viability (Human aortic smooth muscle cells) 98 % Cell targeting achieved at low iron oxide NPs concentration (25 mM) Young's modulus: 1.75 ± 0.3 MPa Tensile strength: 2.22 ± 0.4 MPa Elongation at break: 3.59 ± 0.3 %	[160]
Vascular grafts	Iron oxide core (~10 nm) PEG-coated iron oxide (~26 nm)	n.d.	<i>Ex situ</i> – mixing	Non-toxic: Cell viability (murine endothelial C166) > 80 % Release of iron oxide NPs: < 0.1 %, 14 days	[161]
Cargo-delivery systems, theragnostic, tissue engineering	SPIONs (spherical, 7.1 ± 1.5 nm) AuNPs (spherical, 19 – 47 nm) PtNPs (n.d., 3 nm)	n.d.	<i>In situ</i> – mixing NPs in culture medium Magnetic response in water toward a magnetic field at 10 mm was directly proportional to the SPIONs concentration: [SPIONs] = 2.50 mg mL ⁻¹ – speed of 13.5 ± 1.7 mm s ⁻¹	Superparamagnetic behaviour	[165]

n.d. – not defined.

and interconnectivity that may inhibit chondrocyte proliferation [167]. Numerous approaches have been developed to adjust pore size, similar to the ECM dimensions (ranging from 100 to 500 µm) [168]. These methodologies include laser drilling, photoreticulation, freeze-drying, and incorporating NPs/microparticles during synthesis [123,169,170]. Photoreticulation of a BNC/methacrylate gelatine hydrogel decreased the pore size from 200 to 10 µm, attributed to hydrogen bonding. Notwithstanding the reduced pore size, chondrocyte growth and proliferation were successful [171]. To increase the number of pores in BNC, freeze-drying becomes a practical and cost-effective method to achieve this attribute. Li *et al.* (2017) developed a BNC-chitosan composite (1 wt.%). The combination of the freeze-drying process and the viscosity enhancement, resulting from the presence of chitosan, contributed to a substantial 50 % increase in pore size [172]. Healthy cartilage has a high-water content of about 80 %, so the replacement implant must maintain this property [154]. BNC exhibits a notable inert water retention capacity, rendering it suitable for cartilage replacement applications. Incorporating BNC into cartilage implants must facilitate the progression of new tissue development, a phenomenon referred to as chondrogenesis [173]. Furthermore, BNC hydrogels were modified with alginate and/or hyaluronic acid to achieve this property. The composite BNC/chitosan/alginate/gelatine (80:20 % w/w) was evaluated, promoting cell adhesion, proliferation, and cell differentiation of chondrocytes [174]. The production of printable a 3D hydrogel of BNC and methacryloyl gelatine, demonstrated in Fig. 9 a-g, enhanced mechanical properties. After 24 weeks of *in vivo* implantation, the ear cartilage implant exhibited Young's modulus (1.33±0.13 MPa) similar to the modulus of elasticity of human ear cartilage (1.41±0.67 MPa). Cell viability displayed promising results, with values exceeding 97 % following a 7-day testing [175]. The *in situ* polymerization modification with an additional organic compound, PGA, resulted in a biomaterial that exhibited improved mechanical properties. This modification enhanced bond interactions, amplifying elastic recovery after compression, fracture strength, and tenacity. The composite shows promising properties in cartilage application, including excellent cell viability [176]. Moreover, the application of natural proteins was also

analysed for mechanical improvement. The integration of SF into BNC, followed by the freeze-drying procedure, revealed an enhancement in compressive strength and deformation. This improvement can be attributed to the crosslinking interaction between BNC and SF [177]. All the exemplified examples indicate that utilizing these organic materials did not compromise the non-cytotoxicity of the final composites. The high viability can be attributed to the similarity of BNC to the ECM of hyaline cartilage [168].

Severe cardiovascular conditions require a vascular transplant to replace obstructed pathways. These replacements may involve applying alternative body tissues (from oneself or a donor) or using artificial vascular grafts (e.g., blood vessels or cardiac valves) [178]. Surgically, synthetic materials are employed to replace large-diameter blood vessels (ranging from 6 to 10 mm in diameter), commonly utilizing PTFE and e-PTFE. Nevertheless, these materials are inadequate for replacing small-diameter vessels (with diameters less than 6 mm) due to their susceptibility to clotting and restenosis. This inherent limitation constitutes the main obstacle preventing commercially accessible prostheses designed for this type of vessel [179]. BNC hemocompatibility makes it a promising choice for adequately replacing both types of implants [180]. BASYC® (BACterial SYNthesized Cellulose) is a commercially available BNC-based product that replaces artificial blood vessels. It features a tubular configuration and mechanical properties resembling natural blood vessels [24]. Applying this biomaterial as a cardiovascular tube necessitates the preservation of blood vessel characteristics, including internal roughness and pressure resistance [13]. Furthermore, issues related to limited elasticity and anti-thrombogenic properties must be addressed to establish BNC as a viable product. For this purpose, a crucial modification is required [178]. Fig. 9h illustrates a PVA-modified BNC for application in artificial blood vessels. The D-BNC tube possesses a bigger external diameter and more homogeneous character along the diametric direction and the S-BNC tube possesses denser nano-cellulose close to the inner wall. The hydrogen bonds are notably reduced within the BNC matrix increasing the elasticity/stretch at break. The mechanical properties are displayed in Fig. 9i-m. Higher Young's modulus, tensile strength, and elongation at break are verified

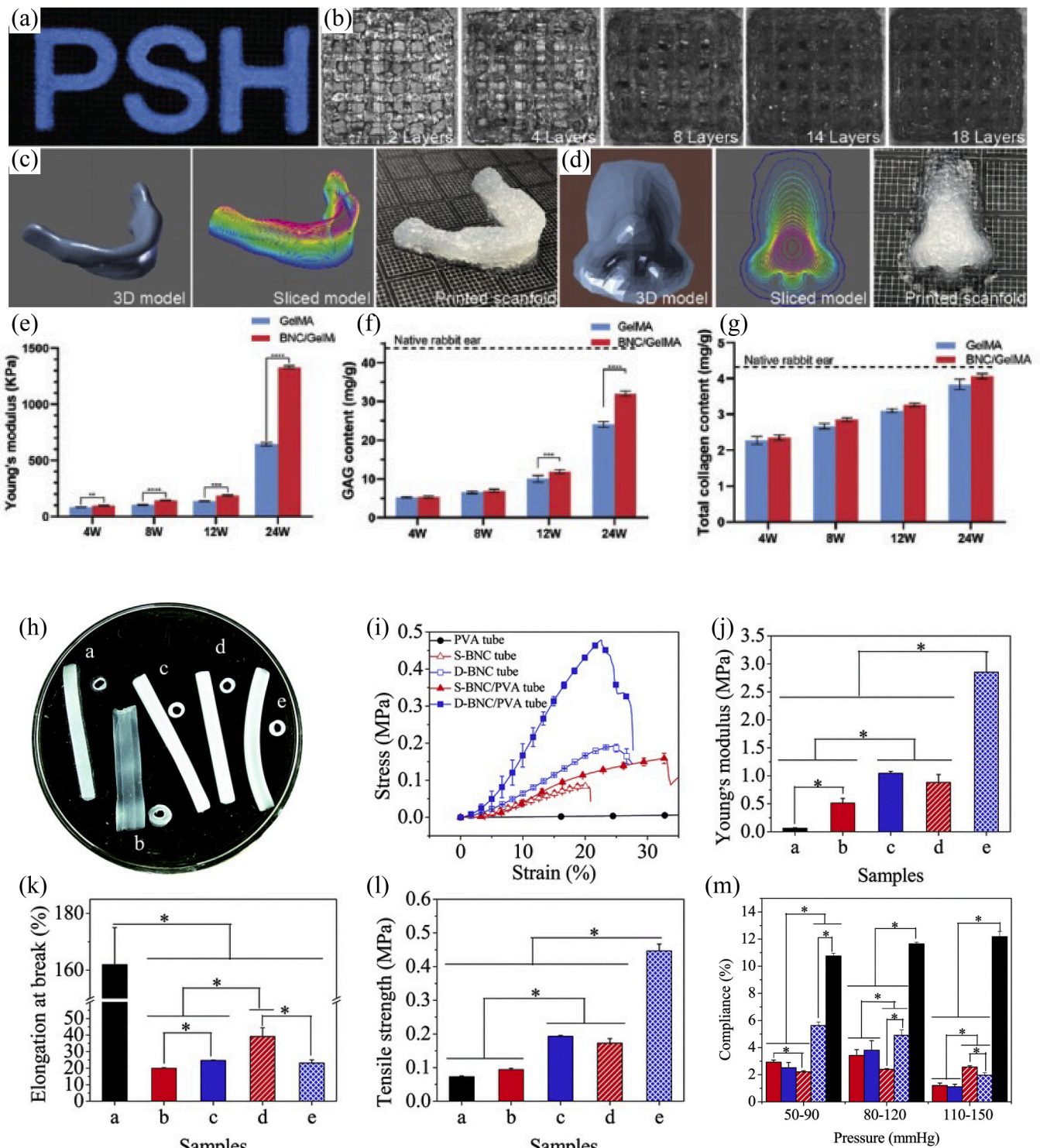


Fig. 9. Printability test of bacterial nanocellulose (BNC)/gelatin methacryloyl (GelMA) hydrogel of (a) PSH characters printed with BNC/GelMA hydrogel, (b) cuboid structure at different layers printed with BNC/GelMA hydrogel, (c) human mandibular model printed with BNC/GelMA hydrogel, (d) human nose model printed with BNC/GelMA hydrogel (e) Young's modulus of regenerated cartilage (n = 3), (f) glycosaminoglycan (GAG) content of regenerated cartilage (n = 3), (g) Total collagen content of regenerated cartilage (n = 3). **P < 0.01, ***P < 0.001, ****P < 0.0001. (h) Macro-morphology of the (h.a) S-BNC tube, (h.b) D-BNC tube, (h.c) S-BNC/PVA composite tube, (h.d) D-BNC/PVA composite tube and (h.e) PVA tube, (i-l) axial stretch mechanical properties of the PVA tube (a), S-BNC tube (b), D-BNC tube (c), S-BNC/PVA composite tube (d) and D-BNC/PVA composite tube (e). Significant differences between groups are indicated (*p < 0.05), (m) circumferential dynamic compliance of the S-BNC tube (red), D-BNC tube (blue), S-BNC/PVA tube (dashed red), D-BNC/PVA tube (dashed blue) and PVA tube (black). Significant differences between groups are indicated (*p < 0.05). Adapted from Ref. [175,181], under the Licence CC BY 4.0, 2023, ACCSCIENCE Publishing and with permission, Copyright 2015, Royal Society of Chemistry, respectively.

for the D-BNC tube as a result of the thicker fibres and a denser nano-fibre network. Permeability attainment was also successful, as no water leakage was detected, and there was an enhancement in burst pressure without any cytotoxic effects [181]. Moreover, ensuring the migration and proliferation of endothelial cells on the implant surface holds extreme importance in achieving biocompatibility. The BNC/chitosan/heparin composites significantly facilitated the adhesion and growth of human saphenous vein endothelial cells (SVEC) and endothelial progenitor cells (EPC). Conversely, the BNC/heparin composite exclusively exhibited these effects in the second cell type, EPC. Both variants hold promise for application in small-diameter vascular grafts, effectively minimizing the potential for thrombogenicity and augmenting biocompatibility [182]. In addition, a chemical crosslinking process was employed to incorporate heparin into BNC, functionalized with SFNPs. The proliferation of endothelial cells, rapid endothelialization, minimal presence of inflammatory cells, and absence of degradability for 4 weeks were observed [183]. Incorporating 1 % w/v fish gelatine resulted in rapid endothelialization, with hemolysis rates remaining below 1 % (demonstrating hemocompatibility) and an enhanced level of biocompatibility [184].

Replacing blood vessels or vascular grafts smaller than 6 mm in diameter presents challenges. However, several multifunctional BNC composites exhibit promising properties. BNC/chitosan composites demonstrated no allergic reactions and effectively suppressed inflammatory responses or excessive growth of regenerated muscle tissue. This fulfils the criteria for biocompatibility and non-cytotoxicity [185]. *In vivo*, blood vessel regeneration was swiftly accomplished through *in situ* modification of BNC with potato starch. Potato starch particles at higher concentrations (2% w/v) expanded during the fermentation process, resulting in an increase in pore size ($\sim 28 \pm 6 \mu\text{m}$) [186].

Corneal dysfunction culminates in vision loss, prompting the adoption of an artificial cornea as the optimal treatment approach. The demand for synthetic alternatives arises from donor scarcity. This transplantation technique demonstrates heightened efficacy owing to its non-vascular nature [187]. The artificial cornea should satisfy the following pre-requisites: biocompatibility, mechanical stability, comfort, rigid structure, transparency, and the ability to enhance cell adhesion and proliferation [188]. In this context, hyaluronic acid can be applied in corneal implants. Anisotropic properties and high transparency (40 % higher than BNC) were achieved due to the incorporation of this agent. The chemically crosslinked BNC/hyaluronic acid composite demonstrated considerable cytocompatibility towards corneal stromal cells and suture strength comparable to the human eye [189]. The integration of this composite into BNC, without requiring a cross-linking agent, displayed several encouraging attributes. Mechanical properties and elasticity, high porosity, and excellent transmittance are some of the characteristics of the composite [190]. Beyond hyaluronic acid, incorporating PVA improves the optical properties through the expansion of interstitial spaces of BNC, promoting increased light transmission. Moreover, this inclusion supports the diffusion of oxygen and essential nutrients crucial for cellular growth while demonstrating minimal immunogenicity [191]. Therefore, the excellent ability to promote light transmission, high water content combined with UV protection establishes PVA as a viable option to replace non-biocompatible polymethyl methacrylate in corneal implants and contact lenses [13].

PP-based implants are applied surgically for the treatment of hernias. Nonetheless, a reduction in physicochemical properties, accelerated degradation in the human body, and its non-inert nature that fosters infections and inflammation decrease the efficacy of employing these synthetic polymers [192]. To mitigate associated biological risks, an inventive biomaterial, BNC, was combined with chitosan and applied to a PP mesh. After 3 months of implantation, the composite favoured tissue remodelling and demonstrated a distinct absence of cytotoxicity. Unlike PP-based hernia meshes, this includes minimal irritation and no allergic reactions [193]. In addition to treating hernias, BNC can serve as

a substitute for a perforated tympanic membrane because of its flexibility, biocompatibility, and transparency. However, no necessity for modifying BNC for this specific application was identified. PVA emerges as a plausible choice for modification to enhance light transmission [147].

Different alternatives have been explored to promote the regeneration of urological tissues, such as the case of urinary conduits - a critical component in managing bladder cancer treatment [147]. Keratocytes were cultured on a BNC/SF bi-layer scaffold. SF encouraged cell proliferation and migration, while BNC supported preserving epithelial cells, which play a crucial role in urethral reconstruction [194].

Repairing nerve injuries is a complex and crucial process characterized by the challenges of limited regenerative potential. An up-and-coming solution lies in applying neural implants, particularly highlighted by the BNC favourable compatibility with Schwann cells [195]. In addition to biological properties, nerve regeneration implants need effective electrical conductivity to facilitate nerve impulses. Thus, Wu *et al.* (2021) developed a 3D bioprinting scaffolds of BNC (0.3 %)/alginate (5 %)/gelatine methacrylate (5 %) loaded with RSC96 cells that showed a good electrical conductivity ($6 \times 10^{-7} \text{ S m}^{-1}$). The effect of introducing RSC96 cells on neural regeneration was assessed, confirming the expression of numerous mRNA genes associated with axon growth, proliferation regulation, migration control, Schwann cell maturation, and myelination. Following 21 days of *in vivo* implantation, no indications of inflammation or toxicity were observed [196]. From a different perspective, rat sciatic nerve regeneration was assessed using an oxidized BNC composite functionalized with chitosan NPs (*in situ*) containing nerve growth factors. The microporous composite exhibited a notable reduction of microbial proliferation, while the oxidation of BNC improved its biodegradability. Schwann cell adhesion and proliferation demonstrated similar results to commercial grafts, and nerve growth factors were responsible for evident nerve repair (10 mm in 4 weeks) [197]. The electrical signal between cells plays an important part in tissue regeneration. As a conductive material, nerve conduits are able to transmit electrical signals between cells in electroactive tissue, thus allowing nerve repair through the promotion of Schwann cells adhesion and proliferation to construct the connected belt when the Wallerian degeneration occurs. In peripheral nerve injury, this is especially important since it allows the bridge between the two ends of damaged nerves. Poly(3,4-ethylenedioxythiophene) (PEDOT), a conductive polymer that has gained a significant interest in tissue engineering due to its biocompatibility and electrical properties, has been used in combination with sulfonated nanofibres (SNFs) and BNC to create a transparent conductive membrane. This composite showed great biocompatibility, high conductivity, and appropriate mechanical strength to be used for peripheral nerve repair, according to *in vivo* tests on Sprague Dawley rats [198].

Overall, composites based on BNC modified with organic compounds present promising physicochemical and biological properties for replacing synthetic soft implants. Table 9 summarizes examples of BNC functionalized with organic compounds for application in soft implants.

3.2.2. Hard tissue implants

Hard tissue implants are medical devices used to support, replace, or repair either bone, cartilage, or dental structures. Typically, biocompatible ceramics and metals are used for this purpose, increasingly allied to biopolymers due to their excellent biocompatibility, adequate mechanical properties, porous structure, and enhanced osteogenic activity [200,201]. Furthermore, BNC is similar to the ECM, having a broad spectrum of applications, including bone in periodontal surgery, cartilage in the temporomandibular joint, skin and oral mucosa, dentin and dental pulp, and has the ability to be engineered into bone tissue. The ability of nanocellulose scaffolds to transfer cells and growth factors to damaged sites is under study. In addition, surface modification to promote the bioactivity of cellulose-based grafts is another area of interest, in which bioactive compounds are linked to BNC to promote bone cell

Table 9

Examples of BNC functionalized with organic compounds for soft tissue implants applications.

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Cartilage	Methacrylated gelatine (n.d.)	n.d.	<i>Ex situ</i> – mixing and polymerization	Porosity: ~47–121 μm Water absorption: ~755–916 % Thermally stable Elastic moduli: ~4–12 $\times 10^3$ % Compressive modulus: ~113–812 kPa Facilitates proliferation and growth of chondrocytes Increased porosity: ~74–87 % Water absorption: ~1000–2000 % BNC/Chitosan 2% Dry compressive modulus: 4.74–9.13 MPa Wet compressive modulus: 9.64 $\pm$ 0.46 MPa Porosity: ~326.6–406.2 μm Compressive strength: ~30–57 MPa Biodegradable Promote cell migration, proliferation, and differentiation of mesenchymal stem cells Non-cytotoxic: Cell viability (hMSCs) >120 % Promotes the production of collagen and other biochemical compounds Thermally stable Porosity: 172.8 $\pm$ 54.19 μm Non-cytotoxic: Cell viability (97.3 $\pm$ 1.45 %) Promotes cell migration Young's modulus: 1.33 $\pm$ 1.33 MPa Promotes collagen production: 24.1 $\pm$ 0.74 mg g ⁻¹ , 24 weeks (1:1 w/w) Toughness: 0.42 $\pm$ 0.03 MJ m ⁻³ Elastic recovery Compression strength: 5.72 $\pm$ 0.30 MPa Fatigue resistance: 1.5 kJ m ⁻³ , 20 cycles High swelling: ~568–662 % Thermally stable Non-cytotoxic Thermally stable Compressive strength (1.49 MPa at a 55.21 % strain) Non-cytotoxic Non-permeable Mechanical properties: Young's modulus: ~1–3 MPa Tensile strength: ~0.2–0.45 Elongation at break: ~20–40 % Reduced suture retention: ~0.37–0.46 N Rupture pressure: ~0.047–0.065 MPa Adequate compliance Non-cytotoxic Fibronectin: Increased cell adhesion and growth (VEC and EPC) Heparin/chitosan: Increased cell adhesion and growth (EPC) Increased contact angle Albumin: Increased cell adhesion and growth (VEC) Hemocompatibility: hemolysis <0.5 %; reduced platelet adhesion; decreased coagulation rate Non-cytotoxic Low inflammatory response Promotes collagen production Facilitates endothelialization Permeable: ~4.2–5.2 mL cm ⁻² min ⁻¹ Burst pressure: ~425–593 MPa Tensile strength: ~0.55–0.85 MPa Young's modulus: ~1.01–1.19 MPa Elongation at break: 36.6–43.9 % Hemocompatible: hemolysis <1 % Non-cytotoxic Non-cytotoxic Non-allergenic: 80 % Non-irritating No inflammatory reactions Cellular adhesion Hemocompatible: hemolysis (0.25 $\pm$ 0.06 %)	[171]
Cartilage	Commercial Chitosan (n.d.)	n.d.	<i>Ex situ</i> – immersion		[172]
Cartilage	Commercial Chitosan/alginate/gelatine (n.d.)	n.d.	<i>In situ</i> – addition of 0.75 % w/v chitosan into the culture medium <i>Ex situ</i> – mixing		[174]
Cartilage (3D-bioprint)	Commercial Gelatine methacryloyl (n.d.)	n.d.	<i>Ex situ</i> – mixing and photopolymerization		[175]
Cartilage	Commercial PGA (n.d.)	n.d.	<i>In situ</i> – mixing		[176]
Cartilage	SiF (n.d.)	n.d.	<i>Ex situ</i> – immersion		[177]
Blood vessels	Commercial PVA (n.d.)	n.d.	<i>Ex situ</i> – injection		[181]
Vascular graft (small-diameter)	Commercial Fibronectin, heparin/chitosan, and albumin (n.d.)	n.d.	<i>Ex situ</i> – immersion		[182]
Vascular graft (small diameter)	SFNPS (n.d., 0.57 μm)	Heparin	<i>Ex situ</i> – injection		[183]
Blood vessels (small diameter)	Commercial Fish gelatine (n.d.)	n.d.	<i>Ex situ</i> – immersion		[184]
Blood vessels (vascular prostheses)	Chitosan (n.d.)	n.d.			[185]

(continued on next page)

Table 9 (continued)

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Vascular graft (small diameter)	Commercial Potato starch (n.d.)	n.d.	<i>In situ</i> – mixing in culture medium	hemolytic activity (0.14 ± 0.03 %) Promotes vascular tissue growth Porosity: 28 ± 6 μm High water content: 97.9 ± 0.07 % Young's modulus: 7 ± 2 MPa Hemocompatible: hemolysis 1 ± 1 %; platelet adhesion Promoted blood vessel regeneration Biocompatible Formation of new capillaries Promotes collagen deposition Transparency: 91.4–96 % Suture strength: 0.081–0.11 N High water content: 99.71–99.86 % Non-cytotoxic Viscoelastic properties Thermally stable	[186]
Cornea	Commercial Hyaluronic acid (n.d.)	n.d.	<i>Ex situ</i> – immersion	Water-holding capacity: >99 % Rehydration ratio: ~25–35 % High swelling: ~1000–3000 % Transparency: 90 % Transparency: 96 % Porosity: 61.68 ± 4.65 % Increased swelling: 160.0 ± 8.4 g g ⁻¹	[189]
Cornea	Commercial Hyaluronic acid (n.d.)	n.d.	<i>Ex situ</i> – dispersion	Promotes cell adhesion and proliferation Biocompatibility Absence of neovascularization Absence of infections Reduced number of inflammatory cells Good mechanical properties	[190]
Cornea	Commercial PVA (n.d.)	n.d.	<i>In situ</i> – mixing in culture medium	Promotes cell adhesion and proliferation Biocompatibility Absence of neovascularization Absence of infections Reduced number of inflammatory cells Good mechanical properties	[191]
Surgical meshes	Commercial Chitosan (n.d.)	n.d.	<i>In situ</i> – mixing in culture medium	Promotes neovascularization Absence of inflammatory response: > 3 months Inhibits allergic reactions and infections Stress at break: 274.6 ± 15.4 kPa Strain to break: 15.7 ± 1.4 % Young's modulus: 6.85 ± 0.26 MPa Suture retention strength: 1.45 ± 0.21 N Porosity: 85 % Promotes cell adhesion and proliferation Biocompatible Compression modulus: 10.9 kPa Porosity: 53.34 % Electrical conductivity: $\sim 6 \times 10^{-7}$ S m ⁻¹	[193]
Urinary conduits tissue (urethra)	SF/keratinocytes (n.d.)	n.d.	<i>In situ</i> – mixing in culture medium	Biocompatible Compression modulus: 10.9 kPa Porosity: 53.34 % Electrical conductivity: $\sim 6 \times 10^{-7}$ S m ⁻¹	[194]
Nerves	Commercial Alginate/gelatine methacrylate/RSC96 cells (n.d.)	n.d.	<i>Ex situ</i> – mixing	Biocompatible Promotes growth and cellular adhesion of RSC96 Proliferation of Schwann cells Prevents cell death (7 days) Young's modulus: 538.5 kPa Stress: > 200 kPa Strain to break: ~50 % Controlled release: maximum of 39 %, 10 days Inhibits bacterial growth: E. coli (99.9 %) S. aureus (99.1 %) Non-cytotoxic Enhances adhesion and growth of Schwann cells Regeneration and recovery of sciatic nerve (10 mm, 4 weeks) Facilitates myelination Conductivity: 6.2×10^{-3} S/cm Polarization: 264 pC at a strain of 50 % Good cell growth and proliferation Inhibition of the expression of pro-inflammatory cytokines TNF- α and iNOS	[196]
Nerves	Chitosan NPs (n.d., >2 μm)	n.d.	<i>Ex situ</i> – injection	Promotes the expression and secretion of anti-inflammatory cytokines IL-10 and CD206 Nerve function index, myelinated nerve fiber density, average diameter of myelinated axons, and myelin thickness of the conductive composite bioelectret close to the Autograft group	[197]
Nerves	Liposome core-shell curcumin loaded microspheres		<i>Ex situ</i> – Immersion		[199]

n.d. – not defined.

adhesion. 3D printing is also able to create tailor-made BNC bone grafts [4].

3.2.2.1. Inorganic compounds in hard tissue implants. Bone has a unique ability to self-heal in the human body, however, the healing process for significant bone damage may be lengthy and challenging. Bone grafting and bone cement have been used to repair bone injury. Unfortunately, these two procedures came with several problems, including short lifetime, lack of available donors, and potential for bacterial and viral transmission. For these reasons, research efforts have been focused on identifying alternative methods for treating bone injuries [202]. On the outside of musculoskeletal joints, there is articular cartilage, which can tolerate complicated mechanical stimuli including pressure and shear stress. Articular cartilage does not contain blood arteries, thus, it must primarily obtain the nutrients it needs through synovial fluid infiltration, which makes it challenging for the cartilage to recover after damage. When not treated, cartilage lesions may develop into post-traumatic osteoarthritis, which can cause movement problems, deformity, and joint discomfort that significantly lower patients' quality of life. Currently, autologous or allogenic cartilage transplantation, microfracture technology, and autologous chondrocyte implantation are the most frequently employed procedures to treat damaged tissues. The majority of the regenerated tissues created by these methods are fibrous cartilaginous, making it difficult to achieve the composition and mechanical properties of natural articular cartilage. As a result, long-term efficacy is not guaranteed, even though these methods can help with cartilage regeneration to a certain extent [203].

The tissue engineering of hard implants, such as those for cartilage, teeth, and bones, incorporating BNC has been extensively studied and is a rapidly advancing field. Here, BNC has shown promising potential due to its unique properties [204,205]. Specifically, its hydrophilicity, non-corrosiveness, toughness, lower inflammatory response, high mechanical strength, high degree of polymerization, and renewability properties have contributed to the development of bone tissue engineering instead of ceramics and alloys [201,206]. Those characteristics permit cell penetration, vascularisation, and adequate nutrient and oxygen diffusion to cells and the unformed ECM. Despite the impressive effectiveness of cellulose and its composites in hard implants, several challenges hinder their application. Specifically, the slow degradation of BNC and inadequate pore size for cell penetration and tissue adhesion are notable issues [207]. For the development of an ideal bone support, the synthetic matrix must be biocompatible and biodegradable, have an appropriate porosity (similar to the replaced tissue), possess antimicrobial activity, and mechanical properties comparable to natural bone (e.g., compressive strength, fatigue resistance, and high Young's modulus) [208].

Nanotechnology has emerged as a promising tool for more effective tissue regeneration namely using nanofibres and NPs. The mechanical robustness of nano-patterned materials and their improved regeneration responses due to their greater surface areas and more effective interaction with cells have been demonstrated. The surface modification of implants with inorganic NPs (e.g., gold, silver, magnetic iron oxide, and magnesium) has been performed to reduce bacterial adhesion and inhibit biofilm formation. It also provides stealth properties to the bio-implant against immunogenic reactions causing enhanced tissue integration, osteoconductivity, and long-term low toxicity facilitating appropriate cellular responses. NPs may act as focal points of tissue adhesion and bone growth through interaction with calcium that regulates the biological responses [209,210]. In this section, works that include the BNC and metal NPs for hard implants applications will be highlighted.

Bone is a calcified tissue composed of 60 % inorganic component (hydroxyapatite), 10 % water and 30 % organic component (proteins) [211]. The replacement of the organic phase of natural bone has been attempted with BNC. In one example, the authors used BNC,

hydroxyapatite, and AgNPs to prepare a composite following two strategies. In the first strategy, the hydroxyapatite was synthesized *in situ* in the BNC matrix with different concentrations of AgNPs using the co-precipitation method. In the second one, hydroxyapatite was obtained separately by the co-precipitation method and later mixed with BNC containing the AgNPs with an ultrasound probe. The results demonstrated a homogenous porous structure, high water absorption capacity (more than 1,000 % w/w) and low degradation rates (< 5 %) in simulated body fluid at 37 °C. The antimicrobial activity against *S. aureus*, *E. coli*, and *C. albicans* was promoted by AgNPs in the polymer matrix. The composite obtained by the *in situ* method showed higher antimicrobial activity (growth inhibition zones ranging 5–11 mm) when compared to the samples obtained by the *ex situ* method (diameter of inhibition zones ranging 5–9 mm). The higher activity in these samples was attributed to the larger porosity, suggesting that the bioactive compounds were absorbed more efficiently and, consequently, present a high release rate [208]. In this project, no biocompatibility studies were found. Another stable and antibacterial biomaterial based on AgNPs was prepared by Yang *et al.* (2023) for temporary cranioplasty with guided bone regeneration containing BNC, AgNPs, and hydroxyapatite. This material was designed to be used between a craniotomy and a cranioplasty, and should have antibacterial properties, adequate mechanical strength, structural stability, low swelling ability, and be biocompatible. Thus, the authors prepared a double-sided functionalized structure with two layers. The first one was dense and covered with AgNPs, and the second one was porous and anchored with hydroxyapatite via mineralization. Once the mineralization period controls the hydroxyapatite deposition rate, which impacts the microstructures and mechanical strength of the composites, the authors studied the optimal conditions. The composites demonstrated remarkable properties, cytocompatibility, and blood compatibility. After 3 days, the leaching liquid of the AgNPs containing hydrogels exhibited continuous proliferative abilities for MC3T3-E1 preosteoblasts cells. *In vivo* tests were performed in a rat cranial bone injury with 5 mm, demonstrating that in the rats without the hydrogel mesh treatment, the defected region was filled with cells and fibrous tissues and no osteoclasts were detected, which hindered bone regeneration. Nevertheless, in the rats treated with BNC composite, a significant reduction of cell infiltration and fibrous tissue adhesion was observed, which revealed optimal guided bone regeneration effects, presented a low swelling ratio, provided long-term mechanical supports for brain tissues, and prevented potential infections, thus being an ideal mesh for temporary cranioplasty [212].

Different NPs can be employed using a similar strategy for scaffolds for bone tissue engineering. Torgbo *et al.* (2019) prepared a nanocomposite with BNC, hydroxyapatite, and Fe₃O₄NPs. Magnetic NPs are able to generate functional bone tissues through osteomimetic architecture, increasing the rate of osteoblast cell differentiation, proliferation, and growth without biocompatibility issues. Moreover, they can be magnetically guided to the interest location. The authors found composites with well-dispersed NPs and a lower crystallinity index (decreased from 82.5 to 62 %). The magnetic field responsive behaviour of the magnetic NPs in BNC samples was studied by vibrating sample magnetometry and showed the influence of BNC and hydroxyapatite in the magnetic field of the Fe₃O₄NPs. A decrease in saturation magnetization of the nanocomposite was observed after the incorporation of hydroxyapatite in the BNC/Fe₃O₄NPs composites from 15.84 to 3.94 emu g⁻¹ at ±10 kOe. Also, the swelling ability decreased after NPs immobilization. The cytotoxicity tests demonstrated that the scaffold is non-cytotoxic for osteoblast MC3T3-E1 and mouse fibroblast L929 cell lines [213].

In other research works, the authors proposed the application of AuNPs with different capping agents as osteogenic agents for facilitating *in vitro* osteogenic differentiation and *in vivo* bone regeneration [201, 214,215]. Different *in situ* methods namely precipitation, oxidation-reduction, and sol-gel can be used to prepare these composites. However, these methods can be laborious, achieve

non-homogenous distribution and reduce the inherent properties of BNC. The incorporation of AuNPs in BNC for rabbit model of femoral defect (Fig. 10a) via the fermentation process was shown to be a suitable strategy since it increases porosity and presents a larger surface area and biocompatibility. Particular attention should be given to the AuNPs' stability during the fermentation process, where ionic species can influence the stability. For this reason, the authors used thiolated PEG as an additional stabilizing agent. Reconstructed 3D and cross-sectional photographs of femurs displayed in Fig. 10b, show a large bone defect in both the control and the neat BNC hydrogel groups. Oppositely, newly formed bone is visible in the femora for the s treated with Au/BNC-III hydrogel group. Fig. 10c shows the bone mineral density (BMD), bone volume fraction (BV/TV), and bone trabecular parameters, including trabecular number (TB.N), thickness (TB.Th), and separation (TB.Sp), essential in the evaluation of the formed osseous tissue differences in defect sites. The values of BMD treated with neat BNC and Au/BNC-III hydrogels indicated that the implantation of neat BNC in defect sites could provide the matrix for osteogenic cells to adhere and further mineralize. The BV/TV, TB.N, and TB.Th values were also impressively elevated after treatment with the Au/BNC-III hydrogel. The results indicated that Au/BNC-III is the best performing hydrogel for bone formation of defect sites. Moreover, mechanism studies suggested that AuNPs increased autophagy response, confirming Au/BNC hydrogels as a proper pathway for osteogenic differentiation of human mesenchymal stem cells. It showed excellent *in vivo* bone repair performance [201].

Magnesium oxide NPs (MgONPs) was also applied in BNC to obtain scaffolds for bone tissue engineering. Ghanbari *et al.* (2023) developed a nanocomposite containing green synthesized MgONPs and BNC. First, the MgONPs were obtained using a *Camelia sinensis* extract and then the BNC was soaked with the obtained dispersion. The functionalized BNC showed increased mechanical strength and porosity. Moreover, osteoblast proliferation tests using human MG-63 cell line showed enhanced results when MgONPs were introduced, as well as higher calcium deposition and alkaline phosphatase (ALP) activity after 21 days [216].

The potential of combining BNC with inorganic NPs to obtain biocomposite scaffolds for bone tissue engineering has been described in these research works. However, the literature contains only a few studies on this topic, and many strategies remain unexplored. The functionalization of BNC or inorganic NPs may change the available functional groups, charge and solubility being able to crosslink with other organic and inorganic substances to improve even more the osteoblasts' adhesion and proliferation [207,217]. A summary is presented in Table 10.

3.2.2.2. Organic compounds in hard tissue implants. Scaffolds consisting of porous microspheres are able to provide the 3D space cells need to maintain morphology, differentiation, and gene expression that are closest to natural tissues, than for example the traditional two-dimensional (2D) cell culture. In addition, their low density, large specific surface area, and porosity, aids in the sustained-release of growth factors [8]. Although the ideal pore size for bone tissue scaffolds is not precisely defined, for effective osteoconduction, it should generally range from 100 to 400 μm [218]. As an alternative to bone tissue implant, a 3D scaffold of BNC with collagen and bone morphogenic protein 2 (BMP-2) presented a rough surface and porous structure with adsorption characteristics typical of mesoporous materials, as suggested by the type iv model adsorption isotherm of N_2 molecules. As illustrated in Fig. 11b, cell viability was unaffected by the treatment of mice MC3T3-E1 cells with BNC, collagen, and collagen/BNC porous microspheres. Besides, these microspheres presented good biocompatibility and were able to promote cell adhesion (90.7 %), proliferation, and osteogenic differentiation of mice MC3T3-EL cells, as shown in Fig. 11c. SEM images displayed in Fig. 11c demonstrate that cell extension over the material and flat-shaped adjacent cells form a continuous cell layer over an extended period [8]. Another option to bone tissue implant is

based on 3D printing technology. By combining the main constituents of bone, hydroxyapatite and type I collagen, with polycaprolactone (PCL), gelatine, and BNC, a scaffold with good cell viability and cell adhesion was obtained [218].

Dental implants are trending and are nowadays a routine procedure. However, there are still constraints that need to be addressed. An example is when the implant needs to be placed in the posterior maxillary region, that due to the common low height of the alveolar process, makes the process more challenging. To circumvent this issue, sinus floor elevation is applied to provide a sufficiently large, safe and secluded space for implants. Cellulose-based membranes have been widely used for regeneration surgery. In fact, Koike *et al.* (2019) grafted BNC into the frontal sinus with BMP-2, a promising osteoinductive substance that facilitates ectopic bone formation, thus allowing alveolar bone augmentation. According to Fig. 11a, the percentage of newly formed bone in the BNC+BMP-2 group was greater than in the other groups at both four and eight weeks. Besides the necessary mechanical and structural properties, BNC is an effective carrier as it is able to control the release of the BMP-2. Histological and immunohistochemical evaluations confirmed the excellent biocompatibility of BNC in a rabbit frontal sinus model. Proliferating cell nuclear antigen and osteocalcin levels were significantly high, $p < 0.05$ [219]. Table 11 provides an overview of BNC functionalized with organic compounds for use in hard tissue implants.

3.3. Cancer therapy

Cancer is one of the leading causes of death among all diseases worldwide. In 2020, the number of new cancer cases reached 19.3 million and the number of cancer-related deaths was almost 10 million [220]. Currently, several treatment methods are used in cancer therapy, including radiation, chemotherapy, immunotherapy, and targeted therapy (see Fig. 12). However, they have some limitations, such as the systemic immunosuppression resulting from the radiation therapy and the growth of drug resistance and large toxic side effects, in the case of chemotherapy [221]. Moreover, when drug molecules are injected, they have to travel a long distance to reach the tumour cells, being very low the dosage that penetrates into the tumour. In this way, one solution is to load the anticancer drugs in biodegradable carriers [222].

The 3D porous structure of BNC facilitates high loading and drug transport capacity, which enhances its application in localized cancer treatment. In the work carried out by Cacicedo *et al.* (2018) to eradicate the side effects of intratumorally administered doxorubicin (Dox), a hybrid biomaterial combining BNC hydrogel and nanostructured lipid carriers (NLCs) as a local drug delivery was developed for localized chemotherapy in cancer. They studied the *in vitro* release profiles of the Dox (neutral (N) or cationic (H) forms) using MDA-MB-231 breast cancer cell line and the *in vivo* performance of a fixed combination of NLCs-H and NLCs-N loaded into BNC and administered intratumorally into an orthotopic breast cancer mouse model. Results showed that NLCs-N displayed over double the encapsulation efficiency of NLCs-H (97 % vs. 48 %) and enabled a sustained drug release. To modulate Dox release kinetics, the mixture 80/20 NLCs-H/NLCs-N ratio was established as the more efficient formulation, with a Dox release of 50 % after 24 h and sustained release after that. Furthermore, the drug release system scaffold assayed *in vivo* revealed a strong efficacy in tumour growth delay and metastasis incidence and, more importantly, no side effects associated with free local drug toxicities, such as inflammation, edema, and necrosis were observed [223]. Aquaroni *et al.* (2020) reported the *in vitro* antibacterial activities and antiproliferative potential of a BNC-based drug delivery system incorporating the metal complex silver-4-aminobenzoic acid. The complex showed a broad spectrum of inhibitory action, namely, on ATCC bacterial strains (Gram negative *E. coli* and *P. aeruginosa*, and Gram positive *S. aureus*, *Bacillus cereus*, and *Mycobacterium tuberculosis*), five uropathogenic *E. coli*, opportunistic pathogen *P. aeruginosa* PAO1, and diarrheagenic pathogens

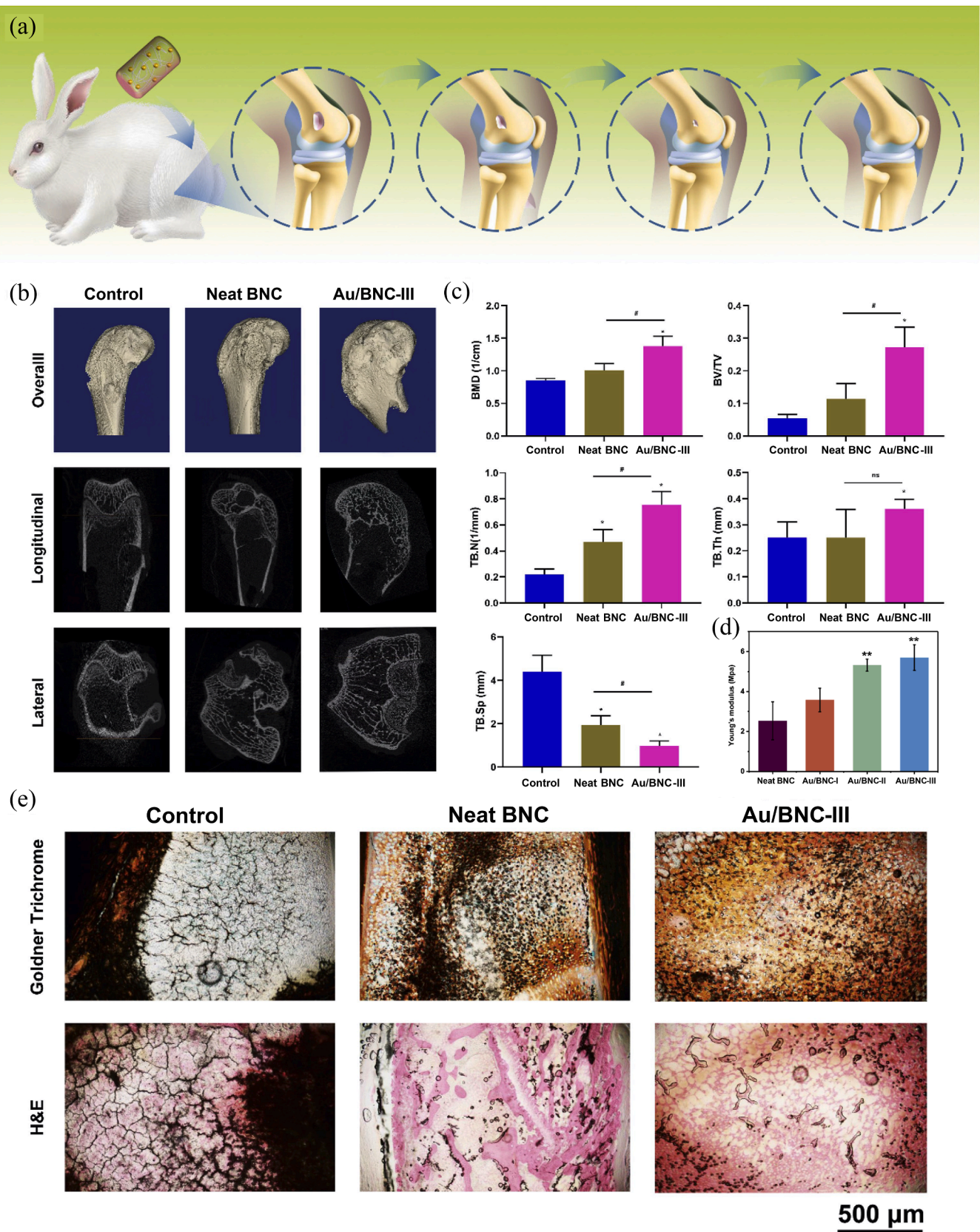


Fig. 10. In vivo osteogenic evaluation of Au/BNC hydrogels. (a) Schematic diagram of experimental procedure. (b) Reconstructed 3D micro-CT and sectional images of femoral condyle after treated with neat BNC and Au/BNC-III hydrogels respectively. (c) Quantitative evaluation of regenerated area by the analyzing parameter of micro-CT: BMD, BV/TV, TB-N, TB.Sp and TB.Th. Asterisk indicates statistically significant differences between control and experience group (*p < 0.05; **p < 0.01; ***p < 0.001). (d) Statistical Young's modulus of stress-strain curves of Au/BNC hydrogels. (e) Goldner Trichrome and H&E staining images of bone defect implanted with neat BNC and Au/BNC-III hydrogels respectively. Adapted from Ref. [201], under the Licence CC BY 4.0, 2023, Elsevier.

Table 10

Examples of BNC functionalized with inorganic compounds for hard tissue implant applications.

Application	Compounds		Incorporation method	Properties	Ref.
	NPs or complex (size; shape)	Drugs or other components			
Bone tissue	AgNPs (spherical, 3–60 nm)	n.d.	<i>In situ</i> – BNC added to calcium nitrate solution followed by reduction <i>Ex situ</i> – mixing	Porosity (~94.5–99 %) Water absorption (~1250–2750 %) Antimicrobial activity against <i>S. aureus</i> , <i>E. coli</i> and <i>C. albicans</i> Reduced crystallinity index: 62 % Decreased swelling ability: 19±4 %	[208]
Bone tissue	Fe ₃ O ₄ (n.d.)	n.d.	<i>In situ</i> co-precipitation on BNC under ultrasonic irradiation	Increased porosity: 81.1 % Increased Young's modulus: 1.85±0.17 GPa Thermally stable Non-cytotoxic Mechanical properties: Young's modulus (5.5 MPa) Higher degradability (11.8–21 %)	[213]
Bone tissue	AuNPs (spherical, 17 nm)	PEG	<i>In situ</i> – mixing in culture medium	Adhesive strength (<0.5 N) The highest Au release ability had the best osteogenic activity Quite high viability and non-cytotoxicity Improved osteoinductive ability Porosity: 89.2±4.85 % Swelling ratio: 88 % Release: 70–80 % over 14 days	[201]
Bone tissue	MgONPs (spherical, 72.18 nm)	n.d.	<i>Ex situ</i> – Soaking	Improved mechanical strength and Young's modulus Increased cell viability Improved cell proliferation Enhanced mineralisation levels of MG-63 cells on BNC scaffolds at 21 days of incubation Increased Young's modulus Lower elongation at break	[216]
Temporary cranioplasty	AgNPs (quasi-spherical, 10–50 nm)	n.d.	<i>In situ</i> – mixing and reduction of the silver salt in basic pH	One side could provide a biomimetic bone structure, and the other side was used to prevent bacterial infections Good antimicrobial properties Non-cytotoxicity	[212]

n.d. – not defined.

(enterohemorrhagic *E. coli* and enteroaggregative *E. coli*). The *in vitro* antiproliferative activity evaluation against eight human tumour cell lines (glioblastoma (U251), breast (MCF-7), multidrug-resistant ovary (NCI-ADR/RES), kidney (786-0), lung non-small cells (NCI-H460), prostate (PC-3), colon (HT-29), and leukemia (K562)) and three human non-tumour cell lines (immortalized keratinocytes (HaCaT), murine macrophages (J774A.1), and lung fibroblasts (MRC-5)) showed that the Ag-4-aminobenzoic acid complex was able to inhibit all the tested cells and with different selectivity. The best results were obtained against leukemia cells (GI50 = 0.96 µmol L⁻¹). Also, the BNC-based scaffold revealed effective capacity to maintain the metal complex release for 120 h, thus showing its potential for application in the treatment of skin, soft tissue, or urinary tract infections, and cancer [224]. More recently, Candido *et al.* (2022) incorporated a silver complex with the antioxidant and anti-inflammatory drug nimesulide (NMS) in a sustained-release device based on BNC membranes for the treatment of skin squamous cell carcinoma (SSCC), one of the most common types of skin cancer. The evaluation of the antiproliferative activity of AgNMS complex on several tumour cell lines showed that it was able to selectively inhibit the proliferation of breast adenocarcinoma (MCF-7), prostate (PC-3), ovarian adenocarcinoma (OVCAR-03), melanoma (UACC-62), and tongue squamous carcinoma (SCC15) cells, without affecting the proliferation of healthy human keratinocytes (HaCat cells). The best results were against melanoma cells (UACC-62) with a total growth inhibition (TGI) of 2.8 µM. Drug release studies showed that the transdermal scaffold was able to keep the release of AgNMS complex for up to 216 h. Further, *in vivo* studies were carried out to evaluate the local and/or systemic toxicity and antitumoral efficacy of the BNC scaffold containing AgNMS topically applied in male Balb/c mice with induced SSCC. No toxicity was shown, and a reduction of almost 75 % in the tumour size was achieved [225]. Saleh *et al.* (2022) developed a novel composite

scaffold with antibacterial, anticancer, and larvicidal activities by casting a mixture of BNC, chitosan, and PVA, loaded with copper oxide (CuO) NPs. The composite material presented antibacterial activity towards Gram positive *S. aureus* and *Streptococcus mutans* and Gram negative *Salmonella typhimurium*, *E. coli*, and *Pseudomonas fluorescens*, though, no activity against *Klebsiella pneumoniae* or *C. albicans* was detected. Additionally, the material showed larvicidal activity with potent lethal effects against all stages of *Aedes aegypti* larval and pupal instars. Regarding the anticancer activity, the *in vitro* cytotoxicity evaluation against colon (Caco-2), hepatocellular (HepG-2), and breast (MDA) cancer cells, showed that the composite has a significant degree of selectivity, with low IC₅₀ for the cancer cells (0.48, 0.27, and 0.33 mg mL⁻¹, respectively) and IC₅₀ of 1.08 mg mL⁻¹ for human skin fibroblast. The morphology of all the studied cancer cells showed noticeable changes with evident nuclear condensation, cell shrinkage, and blebbing. These results suggest that the scaffolds induced the disruption of the cellular membranes and the formation of vacuoles, altering the metabolism and leading to apoptosis, which, consequently, results in cancer cell death [226]. In a study by Chaabane *et al.* (2020), Fe₃O₄NPs were used to develop magnetic BNC scaffolds for antimicrobial and chemotherapeutic activities in cancer treatment. The preparation process involved a multi-step procedure: (i) oxidation of vacuum-dried BNC membranes using sodium periodate to yield dialdehyde BNC (DABNC); (ii) grafting of ethylenediamine (EDA); (iii) grafting of benzyl (Bzl) and iron (II) chloride to obtain [Fe(DABNC/EDA/Bzl)Cl₂]; and (iv) *in situ* co-precipitation of [Fe(DABNC/EDA/Bzl)Cl₂] with iron(III) chloride and ammonium hydroxide by macrocyclic Schiff base reactions to obtain the complex [Fe₃O₄NP/*in situ*/(DABNC/EDA/Bzl)]. *In vitro* evaluation of the magnetic scaffold evidenced cytotoxicity against CT26 colon cancer cells with a low IC₅₀ value (6 µg mL⁻¹). No significant cytotoxic effect was observed towards the normal peripheral blood mononucleocyte

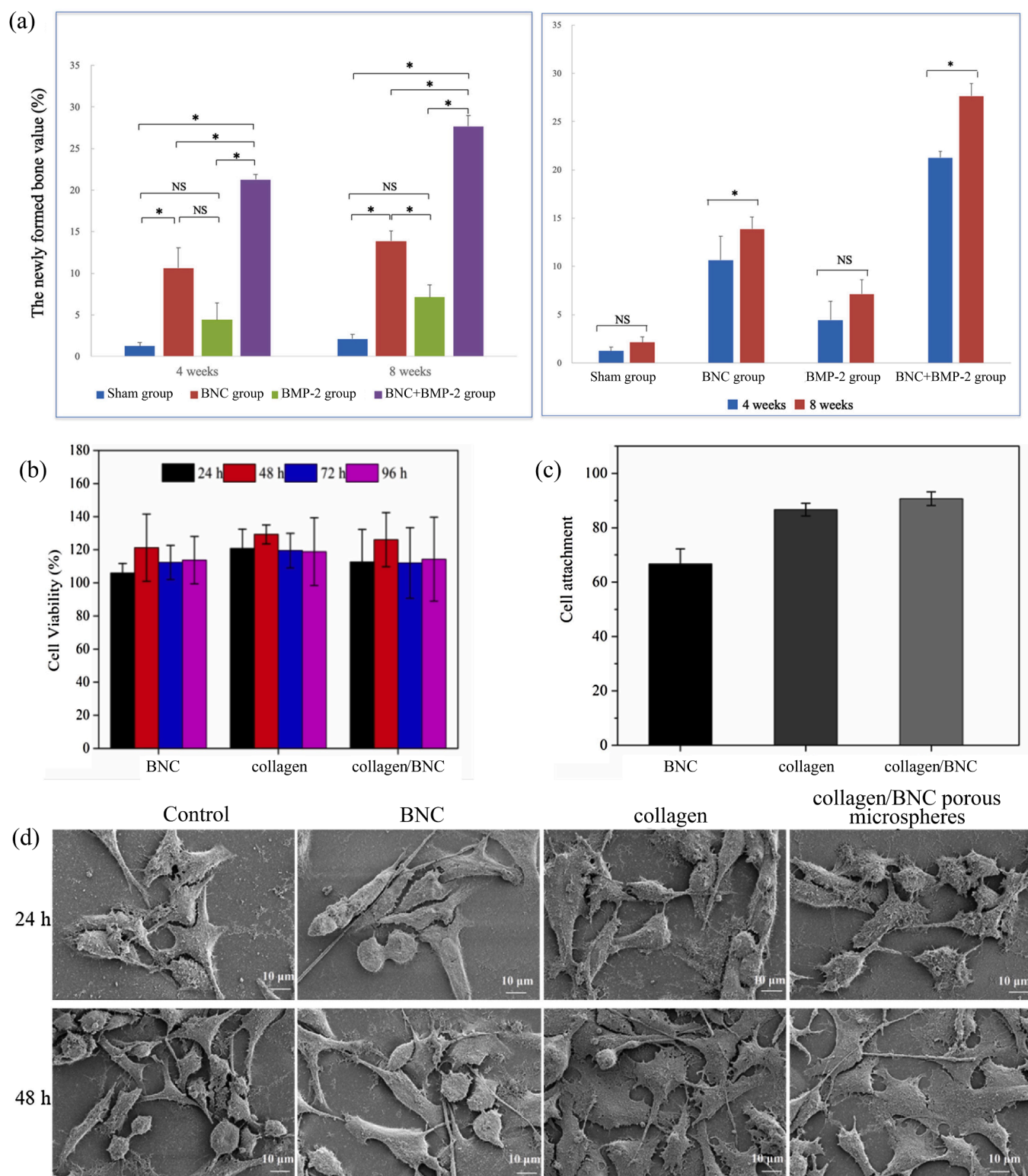


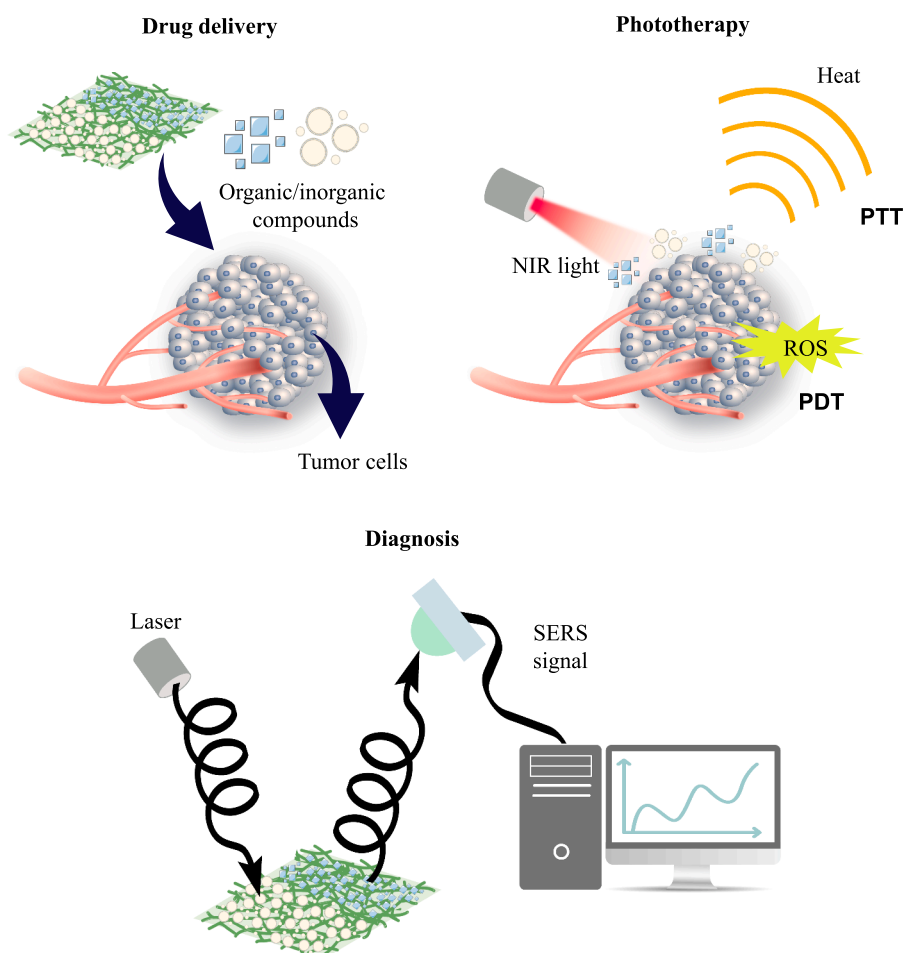
Fig. 11. (a) H&E staining analysis of newly formed bone values: (left) Comparison of newly formed bone values between different groups, analyzed by One-way analysis of variance and LSD-t test. * $p < 0.05$; NS: no significance. Error bars indicate standard deviations. (right) Comparison of newly formed bone values between four and eight weeks, analyzed by independent-samples t-test; * $p < 0.05$; NS: no significance. Error bars indicate standard deviations. Cytotoxic effects and attachment of mice MC3T3-E1 cells on BNC, collagen, and collagen/BNC porous microspheres (b) cell viability, (c) adhesion rate, (d) SEM images of cell attachment. Adapted from Ref. [8,219], with permission, Copyright 2020, Elsevier and under the Licence CC BY 4.0, 2019, MDPI, respectively.

Table 11

Examples of BNC functionalized with organic compounds for hard tissue implants applications.

Application	NPs (size; shape)	Source	Incorporation	Properties	Ref.
Bone tissue	BMP-2 (n.d.)	Collagen	<i>Ex situ</i> – Immersion	Proliferation rate: 105–130 % Good adhesion rate: 90.7 % Normal bone differentiation behaviour Porosity: $314.14 \pm 23.2 \mu\text{m}$	[8]
Bone tissue	Hydroxyapatite (n.d.)	n.d.	<i>Ex situ</i> – Mixing	Tensile strength: $1.58 \pm 0.19 \text{ MPa}$ Strain at break: $3.97 \pm 2.27 \%$ Excellent cell viability	[218]
Bone regeneration	BMP-2 (n.d.)	n.d.	<i>Ex situ</i> – Loading	Healing and bone regeneration remarkably accelerated	[219]

n.d. – not defined.

**Fig. 12.** Representation of the mechanism of drug delivery, phototherapy (PTT and PDT), and diagnosis (SERS) BNC-based implants for cancer therapy.

cells. Moreover, an *in vivo* study performed on CT-26 cells subcutaneously implanted in female Balb/c mice evidenced a significant reduction in the tumour volume after 10 days, validating the high potential of the novel magnetic BNC-based scaffolds for cancer chemotherapy treatment [227].

Photothermal therapy (PTT) and photodynamic therapy (PDT) are alternative treatments that have considerably enhanced the effectiveness of bacterial infections and cancer treatment, overcoming the resistance of antibiotics and anticancer drugs [228]. In PTT, the treatment is based on the local heat generated by photothermal agents under irradiation at a specific wavelength. The increase in the temperature can inhibit the bacteria by destroying the cell membrane, evaporating the cellular fluid, or denaturing proteins and enzymes [229]. The effect of PTT in cancer cells is protein denaturation and temporary cells inactivation, when the tissue temperature increases up to 42 °C, long-term

cells inactivation (43–45 °C), rapid cell necrosis (45–48 °C), and microvascular thrombosis and ischemia (48–60 °C) [230]. In PDT, photochemical reactions occur involving molecular oxygen and specific light wavelengths that stimulate a photosensitizer agent, generating ROS which can destroy target bacteria or cancer cells [231,232]. For example, Ni *et al.* (2021) reported the development of a hybrid material that integrates photodynamic, photothermal, and Ag-ion releasing effects. Porous Coordination Network-224 (PCN-224) metal-organic frameworks (MOFs) NPs were *in situ* grown on the surface of Ti_3C_2 MXene nanosheets and then filtered onto BNC scaffolds, followed by the sputtering of nanosilver using the magnetron sputtering method. The photothermal performance, shown in Fig. 13e, demonstrates that PCN@ Ti_3C_2 -BNC reached up to 58.9 °C after 300 s illumination, which was higher than for PCN-BNC (38.3 °C) and Ti_3C_2 -BNC (53.6 °C). These results suggest that the nanosilver covered on the surface of Ti_3C_2 and

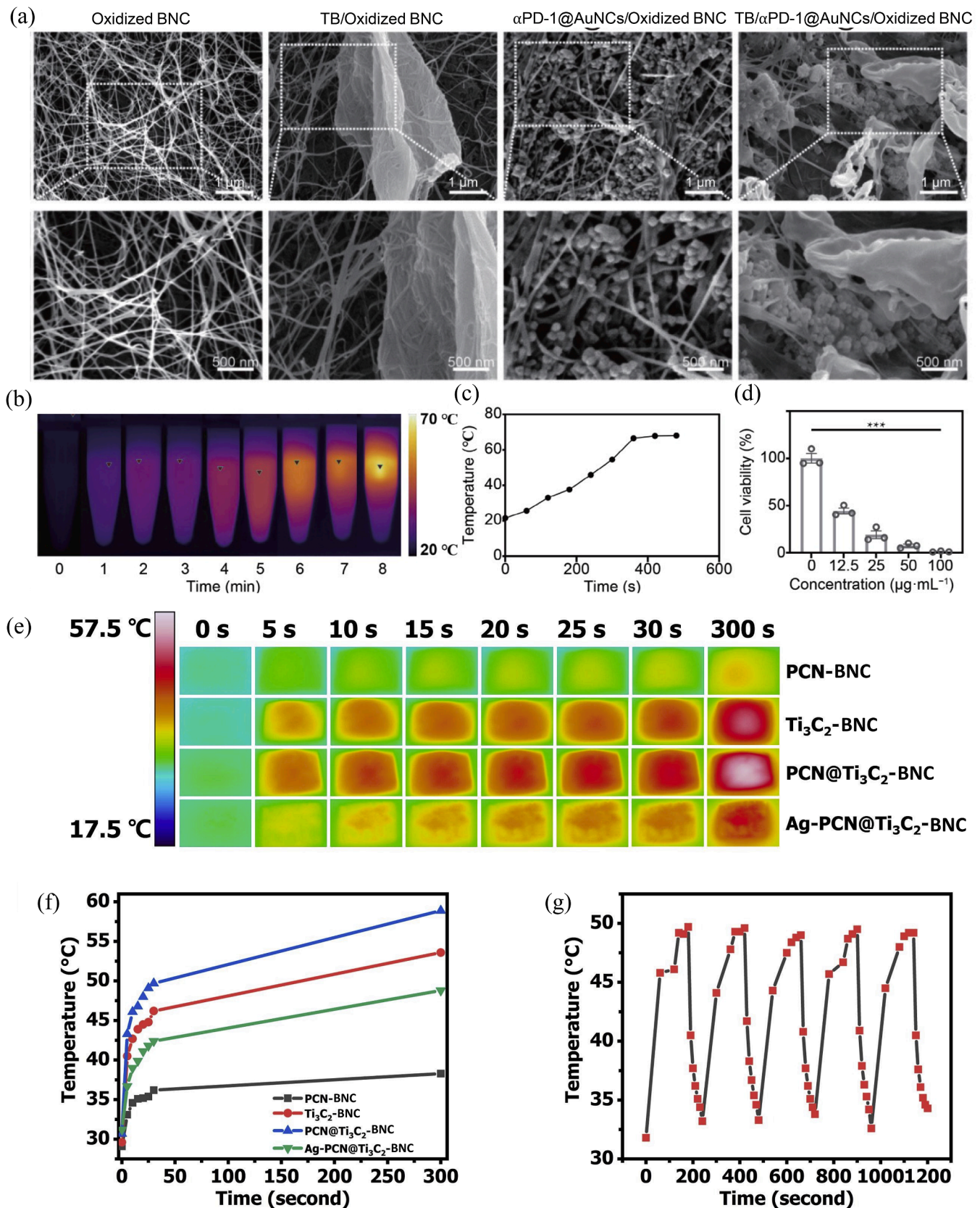


Fig. 13. (a) Representative FESEM images of oxidized BNC, TB/oxidized BNC, α PD-1@AuNCs/oxidized BNC, and TB/ α PD-1@AuNCs/oxidized BNC. (b) Photo-thermal infrared images of α PD-1@AuNCs. (c) The temperature variation curve of α PD-1@AuNCs. (d) Cytotoxicity of α PD-1@AuNCs at different concentrations. (e) Surface thermal images and (f) temperature change of PCN-BNC, Ti_3C_2 -BNC, PCN@ Ti_3C_2 -BNC and Ag-PCN@ Ti_3C_2 -BNC recorded by an infrared camera and illuminated by a 500 W Xe lamp with a 420 nm filter (31.45 W cm^{-2} , 15 cm distance). (g) Photothermal heating curves of Ag-PCN@ Ti_3C_2 -BNC for 5 circles. Adapted from Ref. [233,238], with permission, Copyright 2021, Elsevier and with permission, Copyright 2022, Springer Nature, respectively.

PCN-224, weakened the photon absorption ability of Ti_3C_2 nanosheets. The photothermal stability of $\text{Ag-PCN@Ti}_3\text{C}_2\text{-BNC}$ (Fig. 13g) showed no relevant changes in elevating and declining temperature in 5 circles. Furthermore, they found that under visible light illumination for two rounds of 30 min (Xe lamp, 31.45 W cm^{-2}), the antibacterial efficiency, both against Gram positive and Gram negative, was enhanced due to the synergy between PCN-224 and Ti_3C_2 , by generating 1O_2 and photo-thermal heat, respectively, which in turn boosts the nanosilver oxidation and accelerates Ag^+ ions release. With this sterilization process, it was obtained a 6-log unit and 99.99 % bacteria inactivation which lasted for 6 months of room preservation [233]. In another work, to increase the photodynamic activity of MOFs, carbon quantum dots (CQDs) (energy donors) were encapsulated in the PCN-224 porphyrin-based MOFs (acceptor) during a solvothermal reaction process to construct fluorescence resonance energy transfer (FRET) pairs. Then, the synthesized CQDs@PCN-224 NPs were immobilized into the BNC membrane using an eco-friendly *in situ* biosynthetic approach where a compact sandwich-like structure of the BNC/MOFs membrane was formed by its inversion during the cultivation process. This method prevents MOFs leakage during use as shown by a <1.5 % leaching. The obtained BNC/CQDs@PCN-224 composite showed excellent antibacterial efficacy when exposed to light for 30 min, >99.99 % for both *S. aureus* and *E. coli*, good hemocompatibility (rabbit red blood cells), and low cytotoxicity (L929 cells) [234]. Li *et al.* (2021) incorporated AuNRs into BNC membranes as a photothermal agent, to achieve near-infrared (NIR)-activated bactericidal properties, and further coated with phase-transitioned bovine serum albumin (PTB) film, to improve the blocking efficiency against biofouling, obtaining a dual-functional BNC composite. The resulting BNC scaffold presented antifouling properties against the adsorption of proteins and the attachment of bacteria and mammalian fibroblasts. Moreover, with 5 min of NIR irradiation light, both Gram positive and Gram negative bacterium could be fully eliminated due to the heat generated from the incorporated AuNRs. *In vitro* biocompatibility evaluation showed negligible cytotoxicity and *in vivo* tests using an infected full-thickness skin wound model in Sprague-Dawley rats showed good histocompatibility with the wound being almost fully closed on day 12 post-treatment [235]. Zhang *et al.* (2019) designed a transdermal drug targeting system for breast cancer therapy based on BNC and laser-sensitized magnetic NPs (LMNs). Fe_3O_4 NPs, which served as the magnetic core, were first coated with hydrogels loaded with Dox and hematoporphyrin monomethyl ether (HMME). Then, the LMNs were grafted with folic acid to promote cell-specific recognition and binding. Finally, the NPs complex was loaded onto the BNC scaffold. When irradiated (633 nm laser), this multifunctional theragnostic scaffold produces repeatedly singlet oxygen ($\text{O}^\cdot$) and releases the anticancer drug Dox, thus combining both photodynamic therapy and chemotherapy. The performance of this system was evaluated *in vitro* using human breast carcinoma (MCF-7) and human skin fibroblasts (HSF) cells, and *in vivo* using Balb/c mice bearing MCF-7 cells. For the treatment, cells were irradiated with static magnetic field (100 mT) for 6 h and He-Ne laser (633 nm, 100 mW cm^{-2}) for 10 min. *In vitro* results showed that the MCF-7 cells growth was delayed 50 % in comparison with the non-treated control group and in a scratch/wound healing test the monolayer restoration was delayed by 82 % (24 h). Meanwhile, viability tests indicated no inhibition in the growth of HSF cells. Moreover, *in vivo* chemotherapy and PDT measured on Balb/c mice bearing MCF7 cells with tumour volumes of 100 mm^3 showed, on the 14th day, a decrease of the tumour volume to $40.8 \pm 5.4 \text{ mm}^3$ and tumour growth inhibition rates of 80.38 %, while no evidence of leukopenia or associated toxicity was observed [236]. Chu *et al.* (2018) took advantage of the ability of C_{60} isotopes to generate abundant ROS when exposed to light, to use them in scaffolds for PDT to treat skin cancer. Fullerene C_{60} NPs suspension was first prepared by a solvent exchange method, being afterward incorporated into the BNC by vacuum filtration. Results showed that C_{60} particles of diameters less than 100 nm were uniformly distributed in the ultrafine BNC network and,

when exposed to light, the scaffold exhibited excellent antibacterial performance due to the high destructive power of ROS against bacteria. Moreover, *in vitro* cell experiments showed a high rate of cell death (80 %) toward human epidermoid carcinoma cells A-143, giving rise to a great potential of BNC/ C_{60} scaffolds for treating skin cancer [237]. Recently, Zhou *et al.* (2023) reported a multifunctional therapeutic system to be used as a versatile implant for avoiding the recurrence of head and neck squamous cell carcinoma (HNSCC) after resection, where PTT was used to boost the immunotherapy mediated by the anti-programmed death 1 (PD-1) antibody. The scaffold was prepared by first encapsulating PD-1 into gold nanocages (AuNCs) and then, together with thrombin (TB), they were incorporated into oxidized BNC. The photothermal treatment (Fig. 13b,c), consisting of consecutive NIR laser irradiation (808 nm , 1.0 W cm^{-2}) for 5 min, on SCC7 cells was first evaluated *in vitro*, revealing a noticeable reduction in the cell proliferation (Fig. 13d). The immunotherapeutic system was further evaluated *in vivo* using SCC7 tumour-bearing mice where a tumour was resected after reaching 100 mm^3 , leaving a small amount of tumour behind. Likewise, the NIR laser irradiation treatment inhibited the tumour growth and suppressed the residual tumour recurrence, with no apparent toxicity. This effect is a consequence of the increased intracellular ROS levels that triggered tumour pyroptosis, a type of programmed cell death, induced by the inflammatory Caspase-1 cleavage of gasdermin D protein, the increase in T-cell infiltration, and the decrease in the number of immunosuppressive cells. Field Emission Scanning Electron Microscopy (FESEM) images in Fig. 13a suggest that the properties of the oxidized BNC were maintained since no alterations in its structure were verified after coating with TB. Furthermore, the loading of TB and $\alpha\text{PD-1@AuNCs}$ was confirmed [238].

The early diagnosis of cancer offers a superior chance for a successful treatment. Surface-enhanced Raman scattering (SERS) is an imaging technique with ultrahigh sensitivity, stability, low cost, repeatability, and ease of use. It has the ability to identify with high sensitivity and reliability the main biomarkers which involve different biomolecules, such as proteins, nucleic acids, and lipids, distinguishing tumour cells from normal cells through fingerprint signals [239–241]. In a study on cancer diagnosis, Ferreira *et al.* (2019) developed a low-cost hybrid substrate based on BNC and *in situ* grown AgNPs for direct SERS analysis of exosomes. Statistical principal component analysis (PCA) was used to classify exosome-derived SERS signals obtained from PBS solutions of exosomes isolated from nontumorigenic breast epithelium (MCF-10A) and breast cancer (MDA-MB-231) cell cultures. This label-free diagnostic method allowed a tumoral and nontumoral exosomes discrimination with a 95 % confidence [242]. Similarly, Li *et al.* (2024) reported the development of a SERS substrate based on BNC and *in situ* synthesis of AgNPs, but for detection of glutathione in serum. The monitoring of glutathione can be used in cancer detection as the levels of this tripeptide small molecule are higher in the serum of cancer patients. For SERS measurements, Ellman's reagent (DTNB) was added to colon cancer, gastric cancer, and normal serum diluted with PBS. Higher contents of glutathione were detected in cancer serum when compared with normal serum, being 2.6 times higher in colon cancer and 2.8 times higher in gastric cancer [243]. These works show the feasibility and great potential of BNC/AgNPs substrate for SERS application in real-time and early cancer diagnosis. Table 10 presents examples of the various applications of BNC in cancer therapy.

Table 12

4. Conclusions and future perspectives

The current state of the art regarding BNC in the biomedical field, especially its use as wound dressings and body implants, is described above. According to the literature, the growing interest in BNC is due to its notable intrinsic properties such as excellent mechanical properties, high water retention, easy functionalization, high purity. In fact, the importance of BNC as a substitute for petrochemical-based polymers,

Table 12
BNC composites in cancer therapy applications.

Application	NPs (or complex) (size; shape)	Drugs or other components	Incorporation method	Properties	Ref.
Drug delivery for breast cancer treatment	NLCs (spherical, ~150 nm)	Doxorubicin (Dox)	Ex situ – Immersion	Dox release: 50 % after 24 h and sustained over time <i>In vivo</i> antitumor efficacy (MDA-MB-231 cells) Antiproliferative activity against eight human tumor cell lines (U251, MCF-7, NCI-ADR/RES, 786-0, NCI-H460, PC-3, HT-29, and K562)	[223]
Treatment of skin cancer and soft tissues infected by bacterial pathogens	Ag complex with 4-aminobenzoic acid (Ag-pABA) (n.d.)	n.d.	Ex situ – Immersion	Sustained release of Ag-pABA up to 120 h Antiproliferative activity against adenocarcinoma (MCF-7, TGI = 26.3 μ M, PC-3, TGI = 22.8 μ M, OVCAR-03, TGI = 22.5 μ M, and HT-29, TGI = 41.1 μ M) cell lines Antiproliferative effect at AgNMS concentrations higher than 70 μ M for the other cell lines Inactive free NMS for all human tumors (TGI > 200 μ M)	[224]
Treatment of skin cancer	Ag complex with nimesulide (AgNMS) (n.d.)	n.d.	Ex situ – Immersion	Proliferation of immortalized human keratinocytes HaCaT unaffected by AgNMS complex and NMS Selective inhibition of SCC of SCC15 (TGI = 67.3 μ M) and UACC-62 (TGI = 2.8 μ M) Less active against SCC of FaDu (TGI = 107.2 μ M) and SCC4 (TGI > 400) Sustained release of AgNMS complex for up to 216 h Antitumoral efficacy of the BNC scaffold containing AgNMS (Balb/c mice with induced SCC cells) Antibacterial activity (<i>S. aureus</i> , <i>S. mutans</i> , <i>S. typhimurium</i> , <i>E. coli</i> , and <i>P. fluorescens</i>)	[225]
Drug carrier system with antibacterial, anticancer, and larvicidal activities	CuO NPs (spherical, ~ 48 nm)	n.d.	Ex situ – Solution mixing and casting	Anticancer: Caco-2, HepG-2, and MDA Antilavricidal activity: <i>A. aegypti</i> mosquito in all stages of larval and pupal instar Antibacterial (8 strains) Non-cytotoxic	[226]
Chemotherapy (colon cancer)	Fe ₃ O ₄ NPs (spherical, 8–15 nm)	n.d.	Ex situ – Co-precipitation	Anticancer Antibacterial activity: <i>S. aureus</i> and <i>E. coli</i> (> 99.99 %, 2 h of NIR light exposure)	[227]
Photodynamic and photothermal therapy for microbial disinfection	PCN-224 MOFs@Ti ₃ C ₂ MXene (n.d.)	Nanosilver	Ex situ – Filtration and sputtering	Cytotoxicity: Cell viability (L929 cells) 75.84 and 69.75 %, after 12 and 24 h Thermally stable at 265 °C Breaking stress: 12.5 MPa Elongation at break: 27 %	[233]
Photodynamic therapy for microbial disinfection	CQDs@PCN-224 MOFs (spherical, 99.1 $\pm$ 18.1 nm)	n.d.	In situ – mixing in culture medium + sonication	Antibacterial activity: <i>S. aureus</i> and <i>E. coli</i> (> 99.99 %, 30 min of NIR light exposure) Good hemocompatibility: hemolysis rates < 5 % (rabbit red blood cells) Non-cytotoxic. Cell viability (L929 cells) 88 % Antifouling (FITC-BSA protein, <i>E. coli</i> DH5 α -pBADDs bacteria, and L929 cells)	[234]
Photothermal therapy for microbial disinfection	AuNRs (rod-like, 51 $\pm$ 7x15 $\pm$ 3 nm;)	PTB	Ex situ – Immersion	Antibacterial activity: <i>S. aureus</i> and <i>E. coli</i> (~100 %, 5 min of irradiation of NIR light exposure) Good biocompatibility with negligible cytotoxicity <i>in vitro</i> (L929 cells) Skin wound healing performance <i>in vivo</i> (Sprague-Dawley rats) <i>In vitro</i> – cell growth delayed 50 % (MCF-7 cells) and 82 % in a scratch/wound healing test No inhibition in the growth of HSF cells <i>In vivo</i> – tumour growth inhibition rates of 80.38 % (MCF7 cells) No evidence of leukopenia or associated toxicity	[235]
Drug delivery and photodynamic therapy for breast cancer treatment	Magnetic-hydrogel (Fe ₃ O ₄ -FA-NIPAm-AA) NPs (MHNP) (n.d., ~106 nm)	Dox and HMME	Ex situ – Immersion	Antibacterial activity against <i>E. coli</i> and <i>S. aureus</i> Anti-cancer (human-derived epidermoid carcinoma A-431 cells) <i>In vitro</i> – marked reduction in cell proliferation (SCC7 cells) <i>In vivo</i> – tumour recurrence inhibited without toxicity	[236]
Photodynamic therapy for skin cancer treatment	Fullerene C60 (n.d., <100 nm)	n.d.	Ex situ – Vacuum filtration		[237]
Photothermal therapy and immunotherapy	AuNCs (cubic, 89.0 nm)	PD-1 antibody and thrombin	Ex situ – Immersion		[238]

(continued on next page)

Table 12 (continued)

Application	NPs (or complex) (size; shape)	Drugs or other components	Incorporation method	Properties	Ref.
Cancer diagnosis (SERS)	AgNPs (n.d., 92.1 ± 51.57 nm)	n.d.	Ex situ – Microwave in situ AgNPs synthesis into BNC	Enhancement factors (EF): 10^4 to 10^5 (rhodamine 6G, 10^{-11} M) Differentiation of tumoral and non-tumoral exosomes: MCF-10A and MDA-MB-231 with 95 % confidence	[242]
Cancer diagnosis (SERS)	AgNPs (n.d., 65 ± 10 nm)	n.d.	Ex situ – Soaking in ammonia followed by in situ reduction in silver trifluoroacetate (CF ₃ COOAg) solution	EF: 3.8 ± 10^7 (glutathione in serum of cancer (gastric and colon), 2.9 ± 10^{-8} M)	[243]

n.d. – not defined.

such as polyethylene, polypropylene, polyurethane, polytetrafluoroethylene, and polyether ether ketone, which are prevalent in established biomedical materials, is clear. These polymers often suffer from poor biocompatibility, both short- and long-term, contrary to BNC, which can lead to chronic inflammatory responses and the formation of fibrous collagen capsules around them. This, in turn, can result in pain, restricted movement, and even necrosis.

In turn, the physical characteristics of the BNC's fibrous network are particularly well-suited for wound dressings and implants because they closely resemble the extracellular matrix, especially its component, collagen. As a result, its use in blood vessels and biosensors is, also, of particular interest. In addition, the potential for extensive modifications, functionalization processes, and carrier applications enhances its diverse biomedical uses. In fact, since specific properties can be easily endowed to BNC, tissue engineering seems to be one of the most interesting areas, e.g. by the incorporation of bioactive molecules that promote cell growth leading to tissue repair and regeneration. The major number of studies found are focused on wound dressings and wound healing, highlighting the continued development of BNC for these applications, due to its relevant properties of biocompatibility, high porosity, water holding capacity, and nontoxicity. For soft implant area, cartilage, vascular grafts/blood vessels, and cornea are the most common applications, based on the favourable mechanical properties. Hard tissue implants are majorly based on bone tissue regeneration, that take advantage of its nano scaffold architecture. Inorganic nanoparticles/complexes still play an important role in the functionalization of BNC, despite the growing efforts on using organic nanoparticles/complexes. The displayed separation between inorganic and organic nanoparticles/complexes should assist in highlighting its influence towards BNC and its application goal, envisaging a tendency of the most effective and or less detrimental. Nevertheless, due to non-consistent characterization methodology displayed in the references, it is extremely precarious the establishment of a comparison. Thus, for the development of future applications, for both inorganic and organic nanoparticles/complexes a coherent set of characterizations should be promoted to allow a robust comparison between these two approaches. Again, despite the intriguing physicochemical properties of BNC, there is no clear consensus on testing methodology, especially regarding parameters and assay types. Variations assays, such as whether they are conducted on never-dried, lyophilized, or dried samples, and whether temperature or vacuum conditions are used, are also significant. Therefore, improving the standardization of methodology vs physicochemical properties should focus on defining the applications requirements and treat the material architecture as a nano non-woven material. Furthermore, although cellulases capable of degrading cellulose are not present in the human body, BNC is still exposed to mechanical stress, oxidation, and other factors that could affect its long-term use. Therefore, further investigation is needed to understand and correlate all these concepts in order to take advantage of the excellent properties of this sustainable material.

In conclusion, BNC functionalized with inorganic and organic nanoparticles/complexes clearly display a wide set of relevant functions and activities, that may and should be explored in other areas. Its fibrous

nature clearly drives its use towards the textile industry, in particular for the development of advanced multifunctional technical textiles, that comprise a plethora of applications. The main factor impairing this shift is its recalcitrant high production cost. However, the available funding for the implementation of sustainable industries, should press the development of novel BNC production methodologies and further lead to low-cost culture media use bio-based on waste products. A lower cost BNC will promptly permeate within fibre-based industries such as packaging, automotive, and construction.

CRediT authorship contribution statement

Liliana Melro: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Cátia Alves:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Marta Fernandes:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Sofia Rocha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation. **Behnaz Mehravani:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Ana Isabel Ribeiro:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Sara Azevedo:** Writing – original draft, Validation, Methodology, Investigation. **Vanessa F. Cardoso:** Writing – review & editing, Visualization, Validation. **Óscar Carvalho:** Validation. **Nuno Dourado:** Validation. **António J. Salgado:** Validation. **Andrea Zille:** Writing – review & editing, Visualization, Validation, Supervision. **Jorge Padrão:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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