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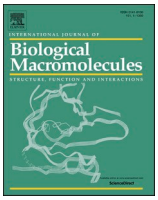

Review

Recent advances and biomedical application of 3D printed nanocellulose-based adhesive hydrogels: A review

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ABSTRACT

Nanocellulose-based tissue adhesives show promise for achieving rapid hemostasis and effective wound healing. Conventional methods, such as sutures and staples, have limitations, prompting the exploration of bioadhesives for direct wound adhesion and minimal tissue damage. Nanocellulose, a hydrolysis product of cellulose, exhibits superior biocompatibility and multifunctional properties, gaining interest as a base material for bioadhesive development. This study explores the potential of nanocellulose-based adhesives for hemostasis and wound healing using 3D printing techniques. Nanocellulose enables the creation of biodegradable adhesives with minimal adverse effects and opens avenues for advanced wound healing and complex tissue regeneration, such as skin, blood vessels, lungs, cartilage, and muscle. This study reviews recent trends in various nanocellulose-based 3D printed hydrogel patches for tissue engineering applications. The review also introduces various types of nanocellulose and their synthesis, surface modification, and bioadhesive fabrication techniques via 3D printing for smart wound healing.

1. Introduction

In conventional medicine, wounds are typically treated by suturing the trauma with a thread and needle or by applying a splint and driving a metal pin to treat fractures. In the case of internal injury to an organ, the affected area is incised, the problematic organ is surgically treated, and the incision is subsequently sutured. Hemostasis is achieved either by directly applying a medication with hemostatic properties or by spraying it onto an open wound. However, these treatment methods are susceptible to infection and necessitate lengthy surgeries along with ongoing wound dressing. To address these drawbacks, bioadhesives have gained significant prominence and experienced remarkable advancements, revolutionizing the field of surgery over the past three decades. Unlike conventional treatment methods involving staplers, metal pins, or sutures, bioadhesives enable direct adhesion to the wound area and have the potential to promote wound healing through minimal tissue damage and various material combinations. Additionally, they can be engineered to possess antioxidant, antibacterial, and anti-inflammatory properties. Moreover, they facilitate faster and safer treatment without the need for incisions in cases of internal injury, utilizing injections instead [1]. Bioadhesives are effective for hemostasis as they cover the wound area. For instance, chitosan biomaterials have been widely utilized in a broad range of biomedical applications, such as drug delivery, wound dressing, and procedures involving hemostasis and sutureless surgery [2]. This paper discusses bioadhesives based on nanocellulose.

Nanocellulose has gained attention as a promising advanced material recently. It is a natural substance that is both biodegradable and renewable. Most human tissues do not contain cellulase enzymes. However, a previous study [3] has shown that crystallinity and manufacturing methods significantly affect in vivo degradation rates. Specific types of nanocellulose exhibit high biodegradability in aqueous solutions compared to other nanoparticles, such as fullerenes and carbon nanotubes. Furthermore, nanocellulose shows higher biodegradability than its macroscopic counterparts in aqueous environments, likely due to its increased surface area [4]. It may be possible to enhance the

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biodegradability of nanocellulose by introducing specific modifications [5]. Considering the potential for developing biodegradable nanocellulose, exploring its applications in various fields, including the medical sector, is worthwhile. Additionally, it is lightweight yet possesses high strength. Ongoing research is being conducted to utilize nanocellulose in various applications, such as lightweight composite materials, packaging, cosmetics, and medical biomaterials. By using nanocellulose, it is possible to create biodegradable adhesives that do not have harmful effects on the body. Hemicellulose is the main component of a plant's cellular structures, aiding in its rigidity and verticality. Consequently, it can be derived from botanical sources, agricultural residues, fauna, and bacterial biofilms. Cellulosic materials can be extracted in various dimensions based on their intended usage. Micro- and nanocellulose represent the typical scales of cellulose employed in industrial settings. When it comes to nanocellulose, it can be broadly categorized into three primary groups: (1) nanofibrillated cellulose (NFC), alternatively referred to as nanofibrils, microfibrils, or microfibrillated cellulose; (2) cellulose nanocrystals (CNC), also referred to as crystallites, whiskers, or rod-shaped cellulose microcrystals [6]; and (3) bacterial nanocellulose (BNC). Researchers have dedicated substantial attention to the development, fabrication, and manipulation of nanocellulose-derived substances, aiming to exploit their prospective applications in the realm of medical science. Moreover, there has been a substantial increase in the quantity of academic papers published since 2015, focusing on key terminology such as "regenerative medicine," "pharmaceutical transportation," and "cutaneous mending" [7]. This paper discusses the ultimate goal of utilizing nanocellulose as a base material to develop and manufacture adhesives using 3D printing technology (Fig. 1).

Three-dimensional (3D) printing technology has significantly enhanced the quality of life and technological advancements. Using a 3D printer, various shapes can be created without the need for separately crafted molds, simply by utilizing different materials. Currently, 3D printing technology enables the construction of houses based solely on blueprints, eliminating the need for construction materials and heavy machinery. This approach significantly reduces construction time and saves costs on labor and materials. Moreover, 3D printing technology has found applications in the field of biotechnology. It can be utilized to create structures such as artificial bones and shaped hydrogels for various purposes. The three commonly used methods in 3D printing include extrusion-based bioprinting, light-assisted bioprinting, and inkjet-based bioprinting. The materials used in 3D bioprinting include alginate, collagen, gelatin, chitosan, and others. With 3D printing, various objects in the field of biotechnology, such as tissue and organ fabrication, bone and cartilage production, and biomaterials for skin and organ wounds, can be easily created. Nanostructured materials, including carbon-based materials, metals, oxides, and nanocellulose, have been extensively investigated for the development of stimuli-responsive shape-memory hydrogels. Among these nanomaterials, nanocellulose has shown promise as an appropriate additive to enhance the shape memory and recovery capabilities of hydrogels. Furthermore, nanocellulose is frequently employed to enhance the mechanical strength of hydrogels [9]. In a recent study, considering the appealing qualities of both nanocellulose and bioactive glass (BG), they were integrated into methacrylate chitosan (CS) to create transparent, 3D-printable, and bioadhesive hydrogels [9]. With the active progress of such research, the day is not far off when actual medical treatments will be conducted using adhesives based on 3D-printed nanocellulose.

2. Nanocellulose: types, synthesis and surface modifications

Apart from traditional fibers such as cotton, wool, nylon, and acrylic, there has been a remarkable interest in a novel form of cellulosic material known as nanocellulose. Fig. 2(a) depicts a schematic diagram of cellulose and its derivatives. Table 1 shows the characteristics of various nanocellulose that change as raw materials and extraction methods change. Nanocellulose possesses distinct attributes that have attracted considerable attention. With minimum dimensions below 100 nm,

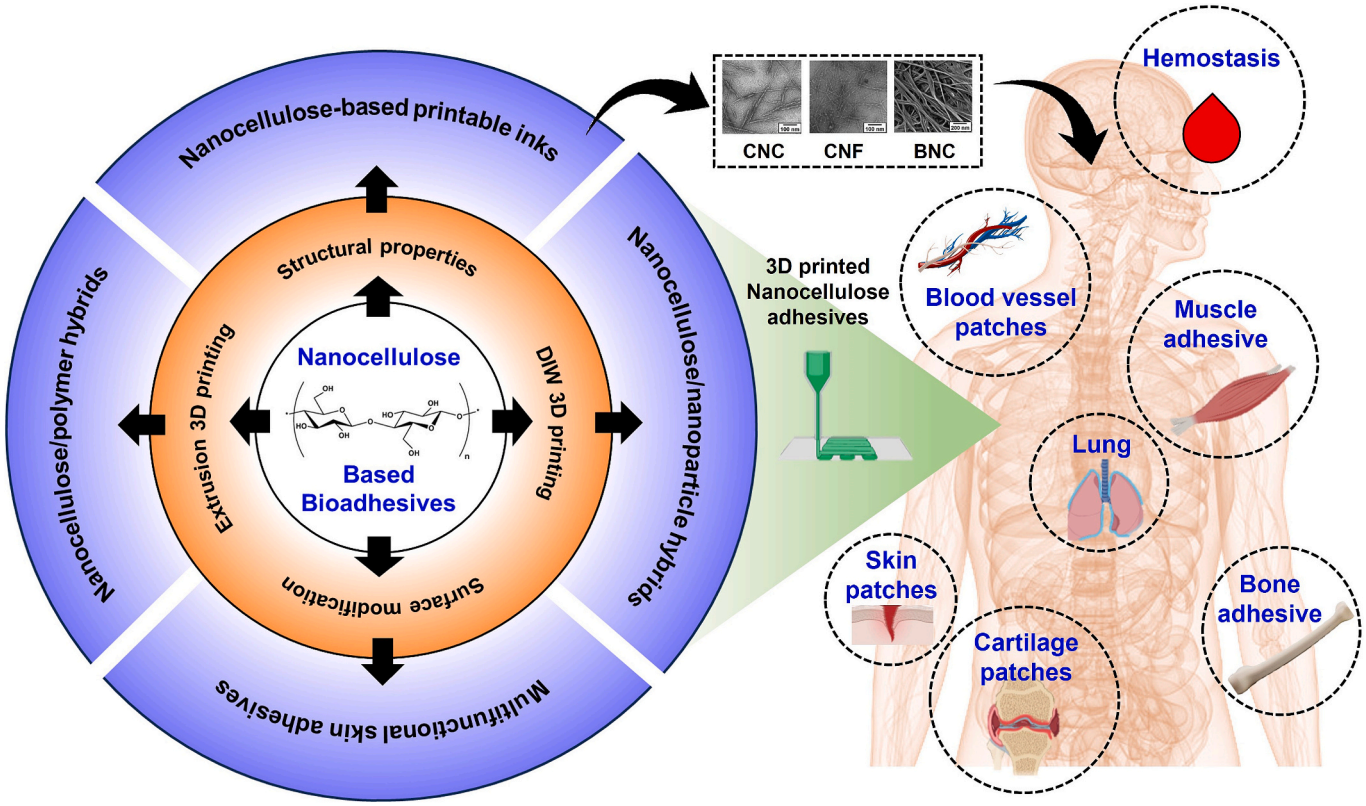


Fig. 1. Schematic overview of the nanocellulose-based 3D printable hydrogels as smart bioadhesives patch for tissue regeneration [8].

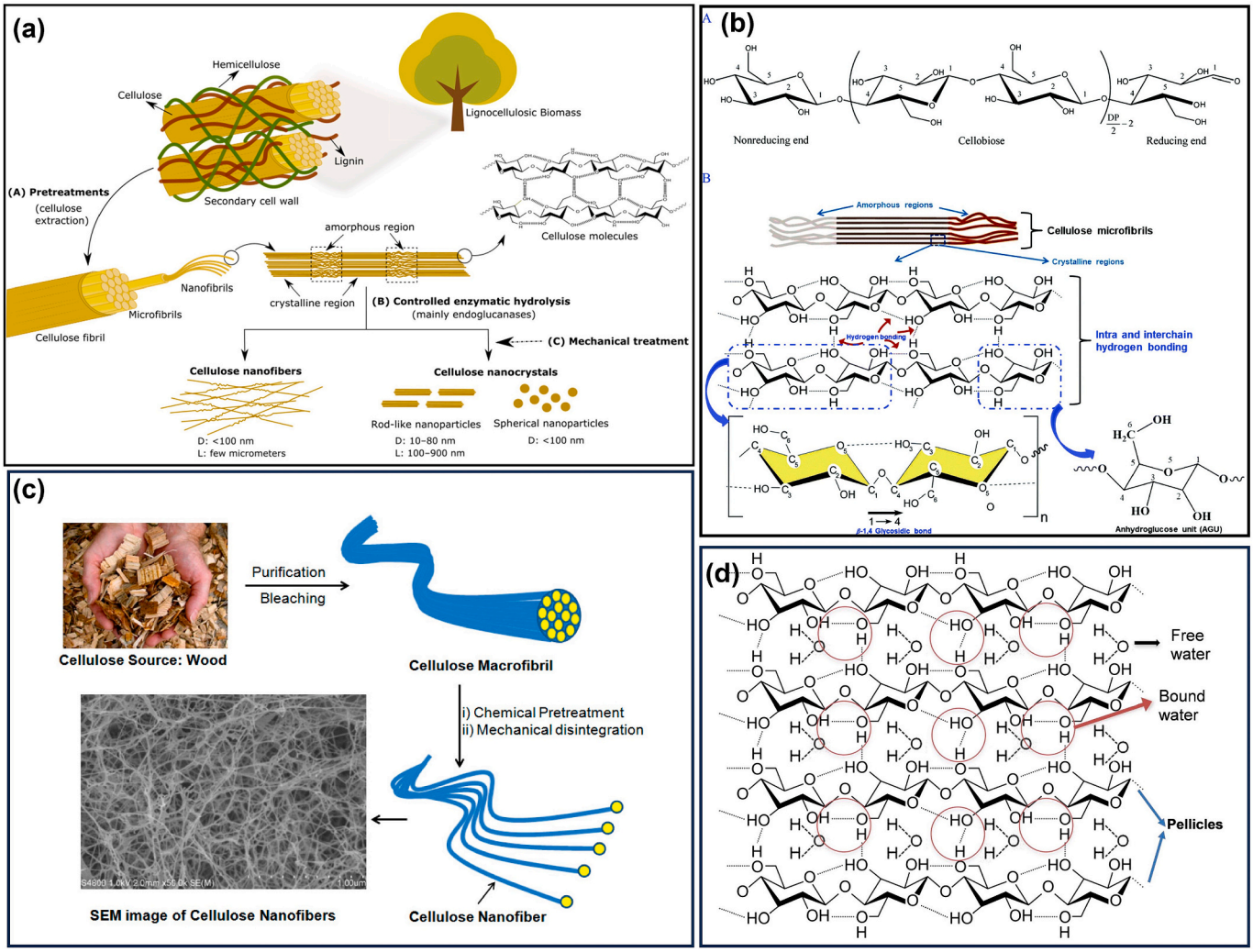


Fig. 2. Nanocellulose's extraction methods and structural insights. (a)Extraction procedure of various types of nanocellulose from the lignocellulosic mass. The native wood can be pre-treated with various alkalis or acids to remove the hemicellulose and lignin moieties. The nanocellulose can be obtained from pure cellulose either by enzymatic degradation, acid hydrolysis, or surface oxidation [11]. (b) Structure of cellulose nanocrystals (CNCs) showing the amorphous and crystalline region of cellulose with β-1, 4 glycosidic bonds and hydrogen bonds [12]. (c) Isolation of cellulose nanofibrils (CNFs) from wood. The CNFs appeared to have a net-like mesh structure under scanning electron microscopy. Scale bar: 1 μm [13]. (d) Structure of bacterial nanocellulose (BNCs) [14].

nanocellulose has a significantly larger specific surface area than conventional cellulose. Despite being categorized as an insoluble fiber, nanocellulose exhibits gel-like behavior under certain circumstances. It can be regarded as a flexible colloidal particle capable of deformation and entanglement. Notably, nanocellulose is nontoxic to humans, compatible with biological tissues, and biodegradable in the environment [10]. This section explores various types of nanocellulose and provides an overview of nanocellulose synthesis and surface modifications.

2.1. Types of nanocellulose

Cellulose exists in the form of microfibrils and can be derived from diverse sources, including wood, flax, cotton, and algae. Through mechanical and chemical processing of these microfibrils, cellulose nanofibrils (CNFs) and cellulose nanocrystals (CNCs) can be obtained. Moreover, bacteria can also serve as a source of cellulose, often exhibiting a more crystalline structure than cellulose extracted from plants [18].

2.1.1. Cellulose nanocrystals (CNCs)

Nanocellulose can be synthesized from fibrous material, comprising a linear chain of β-D-glucopyranose units linked by β-1,4 glycosidic bonds. These bonds lead to robust intra- and intermolecular hydrogen bonding, as illustrated in Fig. 2(b). CNCs are typically produced through various methods such as traditional acid hydrolysis, chemical oxidation, mechanical disintegration, and hydrolysis using ammonium persulfate [19]. CNCs exhibit a rod-like morphology, typically measuring between 100 and 500 nm in length and 1 to 50 nm in diameter. CNCs are highly sought after because of their remarkable properties, including exceptional mechanical strength, colloidal stability, biodegradability, and extremely low cytotoxicity [20]. CNCs have garnered significant attention due to their favorable cost-effectiveness and versatile applications in both industrial and biomedical fields [21]. When incorporated into composite hydrogels along with other ingredients, CNC serves a dual role. Firstly, it enhances the water absorption capability of hydrogels, such as gelatin, by acting as a reducing agent. Furthermore, it decreases the rate of water evaporation from the hydrogel. Hydrogels reinforced with CNC demonstrate the capability to retain a substantial moisture content, even at high temperatures close to the boiling point of water. Conversely, aerogels are materials derived from hydrogels in which the liquid phase is substituted with a gas phase under favorable conditions [22]. Aerogels based on CNC exhibit fascinating attributes, such as exceptionally high porosity (>90 %), low density, and a large surface

Table 1
Characteristics and extraction method of nanocellulose by raw material.

Raw material	Method of extraction	Particle size (nm)	Crystallinity (%)	Ref.
Palm fronds	Acid hydrolysis	108	33	[15]
Palm coir		90	32	
Palm leaves		N/A	30	
Rice husk (short grain)	Acid hydrolysis, centrifugation, and sonication	150–200	58.82	[16]
Rice husk (medium grain)		230–400	62.32	
Rice husk (long grain)		380–650	76.69	
Yerba mate sticks (unmodified)	N/A	N/A	40.62	[17]
Yerba mate sticks	Alkaline treatment Steam explosion Bleaching Acid hydrolysis	105–220	75.99	
	Steam explosion Bleaching Acid hydrolysis	50–91	66.19	
	Alkaline treatment Bleaching Acid hydrolysis	142–295	64.36	

area, which enable them to have excellent liquid adsorption capabilities. However, pure CNC aerogels often suffer from rigidity and brittleness due to the inherent inflexibility of CNC rods, which are unable to dissipate external stresses through deformation. Additionally, the sole connection between CNC rods occurs through hydrogen bonds, making them prone to crack formation. To address the issue of brittleness, CNC-based aerogels can be combined with other non-brittle substances. Examples of such substances include zwitterionic surfactants, neutral or anionic polymers, amino resins, and/or sol-gel crosslinked latex nanoparticles. The incorporation of these materials into the composite aerogels results in improved mechanical properties and enhanced flexibility [23].

2.1.2. Cellulose nanofibers (CNFs)

Nanoscale cellulose nanofibers (CNFs) obtained from biomass possess remarkable biocompatibility, a highly porous structure, abundant hydroxyl groups for chemical reactions, and good machinability. They serve as excellent substrates for wound dressings. Notably, CNFs can form hydrogels on the nanoscale, displaying a unique nanocage-like structure within a complex 3D network as depicted in Fig. 2(c). These nanoscale hollow nanocages, formed by interwoven fibers, resemble resilient hollow microcapsules and coordination nanocage-based nanocomposites. They exhibit an exceptional capacity for drug loading [24]. The nanofibers enclosed within the nanocages display an organized arrangement in multiple dimensions, resembling the structure of a spider's foot. For instance, certain jumping spiders, such as the Exarchal arcuate, have numerous hairs on their feet, with each hair covered by thousands of tiny hairs measuring a few hundred nanometers in width. These tiny hairs enable spiders to effectively adhere to various surfaces. Inspired by this natural phenomenon, CNF-based hydrogels with nanocage structures exhibit exceptional skin adhesion. By emulating the multidimensional structure of spider feet, these hydrogels possess high mobility and shape adaptability, further enhancing their practical applications [25]. Consequently, they offer numerous benefits for addressing irregular wounds [26]. CNFs contain bundles of fibers with diameters in the nanometer range and lengths in the micrometer range. In contrast to CNC fibrils, CNFs exist in both amorphous and crystalline states. CNF production involves various methods, including mechanical, chemical, or a combination of both. Mechanical treatments encompass homogenization, grinding, and milling, while chemical treatments may involve techniques like TEMPO oxidation [27]. The utilization of CNFs has witnessed a remarkable increase owing to their immense potential across a wide range of fields, including packaging, composites, electronic films, drug delivery, and water treatment. Due to their large surface area, CNFs are an excellent reinforcement material for fabricating polymer nanocomposites [28].

2.1.3. Bacterial nanocellulose (BNC)

Bacteria-derived nanocellulose (BNC) is a highly promising natural biopolymer synthesized by certain bacterial species. It is produced as an exopolysaccharide consisting of units of β-D glucopyranose, making it a remarkable natural material of natural origin. Fig. 2(d) shows the chemical structure of typical BNC. Despite its high water content, BNC exhibits exceptional mechanical properties. Its nanostructured morphology and water-holding capacity, akin to collagen—an extracellular matrix protein—make BNC highly compatible with cellular adhesion and immobilization. Moreover, BNC possesses various unique attributes and is classified as a generally recognized safe (GRAS) product, rendering it suitable for diverse applications [29]. Indeed, BNC is synthesized by bacteria in a two-step process involving polymerization and crystallization. Initially, glucose molecules undergo polymerization within the bacterial cytoplasm, forming linear chains composed of β-1,4 glucan. These chains are then secreted into the extracellular environment. Subsequently, the developed chains undergo crystallization, transforming into microfibrils. Certain quantities of microfibrils come together to create a highly pure three-dimensional porous network composed of interwoven nanoribbons. The widths of these nanoribbons typically range from 20 to 60 nm [30]. In contrast to plant cellulose, BNC is synthesized in a pristine form, devoid of lignin, pectin, and hemicelluloses. The ultra-fine structure of BNC presents several notable advantages, such as heightened crystallinity, exceptional liquid absorption capacity, increased degree of polymerization, larger specific surface area, and improved mechanical properties. These characteristics position BNC as a superior alternative to plant cellulose in numerous applications [31]. Moreover, the inherent adaptability of BNC confers significant advantages over plant-derived cellulose. BNC is easily shaped and manipulated during the fermentation process, allowing for the creation of tubes, spheres, or membranes based on specific application requirements. This flexibility in design and customization enables BNC to be tailored to meet the demands of various applications [32]. Another factor to consider is the abundance of hydroxyl groups present in BNC, enabling easy functionalization or blending with other reinforcing substances. This results in the acquisition of novel physical properties by BNC [33], such as antimicrobial activity [34], electroconductivity [35], and multifunctional BNC composites [36]. Consequently, the scope of BNC applications is continuously expanding, encompassing various sectors such as bioprocessing, biomedical and pharmaceutical fields, wastewater treatment, electro-conductive materials, packaging, and the food industry [37,38]. Table 2 depicts various CNC-based nanocomposites and hydrogels used for biomedical applications.

2.2. Synthesis of nanocellulose

Nanocellulose can be employed for enzyme immobilization, synthesis of antimicrobial agents, medical materials, environmental catalysts, biosensing, and drug delivery systems within the body for treatment and diagnosis. Depending on the material from which it is synthesized, nanocellulose can exhibit various effects, and its utilization is actively under research. Some materials are commonly synthesized using nanocellulose. Collagen is a major protein primarily found in human tissues. A synergistic blend of nanocellulose and collagen has diverse applications in the field of biomedicine, including tissue regeneration, wound healing, and the development of artificial tissues [55]. Another material is hyaluronic acid, a major biomolecule widely distributed in the connective tissues of vertebrates. Combining nanocellulose with hyaluronic acid enhances adhesion and biomolecular interactions, making it suitable for applications in artificial joints, skin regeneration, and inflammation reduction [56]. Gelatin, derived from

Table 2
Summary of various cellulosic materials for biomedical applications [39].

Composite material	Formulation	Application	Ref
CNF-hyaluronan-gelatin	Cross-linked hydrogel	3D organoid development	[40,41]
CNF surface adsorption of ODDMAC	Surface-modified film	Antimicrobial film	[42]
CNF-kymene	Aerogel	High mechanical strength scaffold	[43]
CNF-chitosan	Cross-linked hydrogel	3D cell culture scaffold, bone tissue engineering	[44]
CNF-hydroxyapatite	Cross-linked hydrogel	Bone tissue engineering	[45,46]
CNF-alginate	Composite hydrogel	Cell-laden ear and nose cartilage scaffold	[46]
CNF-carbon nanotube	Conductive hydrogel	Neural tissue engineering	[47]
CNF-polyvinyl acetate	Composite polymer	In situ biomimetic implantation	[48]
CNC-fibrin	Cross-linked hydrogel	3D scaffold for tissue culture	[49]
CNC-gelatin	Cross-linked hydrogel	3D scaffold for tissue culture	[50]
CNC-Silk fibroin-CS	Layered assembly	High mechanical strength 3D Structure for tissue culture	[51]
CNC-poly(vinyl acetate)	Cross-linked hydrogel	Transparent 3D structure with good mechanical strength	[52]
CNC-alginate	Cross-linked hydrogel	Microporous hydrogel scaffold	[53]
BNC- carbon source	Glucose	Tissue engineering, wound healing, drug delivery systems, and cell culture scaffolds	[54]
BNC- nitrogen source	Corn steep liquor	Enhanced bacterial growth	[54]
BNC- organic acids	Citric acid	pH regulation, yield enhancement, structural modification	[54]
BNC- phosphates	Na_2HPO_4	Nutrient source, structural modification	[54]
BNC- sulfates	$(NH_4)_2SO_4$	Nutrient source, modulation of nanocellulose properties	[54]

animal connective tissues, is another protein commonly used in the medical field. When combined with nanocellulose, gelatin can be applied in tissue engineering, oxidative wound healing, neural regeneration, and other biomimetic adhesive applications [57,58]. Polyvinyl alcohol (PVA), an artificial polymer widely used as a biomimetic adhesive, has also been used in conjunction with nanocellulose.

The incorporation of nanocellulose and PVA into a composite material enhances both cohesion and durability, making it extremely beneficial for diverse biomedical applications, such as tissue engineering, cell culture, and the production of medical microcapsules [59]. Chitosan is also considered a good synthetic material. Recently, a novel injectable biomaterial was developed to encapsulate preosteoblasts (MC3T3-E1 cells) to facilitate mild bone fracture repair. This biomaterial comprises cellulose nanocrystals (CNC) integrated into a tunable thermo/pH-responsive chitosan hydrogel. The system exhibits thermal sensitivity and rapidly forms a hydrogel within 7 s at 37 °C. This gelation process is attributed to hydrophobic interactions within the chitosan network and hydrogen bonding between chitosan and CNC, which play key roles in the gelation mechanism. By encapsulating preosteoblasts, this injectable biomaterial shows promise for enhancing the repair of mild bone fractures [60]. Only a few biomimetic adhesives have been used in conjunction with nanocellulose. Many other materials are available, and the combination of nanocellulose with different biomimetic adhesives has great potential for various biomedical applications.

2.3. Surface modifications of nanocellulose

Numerous reactions have been explored and specifically designed for the surface modification of nanocellulose. The primary objective behind the surface modification of nanomaterials is to introduce new functional groups or significant biological components onto the nanostructure while avoiding any potential damage to the nanoparticle itself. When it comes to modifying nanocellulose, this aspect is of utmost importance since the core and surface of the material both consist of hydroxyl groups. Hence, it is crucial to ensure that any conversions are limited to the surface functional groups only by carefully controlling the reaction parameters [61]. The surfaces of the CNCs can be modified via reacting them with various acids, alkalis, epoxides, and esters, which is especially useful for industrial and biomedical applications Fig. 3(a, b). Surface modification of nanocellulose, including cellulosic plant fibers, cellulose nanofibrils (CNF) (Fig. 3(c, d)), and cellulose nanocrystals (CNC), is commonly employed. The process of topochemical modification begins and proceeds by interacting with specific sites on the accessible surfaces of the cellulose crystals. These surfaces are abundant in highly reactive hydroxyl groups, providing an opportunity to introduce specific chemical functionalities for diverse applications of nanocellulose. Topochemical surface modification offers the ability to tailor characteristics such as adhesion properties and self-assembly, making it a highly utilized method for controlling the nanostructure properties. This approach enables the fine-tuning of nanocellulose for various novel applications [62]. Surface modification of CNC finds application in a wide range of fields, including biomedicine, drug delivery, tissue engineering, antimicrobial carriers, nano-sensing applications, polymer reinforcement, and nanocomposites [63]. Utilization of nanocellulose via enzymatic treatment is given in Table 3.

3. Fabrication of nanocellulose-based bioadhesives

Nanocellulose-based bioadhesives incorporate nanocellulose as a crucial component. Nanocellulose, derived from cellulose—the most prevalent organic polymer on our planet—can be extracted from various renewable sources, including wood pulp, plants, and bacteria. Nanocellulose-based bioadhesives offer numerous advantages, such as biocompatibility, biodegradability, renewability, and versatile adhesive properties. Due to their environmentally friendly nature and potential for customization and innovation, they show promise in various fields, including healthcare, packaging, and advanced materials. Fig. 4 schematically illustrates the use of CNCs as nanofillers for tuning adhesive properties, showcasing superior mechanical properties, tunable elasticity, and applications in drug delivery (Table 4).

3.1. Types of nanocellulose-based bioadhesives

Nanocellulose-based bioadhesives are extensively used and researched in various fields. These materials offer a diverse array of applications and variations. Some examples of nanocellulose-based bioadhesives; nanocellulose composite adhesives are [85]: Nanocellulose can be incorporated into composite adhesives by blending them with other polymers or fillers. The addition of nanocellulose enhances the adhesive properties, mechanical strength, and elasticity of the composite, and promotes the controlled release of bioactive molecules for tissue regeneration. These adhesive composites find applications in industries like packaging, construction, and automotive industries, where strong and environmentally friendly adhesives are desired. Nanocellulose-based surgical adhesives [86,87]: Nanocellulose can be modified and functionalized to create surgical adhesives that can seal wounds and promote tissue healing. These bioadhesives can be used as alternatives to sutures or staples and have potential applications in various surgical procedures, including internal tissue adhesion and wound closure. Nanocellulose-based drug adhesive patches [86,88]: Nanocellulose can be used to develop adhesive patches for transdermal

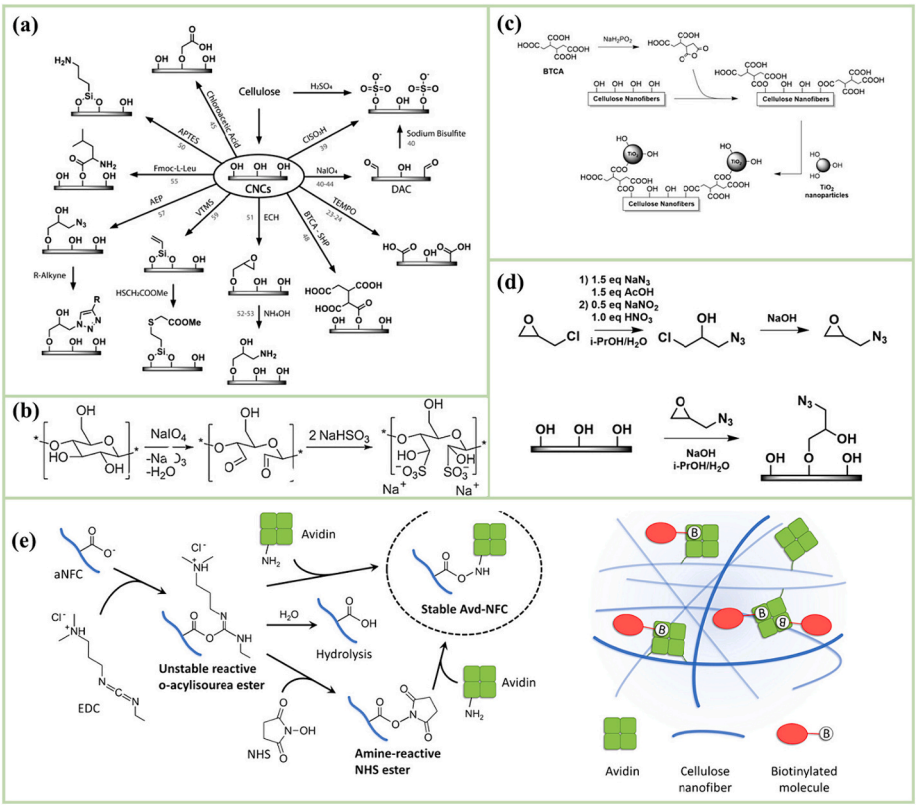
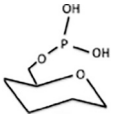
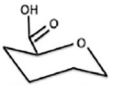
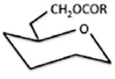
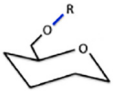
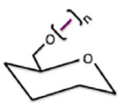


Fig. 3. Surface modification of nanocellulose using various acids, alkalis, epoxides, and esters [61]. (a) The commonly used chemical surface modification types for CNCs. (b) Surface modification of CNC by adding excess sodium periodate to obtain DAC (Dialdehyde Cellulose) (c) A schematic diagram illustrating the chemical conjugation of titanium dioxide nanoparticles onto cellulose nanofibers using the reactivity of BTCA and its anhydride derivatives. (d) Synthetic path for producing AEP and its subsequent attachment onto CNC Surfaces and surface functionalization of nanofibrillated cellulose using click chemistry approach in aqueous medium. (e) The reaction mechanism for the amine group of carboxyl groups and avidin to form a shared bond [71].

Table 3
Surface modification of nanocellulose using enzymes.

Surface modifications of nanocellulose	Enzymes	Functions	Chemical formula	Ref.
Phosphorylation	Hexokinases	Regulating protein function Transmitting signals throughout the cell		[64,65]
Oxidation	Galactose oxidases Laccases	High capacitance		[66]
Esterification	Lipases Subtilisin	Producing cleaner products than the chemical process		[67]
Click Reaction	XET EC 2.4.1.207	Assembly of (bio)chemically active cellulose surfaces Synthesis of 2D crystalline cellulose oligomers High yield and nontoxic		[68]
In situ enzymatic polymerization	Laccases Peroxodases	Environmentally friendly synthetic process of polymeric materials		[69]
Grafting/immobilization of active molecules	Grafting	High selectivity of enzymes towards the substrate No organic solvents and takes place under physiological conditions		[70]

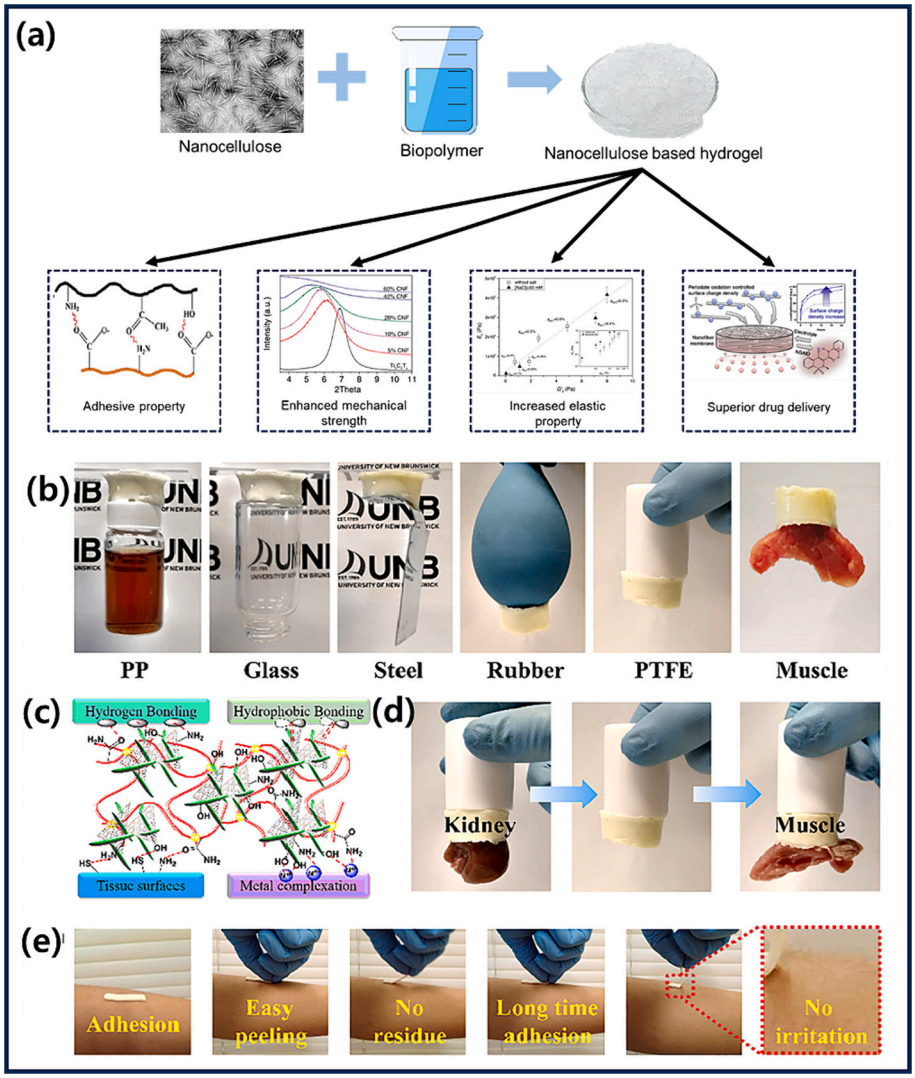


Fig. 4. Nanocellulose hydrogel advancements and applications in biomedical adhesion and controlled release. (a)Nanocellulose-based hydrogel shows better properties in adhesive [72], mechanical strength [80], elasticity [81] and controlled release of bioactive molecules [82]. [83] (b) Adhesive characteristics of the CCNC-C3N4-PAM hydrogel. (c) Mechanism of adhesion of the CCNC-C3N4-PAM hydrogel to various substrates. (d) Sustained adhesion to different organs (kidney and muscle). (e)Long-term adhesion of CCNC-C3N4-PAM hydrogel on arm skin with no evident irritation or residue. [84].

Table 4
Adhesive conjugated agents on nanocellulose.

Adhesive agents	Applications	Tested site	Adhesion strength	Ref.
Methacrylate chitosan modified by sodium alginate and polyacrylamide	Hydrogel patch sensing	Universal Testing Machine	157.5 kPa	[72]
Sodium periodate ($NaIO_4$) + ethylenediamine (EDA) + SiO_2	DAC/ AC@SiO2 adhesive	Plywood	2.05 MPa	[73]
Cottonseed meal	Eco-friendly adhesive	Plywood	2.4 MPa	[74]
Bovine serum albumin and hydroxypropyl cellulose	Bio-adhesives	Aluminum plate	10Mpa	[75,76]
Carboxymethylcellulose and CNCs	–	–	5 MPa	[76]
Mechanically fibrillated CNFs	–	–	2 MPa	[76–78]
Tempo-oxidized CNF	–	–	0.2 MPa	[76]
Lysozyme	–	–	1.5 MPa	[76,79]

drug delivery. The adhesive properties of nanocellulose allow the patch to adhere to the skin, enabling the controlled release of drugs over an extended period. This approach offers a non-invasive and convenient method for drug administration. Nanocellulose-based dental adhesives [89,90]: Nanocellulose can be incorporated into dental adhesives to improve their bonding strength and durability. These adhesives are used in restorative dentistry for bonding dental materials, such as composites or ceramics, to natural tooth structures, providing reliable and long-lasting adhesion.

3.2. *Nanocellulose-based hydrogels as bioadhesives*

Nanocellulose combines with water to form a hydrogel, demonstrating excellent adhesion properties, biocompatibility, and biodegradability. These attributes render nanocellulose-based hydrogels suitable for various biomedical applications, including wound healing, drug delivery, and tissue engineering. Notably, the characteristics of cellulose hydrogels differ from those of most polymer hydrogels due to variations in the solubility of their constitutive polymers. Unlike cellulose hydrogels, which consist of water-insoluble polymers, other polymer hydrogels are comprised of water-soluble polymers. Consequently,

upon introduction to water, these polymer gels dissolve and form solutions. In contrast, CNF, BNC, and CNC are composed of water-insoluble polymers, resulting in colloidal suspensions when dispersed in water [91]. The significance of hydrogels can be underscored in two aspects. Firstly, the critical dimensions of their constitutive units exceed those of other gel types. For instance, bundled cellulose chains can attain lengths up to microns, with diameters ranging from 5 to 50 nm [92]. Secondly, the distinct mechanisms of gel network formation have been investigated. In nanocellulose, a gel network is established through the entanglement of twisted CNFs and chemical crosslinking. Electrostatic stability additionally reinforces the structural integrity of the gel framework [93]. The formation of nanocellulose gels involves various physical interactions, encompassing ionic, hydrogen bonding, electrostatic, and hydrophobic interactions. These diverse forces play crucial roles in maintaining the structural stability of the gel network. However, the reversible interactions can be easily disrupted, leading to the breakdown of the gel structure when the intermolecular forces holding the fibers together are disturbed [94].

3.3. Hemostatic properties of nanocellulose/polymer hybrids

In instances of warfare or accidents, significant bleeding often leads to fatalities [95]. Traditional hemostatic methods, such as gauze or pressure bandages, exhibit limitations, including low efficiency, poor biocompatibility, susceptibility to infection, and inadequacy for severe bleeding [96,97]. Therefore, 3D-printed nanocellulose-based bioadhesives serve as effective replacements for conventional hemostatic methods. Moreover, the use of nanocellulose in creating a hydrogel for hemostasis through 3D printing enables the visual observation of the hemostatic process [98,99]. Nanocellulose hydrogels are typically transparent, providing a clear view of bleeding control and wound management [100]. Conventional methods for assessing hemostasis or inspecting wounds for additional treatment pose infection risks. However, incorporating materials with antibacterial properties into nanocellulose hydrogels used for hemostasis significantly reduces the risk of infection.

A pivotal aspect in achieving hemostasis involves adjusting the surface area and porous structure of the hemostatic device for the adsorption of red blood cells (RBC) and coagulation factors (Fig. 5(a-h)).

The development of 3D biopolymer-based supports, such as cellulose hydrogels produced using 3D printing technology, allows for flexible and customized production of 3D hemostatic supports through combinations of proteins, blood cells, and coagulation factors to enable rapid hemostasis [99,119]. Protein-bound 3D porous supports can mimic cell growth, facilitating strong binding to microparticle factors in the blood and providing better attachment points for essential components of hemostasis, such as platelets and red blood cells [119].

To enhance the hemostatic ability of nanocellulose-based bioadhesives, various materials must be synthesized. Fibrin, thrombin, and chitosan are commonly used for this purpose [100]. Fibrin, a predominantly blood-derived protein, plays a crucial role in clotting. A fibrin-based hydrogel can be created by enzymatically treating fibrinogen with thrombin. Fibrin hydrogels exhibit excellent biocompatibility and biodegradation properties but relatively low mechanical strength [120]. The viscosity of bioinks based on fibrinogen is low, making them suitable for inkjet 3D printers. However, in extrusion-based bioinks, fibrinogen must combine with other biopolymers to enhance bioactivity and mechanical strength after crosslinking [121]. The formation of a fibrin-based gel involves crosslinking fibrinogen with thrombin and incubating the gel at room temperature. Thrombin cleaves fibrinogen, leading to the formation of two symmetrical structures that subsequently aggregate noncovalently [122]. A typical example of a nanocellulose-based hemostat for in vivo applications is represented in Fig. 5(i-k). Additionally, integrating nanocellulose with naturally derived biopolymers, such as chitosan, can further augment attributes related to antifungal and wound-healing properties. Chitosan is well-known for its diverse advantageous biological characteristics, including hemostatic effects, promotion of wound healing, facilitation of cell culture, and drug delivery capabilities [123]. Owing to these exceptional attributes, chitosan is extensively utilized across a range of biomedical domains [124]. However, the utilization of chitosan is somewhat constrained by its inherent limitations, including poor barrier properties and relatively weak mechanical strength [125]. These limitations can be overcome by incorporating nanomaterials into the chitosan polymer matrix. This synergistic approach effectively enhances the mechanical strength and barrier properties of chitosan-based materials [126]. Nanocellulose-based bioadhesives can be combined with hemostatic materials to achieve excellent performance (Table 5).

4. 3D-printed nanocellulose-based bioadhesives

3D printing can address diverse medical research needs, including drug delivery, regenerative medicine, and functional organ replacement. Initially developed in the 1990s through laser-based printing for cell fabrication [64], 3D printing approaches can be broadly classified into four main categories: extrusion-based, droplet-based, SLA (Stereolithography)-based, and laser-assisted printing [65]. Additionally, infinite effects can be achieved through various materials, such as alginate, collagen, chitosan, and gelatin methacrylate (GelMA) [65]. With 3D printing technology, it is currently feasible to fabricate tissues, organs, bones, and cartilage, thereby enabling organ transplantation. It can also be employed for creating hydrogels or bioadhesives for skin and internal organ treatment applications. Fig. 6 schematically illustrates the various types of 3D printing technology used in biomedical engineering.

4.1. Mechanism of 3D printing

Broadly, 3D printing relies on the precise layer-by-layer arrangement of biological components, biochemicals, and viable cells. This is achieved through spatial control, ensuring the accurate placement of functional constituents within the constructed three-dimensional (3D) structure. The mechanism of 3D printing involves several key steps. Firstly, the desired structure is designed using software to create a digital model. Pre-processing then transforms the digital model to be compatible with the bioprinter. A bioink is prepared as a printable material containing cells, such as stem cells or specific cell types, along with a biocompatible scaffold material or hydrogel. Next, following the predefined instructions of the digital model, the bioink is layered and stacked using various bioprinting techniques, such as extrusion-based, droplet-based, or laser-based methods, with precise placement in the desired pattern and location. Subsequently, depending on the materials, curing or crosslinking can be performed using methods such as light, temperature, or chemical reactions. Once the structure is fully formed, it is typically cultured in a controlled environment to promote cell growth and differentiation, facilitating maturation [140].

4.2. 3D printing methods

4.2.1. Inkjet-based 3D printing

Inkjet-based printing is preferred owing to its precise deposition accuracy, high printing resolution, and ability to maintain cell viability. This offers excellent control over the deposition of bioinks with high precision. However, this is limited when it comes to using high polymer and cell concentrations because of the resulting high viscosity [141]. In the late 1800s, Rayleigh studied the instability of jets and the phenomenon of a continuous stream of water breaking into droplets. His groundbreaking work served as the foundation for the exploration of inkjet technologies. Building on this theory, continuous inkjet printing technology was developed, enabling the generation of high-speed droplet flows with adjustable droplet sizes [142]. Subsequently, commercialization was achieved through extensive research conducted

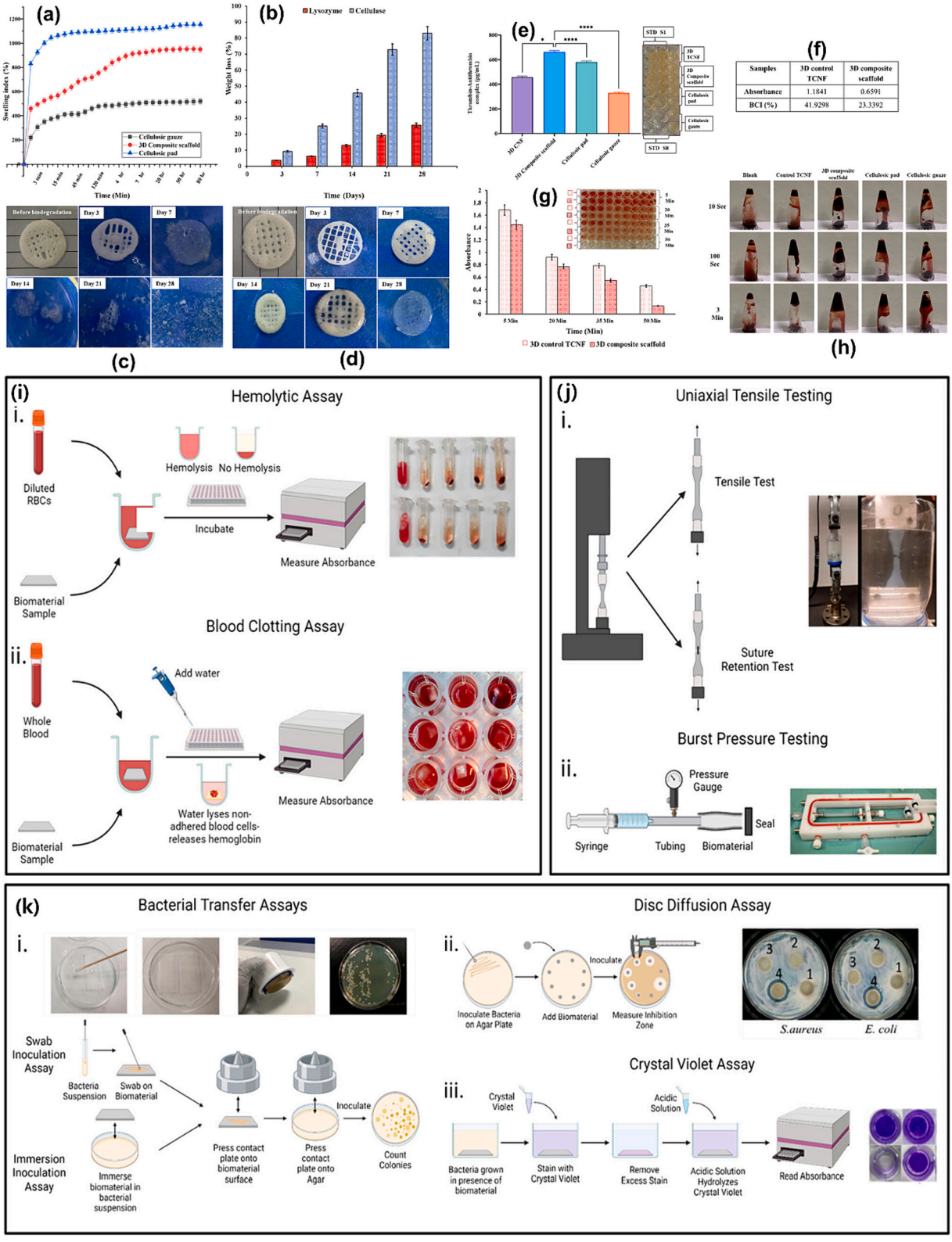


Fig. 5. [101] (a) Swelling characteristics [102–108]. (b) Weight loss. (c) Vitro biodegradation in cellulase and (d) lysozyme [109]. (e) Temporal thrombin production [110]. (f) Table of blood clotting index (BCI) [111]. (g) Whole blood clotting kinetics. (h) blood coagulation. Different testing methods to evaluate biomaterial-blood interactions and applications of modified BNC for hemostatic membranes [112]. (i) (i) Hemolysis test to assess the extent of red blood cell lysis by a biomaterial [113]. (ii) Blood clotting assay to determine a biomaterial's thrombogenicity. (j) (i) Uniaxial tensile testing to evaluate tensile strength and suture retention strength of a biomaterial [114]. (ii) Burst pressure testing is particularly crucial for assessing the strength of vascular grafts [115]. (k) (i) Bacterial transfer assays, including swab inoculation and immersion inoculation, to assess bacterial adhesion and transmission by a biomaterial [116]. (ii) Disc diffusion assay to determine a biomaterial's antibacterial properties [117]. (iii) Crystal violet assay to evaluate biofilm growth and formation on a biomaterial [118].

Table 5
Hemostatic materials that can be mixed with nanocellulose and their effects.

Hemostasis substances	Type of NC	Applications	Blood clotting time	Ref
Andrias davidianus (SSAD)	CNC, CNF	Injectable hemostatic sponge	30(s)	[121]
Chitosan + Oxidized regenerated cellulose (ORC)	Oxidized bacterial nanocellulose (OBC)	OBC/COL/CS sponge	86(s)	[99]
Thrombin+silk fibroin	TEMPO-oxidized cellulose nanofiber (TOCN)	TOCN-SF5-Th scaffolds	100(s)	[127]
Oxidized BNC	BNC	BNC oxidized membrane dressings	Within 25 (min)	[128]
Antibacterial lawsone + chitosan	tempo-oxidized nanocellulose (TOCN)	Freeze-dried wound dressings	60(s)	[100]
Crosslinking with Ca^{2+}	CNF	Advanced dressings with tuned wound healing properties.	6–8(min)	[98]
γ-glycidoxypropyltrimethoxysilane (GPTMS), chitosan, vinyl trimethoxy silane (VTMS)	CNF	hemostatic wound dressing construct sponge	83 ± 15(s)	[129]
Cinnamon extract	CNC	ciMFC/cCNC composite sponge as hemostatic wound care dressing	45(s)	[130]
Carboxylate sodium	ORC	Woven/Nonwoven	102–138(s)	[131]
N,O-carboxymethyl chitosan	ORC	Woven/Nonwoven	90(s)	[132]
CNT	ORC	Woven/Nonwoven		[133]
Thrombin	ORC	Woven/Nonwoven	80(s)	[134]
Thrombin+silk(TOCN-SF5-Th)	Oxidized cellulose nanofiber	Sponge	100(s)	[127]
Alginate	Oxidized cellulose nanocrystal	Film		[53]
Gelatin (Gel-NaORC1)	ORC	Powder	120(s)	[135]
Polyethylene glycol/zinc oxide (ZnO in TOCN-10%PEG)	Oxidized cellulose nanofiber	Sponge	150(s)	[136]
ci(*Cinnamomum*) MFC(microfibrillated cellulose)	carboxylated cellulose nanocrystals	Sponge	~45(s)	[130]
Montmorillonite	Ca2+ crosslinked Carboxyl nanocellulose	Sponge	60–270(s)	[137]

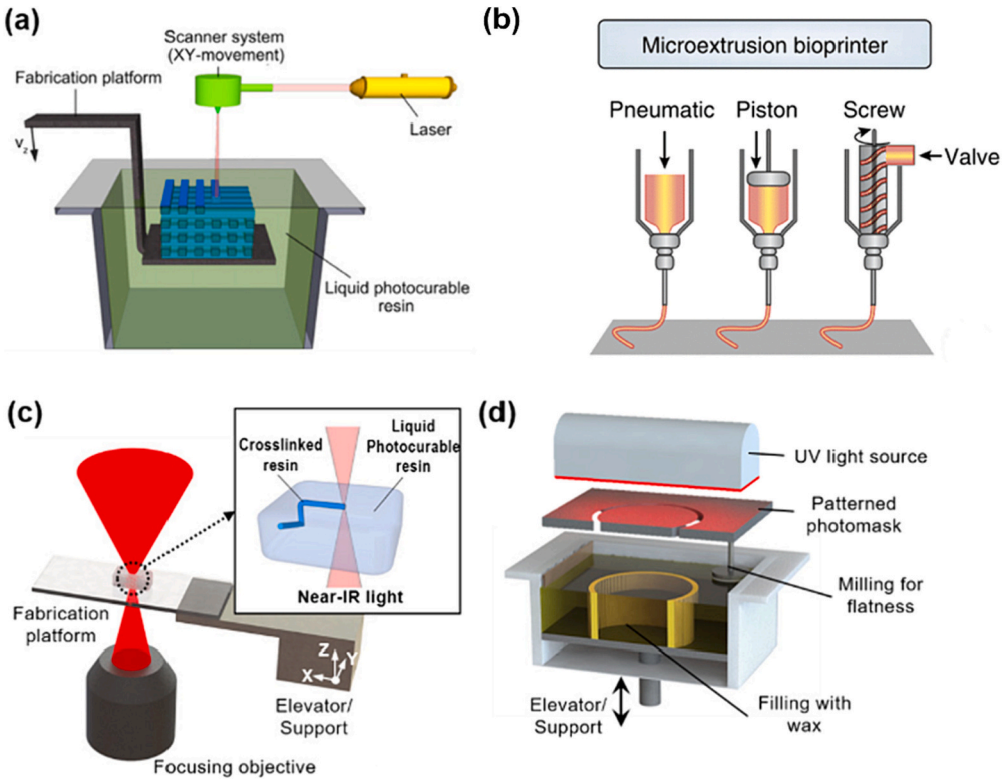


Fig. 6. Types of various 3D printing technology for the fabrication of nanocellulose-based hydrogels. (a) Laser-assisted 3D printing [138], (b) Extrusion-based 3D printing [139], (c, d) Light-assisted 3D printing [138].

by various major corporations. As inkjet printing technology advanced, the nozzle diameter was reduced to the microscale, enabling printing with ultrafine precision.

The droplet size achieved through inkjet printing is comparable to the size of the cells, which has paved the way for cell printing. By leveraging these unique capabilities, inkjet bioprinting has been established in the field of life sciences since the latter half of the 20th century [142]. There are several methods for inkjet 3D printing: (1) Thermal

inkjet technology, which involves heating the local ink to generate hot bubbles and expelling liquid droplets. It utilizes thermal energy to create pressure for droplet ejection. (2) Piezoelectric inkjet technology: This method uses variations in piezoelectric actuators to squeeze and discharge ink droplets. Piezoelectric materials change shape when an electric field is applied, allowing precise control over droplet formation. (3) Electrostatic inkjet technology: This method utilizes variations in electrostatic plates to squeeze and expel ink droplets. The ink was ejected as droplets by manipulating the electrostatic forces. (4) Electrohydrodynamic jet technology: This method uses high-voltage electric fields to create liquid jets using Taylor cones. The electric field induces the formation and ejection of liquid droplets [142]. These methods represent the different techniques employed in inkjet bioprinting, each with its own advantages and applications. The characteristics of bioinks, such as rheological behavior, mechanical strength, temperature sensitivity, and crosslinking capabilities, are key factors determining their suitability for implant applications. Natural polymers such as fibrin, alginate, and gelatin have been extensively investigated and proven to be exceptional biomaterials for 3D inkjet printing, demonstrating their potential as bioinks. Furthermore, gelatin methacrylate (GelMA) bioinks, synthetic polymers, and composite bioinks have also demonstrated excellent performance as bioinks [143]. In a recent study [144], a therapeutic hydrogel was developed using an inkjet-based 3D printing method that combines bacterial nanocellulose and gelatin. They utilized dBNC as a dialdehyde-infused nanoreinforcement to crosslink and fortify a Gel/CMCh amino-filled framework by creating imine bonds. The hydrogel demonstrated shear-thinning, self-restoring, and self-repair characteristics. Furthermore, they assessed the printability and the mechanical and structural attributes of the prepared nanocomposite double-network (ncDN) gelatin hydrogels. The biocompatibility of the ncDN gelatin hydrogel was examined to determine its suitability as an ink for tissue engineering scaffolds. The thermo-mechanical traits, 3D printability, and biocompatibility of our hydrogel indicate its potential utility as an additive-generated scaffold for tissue engineering, and it is noteworthy that this self-healing ncDN gelatin hydrogel has not yet been employed as an ink for 3D printing [144].

4.2.2. Extrusion-based 3D printing

A commonly used method such as inkjet-based bioprinting is extrusion-based 3D printing. Extrusion-based 3D printing involves loading high molecular weight substances into a syringe and applying pressure to create polymeric scaffolds or hydrogels [145]. Extrusion-based printing may be pneumatic, piston, or screw-valve. In extrusion-based bioprinting, many bioinks exhibit shear-thinning behavior, where their viscosity decreases under shear stress. However, at high nozzle speeds, the viscosity of bioinks can be further reduced, resulting in increased surface tension. Typically, when filaments are deposited at high flow rates, they tend to have lower viscosity and display smoother surfaces upon exiting the nozzle owing to their higher surface tension [146]. Several approaches can be employed to manipulate the surface tension and energy of a substrate to enhance or restrict bioink spreading. One method involves treating a printed substrate with a hydrophobic material that creates a low-energy surface. This treatment prevents bioinks with a low surface tension from spreading excessively. The surface treatment of a substrate has proven to be an effective technique for modulating its surface energy [147]. High-viscosity bioinks can be employed to minimize spreading. However, bioinks with high viscosities are not suitable because of their potential negative impact on cell viability caused by the high extrusion pressure required. Additionally, faster crosslinking or drying rates can enhance the printing resolution and improve the overall printability of bioinks [144]. Faster crosslinking or drying rates can be accomplished by adjusting the crosslinking mechanism of the scaffold or by increasing its porosity (as higher porosity facilitates faster crosslinking). Another critical factor to consider in printability studies is the type of the needle used. The interaction between the bioink and needle should consider the surface energy of the needle [148]. Extrusion-based bioprinting offers several advantages over other 3D bioprinting techniques. They can process a broad range of biomaterials and cell types, thereby providing versatility in material selection for specific applications. Additionally, extrusion-based bioprinting causes less process-induced cell damage and preserves cell viability during printing. This makes it the preferred choice for applications in which maintaining cell integrity is crucial [149,150]. Extrusion-based bioprinting bioinks typically consist of naturally derived materials such as collagen, fibrin, gelatin, hyaluronic acid, chitosan, alginate, and agarose. Additionally, synthetic biomaterials, such as polycaprolactone, polyethylene glycol, and polylactic acid, are commonly used in extrusion-based bioprinting. The broad selection of biomaterial options in extrusion-based bioprinting facilitates the customization of bioinks to meet the specific demands of tissue engineering and regenerative medicine [151]. These bioinks, typically composed of a combination of high-molecular-weight polymers, exhibit shear-thinning properties, making them suitable for extrusion through printing nozzles. Furthermore, bioinks can be crosslinked to provide stability and structural integrity [152]. By precisely adjusting the biophysical and mechanical properties of bioinks and incorporating functional growth factors or drugs, extrusion-based bioprinting approaches can effectively promote cell growth and guide the cell fate for targeted regeneration applications. This capability opens up possibilities for tailored and controlled tissue engineering strategies [153,154]. In one particular study, a high-viscosity hydrogel with enhanced printability of this hydrogel system was developed by incorporating cellulose nanofibers and employing covalent cross-linking after extrusion through a bioorthogonal reaction. This innovative approach facilitates convenient bioprinting while supporting cell proliferation [155].

4.2.3. Light-assisted 3D printing

Recently, there has been a significant surge in the interest in stereolithographic 3D printing using digital light processing (DLP). This cutting-edge technology enables the production of complex structures with remarkable precision and rapid printing pace. Owing to its ability to achieve high spatial resolution, DLP-based stereolithographic 3D printing has attracted considerable attention and has been widely explored in various fields, including manufacturing, research, and development [156]. Despite the growing popularity of stereolithographic 3D printing via digital light processing (DLP), the adoption of bioinks has been relatively low compared to other bioprinting methods that utilize hydrogel inks. DLP printing requires photosensitive polymers as bioinks, resulting in a limited selection of materials. Currently, only a few options such as PEGDA, GM-HA, and GelMA have been successfully employed as bioinks for DLP printing. This highlights the need for further research and development to expand the range of materials applicable to DLP bioprinting [157]. Light-based 3D printing can be categorized as laser-or ultraviolet (UV) light-based printing. The printing technique was operated in an upward or downward configuration. DLP technology offers numerous benefits compared to the aforementioned methods [158]. First, the projection technology enables polymerization to occur sequentially, layer-by-layer. As a result, the printing time is accelerated (~30 min, $mm^3 \cdot s^{-1}$) compared to other printers that utilize a line-by-line approach (such as extrusion, injection, and laser micro-stereolithography) [159]. Irrespective of the complexity or size, each layer required the same amount of time for printing and depended solely on the thickness of the structure. This rapid printing process, devoid of nozzles, leads to remarkably high cell viability (85–95 %). Digital micromirror devices (DMD) contain multiple mirrors that tilt individually in an on/off state and serve as dynamic masks. This feature allows for achieving a resolution as fine as 200 μm [160]. A novel, fully bio-based resin formulation for DLP printing was developed in a particular paper using biocompatible polymers and extremely low concentrations of fluorescent TEMPO-CNC (T-CNC). The 3D bioprinted GPCD hydrogel they developed was tested for biocompatibility with “skin” and “vascular” prototypes of the entire thickness, demonstrating

higher expression of cell differentiation markers. They asserted that the developed GPCD resin is nontoxic, exhibits ideal rheological properties for DLP printing, and can successfully replicate the microenvironment of bioinspired skin and vascular models [161].

5. Application of nanocellulose-based bioadhesives

5.1. Skin tissue regeneration

The development of scaffolds for skin tissue engineering and the subsequent evaluation of the human skin cell response were carried out by incorporating cellulose nanocrystals (CNC) into hydrogels based on polyvinyl alcohol (PVA) [162]. Polyvinyl alcohol (PVA) is well known for its biodegradability and biocompatibility, making it an ideal choice for various biomedical applications. The three-dimensional (3D) configuration of PVA hydrogels has attracted considerable interest in the field of tissue engineering because it holds promise for replicating the intricate architecture of extracellular matrices found in biological tissues [163]. The incorporation cellulose nanocrystals (CNCs) into the PVA hydrogels had a noticeable impact on the porous structure of the resulting nanocomposites. All nanocomposites composed of PVA/CNC demonstrated a prominently porous structure with irregular and interconnected pores, varying in size from around 37 to 164 μm. In addition to porosity, the addition of CNCs led to significant improvements in

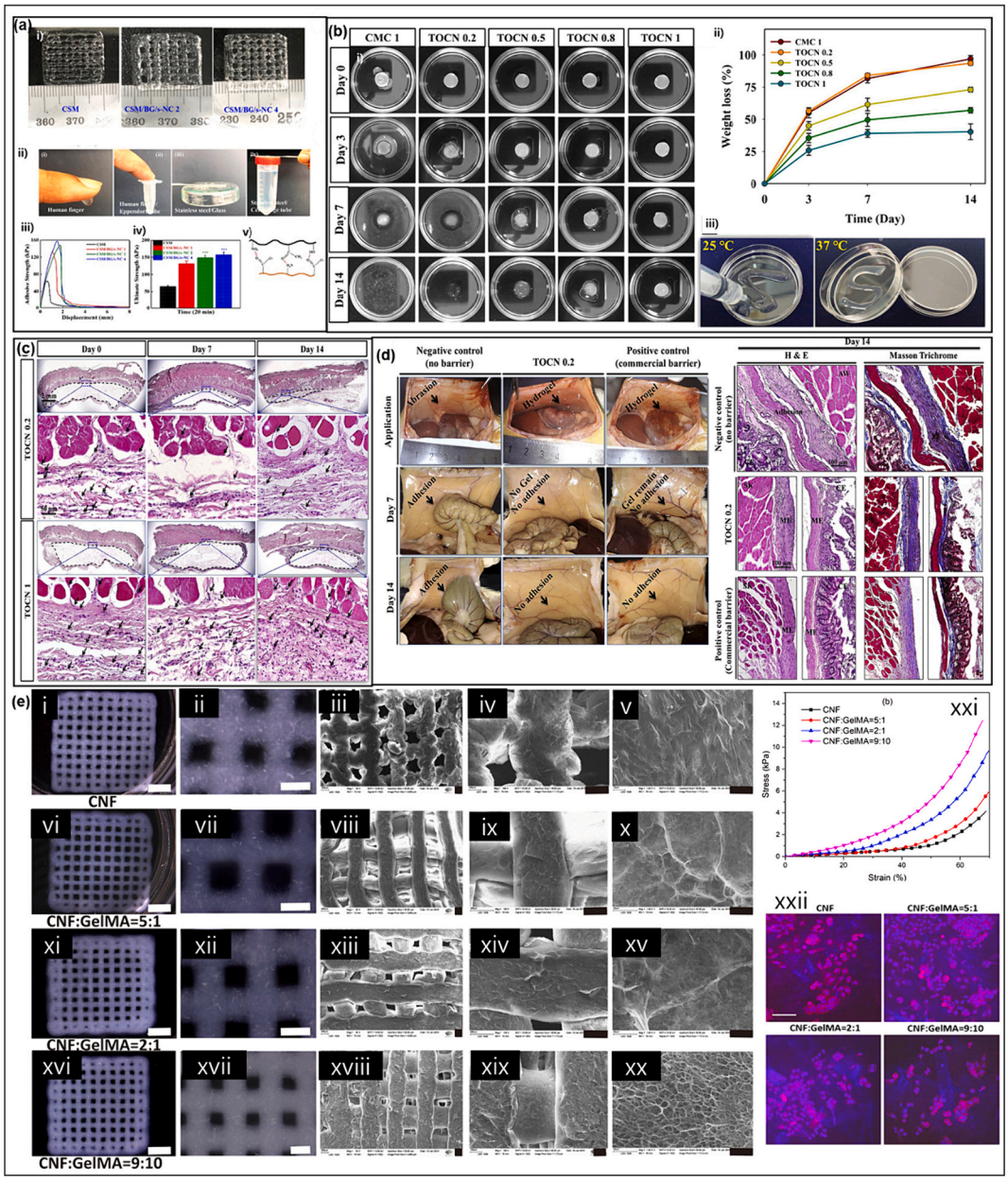


Fig. 7. 3D printed nanocellulose-based adhesive hydrogels for skin tissue engineering. (a) 3D-printed hydrogel images, visual representations of hydrogels adhered to various surfaces, and adhesive strength-displacement curves [72]. (b) i) The significant degradation of CMC 1, TOCN 0.2, TOCN 0.5, TOCN 0.8, and TOCN 1 hydrogels in SBF at 37 °C. ii) The weight loss of hydrogels in simulated body fluid (SBF) at 37 °C showed an inverse relationship with the concentration of TOCN. iii) Sol-gel transition was observed for TOCN 0.2, TOCN 0.5, TOCN 0.8, and TOCN 1 hydrogels. (c) The in vivo biodegradation and biocompatibility of the hydrogels in rats. (d) The effective anti-adhesion properties of TOCN 0.2 hydrogel. The negative control group (normal saline) demonstrated increased adhesion by day 14 compared to the positive control group (commercial gel) [167]. (e) SEM images of scaffolds printed with CNF hydrogel and CNF/GelMA hydrogel inks (i ~ xx), representative confocal images (xxi, xxii) [147].

various properties, including mechanical strength, thermal stability, and swelling behavior. Remarkably, PVA/NCC scaffolds with NCC contents of 4 wt% and 6 wt% displayed significantly higher equilibrium swelling ratios, reaching 853 % and 850 % respectively. Furthermore, an in vitro assessment of cytotoxicity and cell culture conducted with PVA/CNC nanocomposites containing 10 % CNC demonstrated no cytotoxic effects, as human fibroblast skin cells exhibited robust attachment, proliferation, and infiltration within the scaffold. These findings underscore the biocompatibility and favorable cellular response of PVA/CNC nanocomposite scaffolds [162]. Hydrogel constructs comprising gelatin,

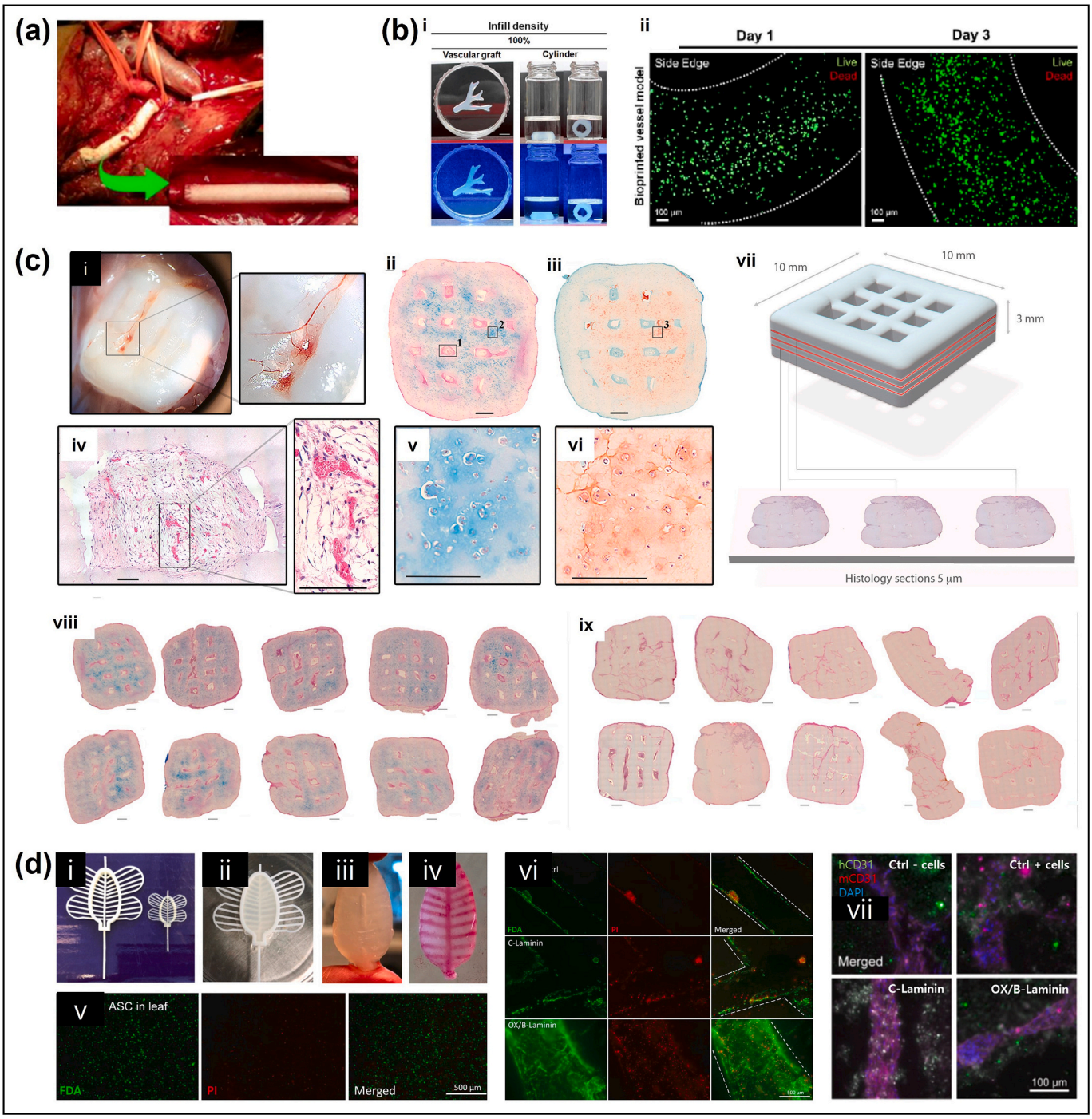


Fig. 8. Biochemical processes and applications of nanocellulose composites. (a) In a patient diagnosed with Multiple Endocrine Neoplasia 2B (MEN 2B), vascular prostheses composed of nanocellulose-polyurethane were implanted between the brachiocephalic trunk and the right common carotid artery [173]. (b) i) Digital photographs of 3D printed GPCD hydrogels under visible and ultraviolet (UV) light, indicating the fluorescent nature of the hydrogels. ii) representative fluorescent images of the surface and cross-sectional views. [161] (c) i) Macroscopic view of a representative cell-containing construct. ii) and iii) Core histological sections were stained with ABvG and Safranin-O. iv) Magnified image of grid holes containing blood vessels and erythrocytes. v) Chondrocytes surrounded by GAGs (cloudy blue) in the extracellular matrix. vi) Proteoglycans (cloudy orange) in the extracellular matrix surrounding the chondrocytes. vii) 3D printing model. viii) and ix) In some of the cell-free control groups, the shape and grid structure were lost. [174] (d) i) ~ iv) Fabrication of a mold for casting a leaf. v) Viability of the ASCs in the leaf device. vi) Evaluate of endothelialization and cell viability in the different experimental groups at day 14. vii) Transplantation beneath the skin, retrieval, and immunofluorescent staining of the semi-circular scaffolds. [175].

hyaluronic acid, and cellulose nanocrystals were synthesized using a freeze-drying method. The prepared hydrogels were evaluated for their ability to facilitate the attachment, growth, and proliferation of fibroblast cells. Each component of the hydrogel system serves a distinct function owing to its unique characteristics. Gelatin (GA) creates an environment that closely resembles the extracellular matrix, providing a suitable substrate for cell adhesion, growth, and proliferation [164]; Hyaluronic acid (HA), renowned for its remarkable ability to bind water, plays a vital role in tissue hydration and promotes effective wound healing without leaving noticeable marks [165]; Cellulose nanocrystals (CNC) embedded within the hydrogel act as reinforcing agents, effectively enhancing its properties. They play a critical role in influencing the rheological behavior and swelling characteristics of hydrogels. The GA-HA-CNC hydrogels display a smooth surface with pore sizes ranging from 80 to 120 μm, providing ample volume and surface area for cell growth. To evaluate the ability of the hydrogels to support cell viability and growth, NIH-3 T3 cells were cultured in various hydrogel compositions, including GA, HA, HA-CNC, GA-CNC, GA-HA-CNC, and GA-HA. The results revealed that the GA-HA-CNC hydrogels exhibited significantly higher cell viability than the other hydrogel formulations at 1, 4, and 7 d. This positive outcome can be attributed to the combined effects of the three constituents, which facilitate the diffusion of tissue fluids and nutrients, and create an optimal environment for cell survival and growth. Moreover, the GA-HA-CNC hydrogel provided a similar extracellular matrix environment, minimizing foreign body response. Based on these findings, it can be inferred that the GA-HA-CNC hydrogel has significant potential for use in skin wound treatment, offering promising prospects for wound repair applications [7,166]. Examples of nanocellulose-based bioadhesives used for skin tissue regeneration are shown in Fig. 7.

5.2. Blood vessel reconstruction

The intricate network, known as the cardiovascular system or blood circulatory system, comprises the heart, heart valves, and blood vessels, including arteries, capillaries, and veins. Dysfunction or abnormalities in these organs can lead to various cardiovascular diseases. Synthetic grafts have emerged as potential treatment options for some of these conditions. The materials used in the fabrication of synthetic grafts or implants must possess specific characteristics such as oxygen uptake, response to shear stress, anticoagulation properties, and biocompatibility. [168]. Because of its biocompatible and immunocompatible properties, nanocellulose is a promising candidate for the advancement of various cardiovascular devices, including artificial blood vessels, heart valves, aortic grafts, and prostheses. In a previous study conducted by Millon and Wan [169], a nanocomposite of cellulose nanofibrils (CNF) and polyvinyl alcohol (PVA) was developed, which exhibited exceptional mechanical strength, comparable to that of natural heart valves and aortas (Fig. 8(a,b)). In another notable study, scientists successfully fabricated a tubular architecture using bacterial nanocellulose (BNC), which demonstrated remarkable capabilities to promote the attachment and growth of HUVECs (human umbilical vein endothelial cells), smooth muscle cells (SMCs), and fibroblasts. This significant achievement signifies the potential suitability of BNC-based tubular structures for the development of artificial blood vessels [170]. Until now, several nanocellulose-based products, such as BASYC®, Securian®, and SyntheCel®, have been successfully brought to market for their use as cardiovascular implants [171,172]. Applications of nanocellulose-based adhesive hydrogels for blood vessel reconstruction and hemostasis are shown in Fig. 8(c,d).

5.3. Visceral tissue regeneration

Although the global demand for organ transplantation is growing rapidly, there is a significant shortage of available organ donors. To address these challenges, 3D bioprinting technologies have been actively researched and developed. In tissue engineering, 3D bioprinting is a widely used and advanced technique. It combines the principles of materials science and biology to fabricate frameworks for organs and tissues. This technology offers great potential for the development of functional and customized organ replacements [176]. The primary aim of additive manufacturing in tissue engineering is to restore and regenerate damaged tissues or organs by closely replicating the intricate structure and environment of native biological tissues, which is known as the cellular niche. In this process, it is necessary to create connections between the cells, scaffolds, and growth factors. These scaffolds provide supportive structures for cell growth and development in the presence of growth factors [176]. Considering the requirements for hernia repair implants, the bio-based polymer bacterial nanocellulose (BNC) can be regarded as an excellent choice for enhancing tissue application [177]. BNC produced through biotechnology are insoluble networks of nanocellulose fibrils that can retain a significant amount of liquid while demonstrating outstanding mechanical properties [178]. Because of these characteristics, BNC have a growing range of applications in the field of biomedicine, particularly in wound dressing and drug delivery [178]. Additionally, BNC is emerging as a high-performance substitute for addressing various issues for which non-biodegradable implants are preferred. Recently, a combination of BNC with PP-meshes meshes was described as a hybrid biomaterial for soft tissue reinforcement, showing low adhesion properties in vitro [179]. In a study comparing the BNC and PP meshes, Zharikov et al. found that BNC-implanted dogs exhibited fewer adhesions and no infections [180]. However, that study used wet native BNC pellicles and did not conduct mechanical tests. In a particular experiment, Rauchfuß et al. conducted two tests to implant bioink into the abdominal cavity of rats [181]. They observed the formation of adhesions, along with minor tissue reactions. Mechanical testing of the BNC after its removal showed variable results, suggesting the need for further investigation of its mechanical properties for hernia repair. Although these studies are preliminary, they demonstrated the absence of significant postoperative complications and emphasized the untapped potential of BNC in the field of herniology [178]. Application of 3D printed nanocellulose-based hydrogels for visceral tissue regeneration is shown in Fig. 9.

5.4. Bone tissue regeneration

BNC, a non-toxic biopolymer, have demonstrated minimal inflammation and toxicity at the genetic and cellular levels, even with long-term use. Consequently, there is a growing interest in utilizing BNC-based scaffolds for diverse medical purposes. One notable application is commercially available BNC-based wound dressings. Additionally, BNC implants are currently undergoing clinical studies as an innovative approach to address unresolved challenges in regenerative medicine [183]. Research has indicated that BNC-based scaffolds play crucial roles in bone and cartilage regeneration [126], including drug delivery systems [184,185], biosensing platforms, diagnostic tools [186], and several other areas [187,188]. The incorporation of nanomaterials into BNC offers a practical solution for addressing various clinical issues. Nanocellulose has versatile applications as a wound-dressing biomaterial, broad-spectrum antimicrobial agent, surface disinfectant, and nano drug for treating various diseases [189]. Nanocellulose holds promise in the field of bone regeneration because it can be utilized as a bone substitute, provides a matrix for impregnated nanomaterials, and acts as a drug carrier for targeted delivery in bone-related therapies [190]. An example of utilizing nanocellulose in bone regeneration is bisphosphonate-modified nanocellulose (pNC). This material can be used as a substitute to stimulate bone regeneration. pNC is an injectable bone material that regulates osteoclast formation and enhances osteoblast differentiation. Its unique properties make it a promising candidate for the treatment of various bone diseases [191]. Despite the relatively lower mechanical strength of nanocellulose compared to that of natural bone tissues, it can be enhanced through methods such as cross-linking.

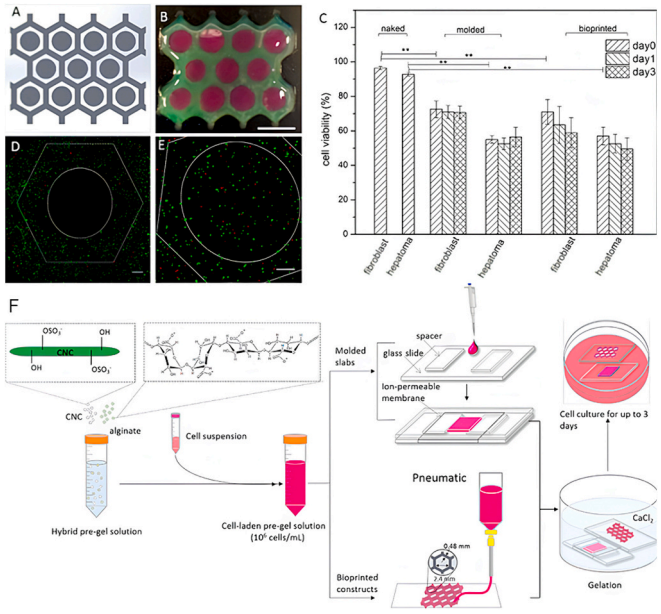


Fig. 9. Engineering liver-mimicking tissues with 3D-printed nanocellulose hydrogel. (A) Schematic bird's-eye view of the liver-mimicking engineered tissue constructs. (B) 3D printed structures using bioink 20/40, with the distinction between fibroblast-laden bioink (green) and hepatoma cell-laden bioink (purple) made using food dyes. (C) Statistical analysis of cell viabilities (proportion of live cells to total cell population) for fibroblast and human hepatoma cells on days 0, 1, and 3. (D&E) Representative fluorescent images showing live/dead cells in bioprinted structures, with (D) fibroblast-only and (E) fibroblast combined with human hepatoma cells. The dashed lines represent the boundaries of the designed structure. Calcein-AM and ethidium homodimer-1 stains were used to visualize live (green) and dead (red) cells. Scale bars are 5 mm in (B) and 250 μm in (D) and (E). (F) The 3D printing process is used to create a liver-like structure using alginate/NCC hybrid bioink. [182].

One study utilized sulfuric acid hydrolysis to develop mechanically stable scaffolds composed of crosslinked cellulose nanocrystals (CNCs). These scaffolds exhibited notable compressive strength and porosity and demonstrated in vivo osteoinductivity. They successfully facilitate bone formation at the site of injury [192]. Moreover, nanocellulose can be modified by incorporating anti-osteosarcoma metals or nanoparticles, such as selenium, strontium, and arsenic nanoparticles. This enables nanocellulose to serve as both a drug carrier and substitute for damaged bone tissues, providing potential therapeutic advantages [190]. Selenium and arsenic have been extensively studied for their abilities to combat cancer. When combined with hydroxyapatite, selenium has demonstrated effectiveness against osteosarcoma. Further studies are needed to explore the potential of As and Se in the treatment of various types of cancers [190,193]. Consequently, the integration of these metals with nanocellulose could enhance their applications in the treatment of bone-related disorders [194]. Application of 3D printed nanocellulose-based polymer hydrogels in bone tissue engineering is shown in Fig. 10 (a-e).

5.5. Cartilage regeneration

Native cartilage is a resilient tissue with a limited ability to repair itself and is prone to failure of the subchondral bone during excessive impact or trauma. The current surgical options for osteochondral repair can be uncomfortable and lead to persistent pain. Hydrogels with properties resembling the extracellular matrix (ECM) of native cartilage have emerged as potential alternatives for engineering skeletal tissues. Therefore, they have been proposed as an alternative therapeutic strategy. The combination of crosslinked sodium alginate and nanocellulose has recently gained attention in cartilage tissue engineering, particularly for applications involving articular and nasal reconstruction [195]. The chondrogenic capability and biocompatibility of these composite hydrogels were investigated through in vitro and in vivo studies using bacterial nanocellulose [196]. In a recent study, Müller et al. employed 3D bioprinted hydrogels composed of alginate, nanocellulose, and bovine articular chondrocytes to investigate their performance. This study demonstrated exceptional cell viability, robust proliferation, and substantial deposition of type II collagen after 28 d of culture [197]. Nguyen et al. conducted a study in which composite hydrogels were utilized as scaffolds to facilitate the differentiation of human-induced pluripotent stem cells (iPSCs) into chondrogenic lineages. These findings demonstrated a notable increase in the expression of RNA markers associated with chondrogenesis and matrix deposition. This enhancement was further validated by histological staining and immunohistochemical analysis after a 5-week differentiation period [198]. In a study conducted in 2015, Martínez-Avila et al. demonstrated the in vivo formation of new cartilage using co-cultures of human nasoseptal chondrocytes and bone marrow mononuclear cells. The cells were embedded in bilayer hydrogels composed of alginate and nanocellulose [199]. In summary, the implanted constructs, placed subcutaneously in nude mice, elicited a non-pathological foreign body reaction. Eight weeks after implantation, the constructs demonstrated significant deposition of proteoglycans and type II collagen. Additionally, an increase in the instantaneous modulus was observed, indicating improved mechanical properties of the constructs [195]. Fig. 10(f-j) shows examples of nanocellulose-based 3D printable hydrogels for cartilage tissue regeneration.

5.6. Muscle tissue regeneration

Nanocellulose-based bioadhesives can be used for muscle therapy in various ways. These adhesives can be applied to the surface of injured muscle tissue to enhance the bonding between the tissues. This can improve the healing rate of the muscle tissue and support the regeneration process. Nanocellulose can be used to deliver functional growth factors into cells (Table 6). When incorporated into a nanocellulose matrix, growth factors or cells can be stably supplied to the tissue, promoting recovery and regeneration of muscle tissue. Nanocellulose-based bioadhesives are being developed in various forms, such as biotissues and functional nano-patches, and have great potential in the field of muscle therapy and regeneration. These adhesives can be used to treat muscle injuries, tissue transplantation, and regeneration. Further research and development are expected to lead to advancements in this field [200]. Examples of 3D-printed nanocellulose-based hydrogels for muscle tissue regeneration are shown in Fig. 10(k-r).

6. Summary and future outlook

This study explored the potential of nanocellulose-based tissue adhesives for rapid hemostasis and effective wound healing. Furthermore, the application of 3D printing approaches in the manufacturing process of these adhesives was examined. By utilizing nanocellulose as the primary material, biodegradable adhesives that minimize adverse effects on the body can be developed. Moreover, the incorporation of nanocellulose into bioadhesives opens up possibilities for a wide range of applications in the medical field including surgery, regenerative medicine, and tissue engineering. Looking to the future, further research and development are warranted to enhance the performance and functionality of nanocellulose-based tissue adhesives. This includes optimizing the formulation, investigating long-term effects and biocompatibility, exploring novel fabrication techniques, and conducting preclinical and clinical studies to validate their efficacy and safety. By advancing the field of nanocellulose-based tissue adhesives, we anticipate improved patient outcomes, reduced surgical complications, and accelerated wound-healing processes. Additionally, the use of nanocellulose-based tissue adhesives offer the advantages of cost-effectiveness and

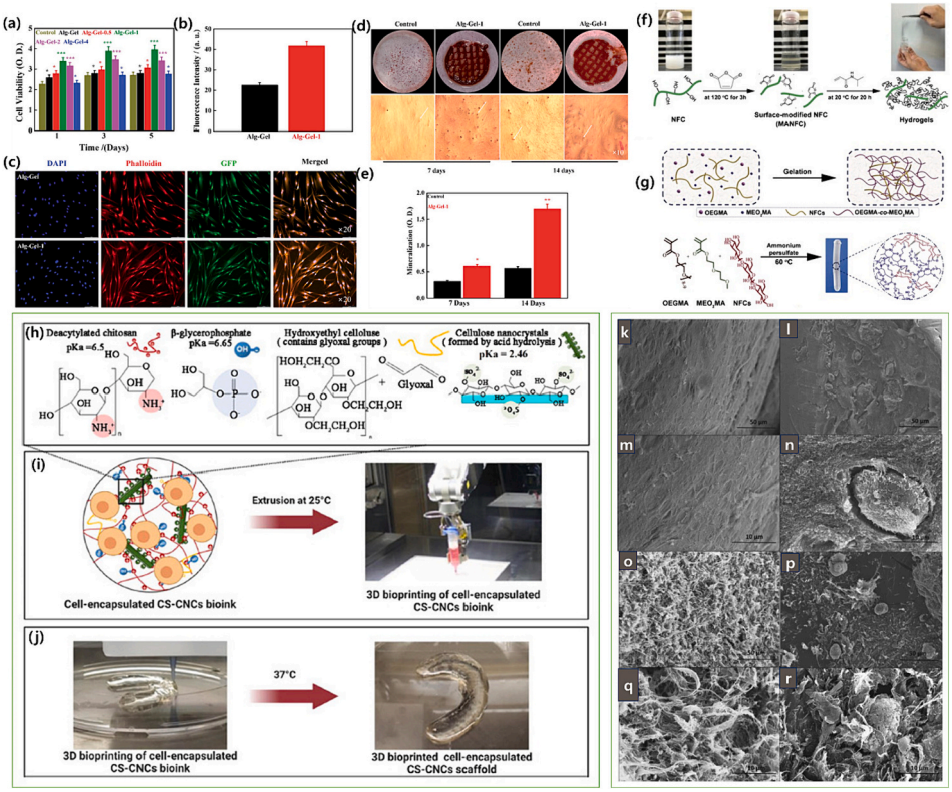


Fig. 10. Examples of 3D printed nanocellulose and its composite hydrogels for bone tissue engineering. (a) Cell viability results for hBMSCs treated with the mentioned scaffolds at different time intervals. (b) Fluorescence intensity of hBMSCs treated with the specified scaffolds after 5 days. (c) Fluorescence microscopy images of hBMSCs. (d) Alizarin Red-S staining process to assess mineralized nodule formation, along with corresponding optical images showing nodule formation at various treatment durations (arrow indicates formed nodules). (e) Quantitative analysis of mineral formation. The control group consisted of media without any samples. [206]. Schematic illustrations of nanocellulose (NC)-based hydrogels for skeletal tissue regeneration. (f) Surface modification of nanofibrillated cellulose (NFC) and subsequent hydrogel formation through in situ polymerization [207]. (g) The process of preparing thermosensitive hydrogels [208]. The examples of the nanocellulose-based 3D printable hydrogels for cartilage tissue regeneration. (h) Schematic representation of the 3D bioprinting process. The bio-ink is composed of CS (chitosan), NCC (nanocellulose), BGP (bioactive glass particles), and HEC (hydroxyethyl cellulose). (i) 3D bioprinter printing cell-loaded CS-NCC bio-inks. (j) Rapid gelation of the 3D-bioprinted knee meniscus at 37 °C. The right figures depict scanning electron microscopy (SEM) images after bioprinting Alg (alginate) and nanocellulose blend ink. (k and l) Alg bio-ink without cells. (m and n) Alg bio-ink loaded with human nasal septal chondrocytes after 3 weeks of culture. (o and p) NCB-Alg bio-ink without cells. (q and r) NCB-Alg bio-ink loaded with human nasal septal chondrocytes after 3 weeks of culture. [209].

Table 6
Various tissue regeneration applications utilizing 3D printing methods and bioinks incorporating nanocellulose.

Application	3D printing methods	Bioink	Nanocellulose	Cross linking	Ref.
Skin	Extrusion-based 3D printing	Alginate	TEMPO-oxidized cellulose nanofibrils	Calcium chloride	[201]
		4-Arm poly(ethylene glycol) (PEG)	TEMPO-oxidized cellulose nanofibrils	Blue light (460 nm and 25 mW cm^{-2}) for 3 min	[202]
	Direct ink writing (DIW)	Gelatin methacrylate (GelMA)	Cellulose nanofibrils	Calcium chloride, UV (365 nm)	[147]
Blood vessel	Extrusion-based 3D printing	Polylactic acid	Bacterial nanocellulose	–	[203]
Visceral		Collagen	TEMPO-oxidized cellulose nanofibrils	Cationic crosslinked	[204]
Bone		Alginate	Nanofibrillated cellulose	Calcium solution	[205]
		Chitosan	Cellulose nanocrystals	Glyoxal groups in HEC	[60]
Cartilage		Alginate	Cellulose nanofibrils	–	[46]
		Sodium alginate	Cellulose nanocrystals Cellulose nanofibrils	Calcium chloride	[195]
Muscle		Carboxymethyl chitosan/gelatin	Dialdehyde-bacterial nanocellulose	Imine network	[144]

scalability, making them more accessible for widespread medical use. The development of standardized manufacturing processes and establishment of regulatory guidelines are crucial for ensuring the consistent quality and safety of these adhesives. With continued advancements in nanocellulose research and collaboration between academia, industry, and healthcare professionals, the potential for transforming wound care and tissue engineering practices is vast, leading to better healthcare outcomes and an improved quality of life for patients.

CRediT authorship contribution statement

Hojin Kim: Writing – original draft. **Sayan Deb Dutta:** Writing – review & editing. **Aayushi Randhawa:** Writing – review & editing. **Tejal V. Patil:** Writing – review & editing. **Keya Ganguly:** Writing – review & editing, Conceptualization. **Rumi Acharya:** Writing – review & editing. **Jieun Lee:** Writing – review & editing. **Hyeonseo Park:** Writing – review & editing. **Ki-Taek Lim:** Supervision.

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.

Data availability

Data will be made available on request.

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