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Review

Recent advances in 3D printed cellulose-based wound dressings: A review on *in vitro* and *in vivo* achievements

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ABSTRACT

Chronic wounds, especially diabetic ulcers, pose a significant challenge in regenerative medicine. Cellulose derivatives offer remarkable wound management properties, such as effective absorption and retention of wound exudates, maintaining an optimal moisture environment crucial for successful chronic wound regeneration. However, conventional dressings have limited efficacy in managing and healing these types of skin lesions, driving scientists to explore innovative approaches. The emergence of 3D printing has enabled personalized dressings that meet individual patient needs, improving the healing process and patient comfort. Cellulose derivatives meet the demanding requirements for biocompatibility, printability, and biofabrication necessary for 3D printing of biologically active scaffolds. However, the potential applications of nanocellulose and cellulose derivative-based inks for wound regeneration remain largely unexplored. Thus, this review provides a comprehensive overview of recent advancements in cellulose-based inks for 3D printing of personalized wound dressings. The composition and biofabrication approaches of cellulose-based wound dressings are thoroughly discussed, including the functionalization with bioactive molecules and antibiotics for improved wound regeneration. Similarly, the *in vitro* and *in vivo* performance of these dressings is extensively examined. In summary, this review aims to highlight the exceptional advantages and diverse applications of 3D printed cellulose-based dressings in personalized wound care.

1. Introduction

Diabetes affects approximately 9.8% of the world's population and it is estimated that over 592 million people will suffer from diabetes in 2035, with around 90% of them with type II diabetes (Saeedi et al., 2019). The rising prevalence of type II diabetes and obesity in recent years has resulted in an increased incidence of diabetic ulcers and chronic wounds. The care and repair of diabetic ulcers entails high costs for healthcare systems and patients (Macdonald, Boeckh, Stacey, & Jones, 2021). The most common ulcers occur on the feet due to peripheral neuropathy and vascular ischemia (Edmonds, Manu, & Vas, 2021). Nerve damage in foot muscles disrupts the balance between flexion and extension, leading to deformities, bone prominences, pressure points, and subsequent skin deterioration and ulceration. Peripheral vascular disease exacerbates ulcer formation through ischemia caused by blood vessel calcification and reduced blood circulation (Hilaire, 2022).

The current approach for diabetic ulcer treatment is limited to wound care, antibiotic therapy and, in severe cases, amputation (Macdonald et al., 2021). Conventional clinical approaches for chronic wounds include debridement, negative pressure wound therapy, topical growth factors, skin substitutes, and hyperbaric oxygen therapy (Dayya et al., 2022; Han & Ceilley, 2017; Las Heras, Igartua, Santos-Vizcaino, & Hernandez, 2020; Wong et al., 2022). The primary challenge in wound management is creating an optimal environment to support uninterrupted healing and achieve wound closure in a short timeframe. An effective wound dressing should not only fulfill essential functions such as maintaining a moist environment, providing thermal insulation, facilitating gaseous exchange, and aiding in drainage and debris removal (Fig. 1), but it should also possess the ability to be easily removed without causing further damage (Morin & Tomaselli, 2007; Wittaya-areekul & Prahsarn, 2006). Hydrogels, hydrocolloids, foams, and films are commonly used in managing diabetic wounds, addressing specific needs. Hydrogels, in particular, possess a structure and porosity

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that closely resemble the microenvironment of healthy skin, making them suitable as temporary or permanent dressings for supporting wound regeneration (Boateng, Matthews, Stevens, & Eccleston, 2008).

Traditional wound dressings often provide a one-size-fits-all approach, which may not effectively address the unique requirements of individual patients or specific types of wounds. Ulcers exhibit significant variations in terms of size, shape, and severity, rendering a standardized approach insufficient for achieving optimal outcomes in wound healing. Recently, 3D printing has revolutionized wound tissue engineering by enabling the creation of patient-specific structures with tailored shapes and properties (Uchida & Bruschi, 2023). This customization considers the specific characteristics of a patient's wound, such as depth, irregularity, or location, thereby ensuring a more precise fit and enhancing the therapeutic efficacy of the dressing. In contrast to traditional film/block-like wound dressings with fixed compositions and limited customization options for end-users, 3D printing offers a distinct advantage by enabling the incorporation of diverse materials with varying properties into dressings having very versatile internal architectures and external morphologies (de Oliveira, Fantaus, Guillot, Melero, & Beck, 2021). This advanced capability allows for the development of active dressings, which involve the integration of bioactive molecules into products prepared with a fine control of pore size and pore interconnectivity. As a result, active dressings not only regulate the fluxes of exudates and oxygen in the wound but may also effectively regulate the release of auxiliary substances and drugs that expedite the wound healing process. Passive dressings primarily serve the purpose of providing physical protection while also regulating exudate levels and maintaining optimal moisture content (Tantillo et al., 2021). 3D printing has already enabled the manufacturing of personalized dressings for drug delivery and transdermal administrations (de Oliveira et al., 2021). 3D printed dressings may provide effective topical drug delivery, with the advantages of locally administered treatments over those administered systemically, as it avoids exposure of healthy tissue to the drug and off-target effects (Las Heras et al., 2020; Sun, Juncos Bombin, Boyd, Dunne, & McCarthy, 2022).

In addition to the manufacturing technology, the components of the wound dressing should be carefully chosen. Cellulose is gaining increasing attention since its peculiar combination of amorphous and crystalline regions can be tuned through versatile chemistry approaches and exploited through the recent 3D printing technologies. Crystalline regions confer stiffness and elasticity, while the amorphous region is responsible for flexibility and plasticity. Crystalline regions serve as structural reinforcements of the inks allowing for better control of the printing process. Indeed, typically highly crystalline celluloses, such as cellulose nanofibers (CNFs), have been shown to enhance the mechanical properties of fused filament fabrication (FFF) 3D printed materials acting as fillers and also as nucleating agents that facilitate the crystallization of the extruded polymers (Ambone, Torris, & Shanmuganathan, 2020) Additionally, the inherent viscoelastic and thixotropic properties of semi-crystalline celluloses (*e.g.*, cellulose ethers) facilitate the printing process through fine nozzles and contribute to the shape fidelity of the printed scaffolds (Diaz-Gomez et al., 2022), ensuring that the desired structure is accurately reproduced. The balance between crystallinity and amorphicity makes cellulose a very interesting material for 3D printing due to the mechanical properties that it provides to the inks in terms of thickening capability, and to the printed scaffolds, such as shape fidelity (Blessy, Sagarika, Sabu, Kalarikkal, & Thomas, 2020). As for any other semi-crystalline polymers, the printing conditions and adjuvants should be carefully chosen to preserve the original cellulose properties or even to tune the crystallinity of the final product, which in turn determines its response under stress and the chemical and enzymatic degradation rate among other relevant features (Vaes & Van Puyvelde, 2021).

Cellulose-based dressings offer unique advantages compared to synthetic materials, including excellent biocompatibility, biodegradability, and high-water absorbance. They can effectively manage moisture levels, promote healing by creating a moist environment, and provide a protective barrier against external pathogens. Additionally, cellulose exhibits remarkable versatility, enabling its combination with various polymers, bioactive compounds, or therapeutic agents, thereby enhancing the efficacy of dressings for ulcer healing. Furthermore, cellulose-based dressings can be tailored for controlled drug release, allowing for localized delivery of therapeutic agents to the wound site (Momin, Mishra, Gharat, & Omri, 2021; Zheng, Li, Luo, & Wang, 2020).

A challenge in the use of raw cellulose in wound dressing applications is its insolubility in aqueous medium. However, the reactivity of the hydroxyl groups in cellulose presents potential for modifying its properties. Recent advances in methods for chemical modification of cellulose have allowed to produce a wide range of cellulose derivatives that are water-dispersible and can be processed into various forms such as hydrogels, aerogels, and foams (Bacakova et al., 2019). Cellulose derivatives can be produced in a versatile way to suit the demands of various biomedical applications, including wound healing, as they allow for the adjustment of their physical, chemical, and biological attributes. Notably, these derivatives are still biocompatible and cost-effective (Abazari et al., 2021). The remarkable biocompatibility and hemocompatibility of cellulose derivatives have stimulated research into their potential utilization as inks for the biofabrication of personalized wound dressings and tissue engineering scaffolds in the field of regenerative medicine (Blessy et al., 2020). Some commercial sources of cellulose derivatives used for wound dressing are compiled in Table S1 (Supplementary material).

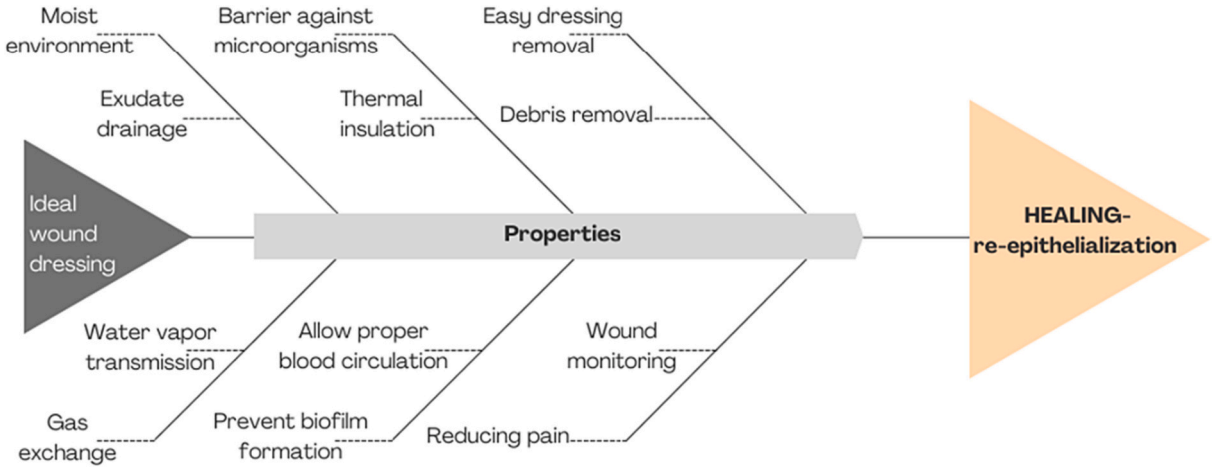

Fig. 1. Scheme of the properties of an ideal wound dressing.

This review focuses on the most recent *in vitro* and *in vivo* achievements using cellulose derivatives as the primary components of 3D printing inks for creating personalized wound dressings, specifically for the purpose of healing diabetic ulcers. Recent reviews have discussed the potential of cellulose derivatives as inks for 3D printing, including rheological and printability aspects (Wan Jusoh, Sajab, Mohamed Abdul, & Kaco, 2022; Wang et al., 2018), the tedious procedure of obtaining porous structures from biodegradable or reusable raw materials, aiming to decrease reliance on fossil resources (Ferreira et al., 2023), or the characteristics and applications of 3D printed dressings (Fahma et al., 2022). Nevertheless, the significance of this review lies in its aim to advance beyond the current state of cellulosic derivatives in this domain. Rather than concentrating solely on the manufacturing process, this work delves extensively into the accomplishments of the developed dressings and the tested combinations, offering a detailed examination of the possible clinical applications of the conducted studies. By highlighting trials conducted *in vitro* and *in vivo*, this review emphasizes the progress achieved as a foundation for bridging the gap between tissue engineering and patient care, ultimately bringing us closer to practical applications in the clinical setting.

The terms "3D printing", "cellulose", and "wound" were searched in the Web of Science Core Collection data base, PubMed and Scopus refined with a range of publication dated from 2018 to May 2023, resulting in 133 outcomes. Of these, 62 were discarded because they were duplicated across databases. Publications differently from original articles were discarded too. Finally, 22 original research papers were selected. Although the development of cellulose inks for wound healing is still emerging, the field is rapidly evolving, and the number of papers published in the last two years has doubled from the previous three years. The selected publications were analyzed in terms of materials, methods and results obtained to identify the novel aspects and their potential clinical impact.

2. Nanocellulose-based 3D printed wound dressings

Nanocellulose is a term used to describe a cellulose material that has been isolated or fragmented into nanoscale particles or fibers. These nanomaterials can be classified into two distinct categories according to their particle size: i) cellulose-derived materials based on cellulosic nanostructures, including microcrystals and microfibres (Zhang, Shen, Li, Yang, & Lin, 2023), and ii) cellulose nano-objects, which consist of nanofibers and nanocrystals. Although most cellulose derivatives are obtained from vegetal sources, certain cellulose nanomaterials are derived from bacteria, as depicted in Fig. 2 (Du et al., 2019; Kargarzadeh et al., 2018). Recently, nanocellulose has been tested for tissue engineering applications, particularly in the context of chronic wound healing. However, to ensure its suitability for such purposes, it is crucial to effectively regulate and control its physical, chemical, and biological properties (Bacakova et al., 2019).

Cellulose nanofibers (CNFs), cellulose nanocrystals (CNCs), and bacterial cellulose (BC) are particularly prominent in the field of wound healing due to their high water retention capacity (up to 99 %) and hydrophilicity, thermal stability, and cost-efficacy, among others (Huo et al., 2022). Obtaining various nano-derivatives from cellulose involves employing different techniques to modify the three-dimensional crystalline structure of naturally derived cellulose fibers. Acid digestion, bacterial generation, and mechanical defibrillation are commonly employed methods (Carter, Grant, Dewey, Bourque, & Neivandt, 2021). Acid digestion leads to the degradation of accessible areas of the cellulosic fibers, including the crystalline regions. The extent of degradation depends on factors such as acid concentration, reaction time, and temperature. Extended exposure to acid leads to the complete hydrolysis of cellulose, resulting in its breakdown. Conversely, shorter exposures preserve higher degrees of polymerization, primarily due to the presence of large, undispersed fibers in the cellulose structure. Reaction time is inversely related to reaction temperature. Following the hydrolysis of cellulose and a subsequent dialysis purification process, small crystalline rod-shaped particles suspended in water are obtained (Börjesson & Westman, 2015).

Mechanical treatment involves refining cellulose pulp, followed by high-pressure homogenization to produce individual cellulose nanofibers. To facilitate the mechanical treatment and reduce energy consumption, the fibers can be chemically or enzymatically treated before the mechanical process. This chemical or enzymatic treatment modifies the surface of the fibers, making defibrillation easier. The refining process can be carried out using a refiner or through cryocrushing (Börjesson & Westman, 2015). Refining and cryocrushing processes enable the separation of the fiber wall, exposing the cell wall fragments for further treatment. Homogenization is achieved through the application of pressure, shear forces, and particle collisions, and leads to a high degree of microfibrillation and the production of cellulose nanofibers (Patil et al., 2022).

Bacterial generation involves a static fermentation process. Initially, cellulose-producing bacteria such as *Gluconacetobacter xylinus* are cultured in Petri dishes. Under favorable conditions, these bacteria produce cellulose within their membrane pores, which is then excreted in the form of microfibers. In static culture conditions, these microfibers assemble to form a film-like structure on the culture surface. If the conditions are not static, irregular BC granules with lower crystallinity and inferior mechanical properties are formed instead of a film. A purification step is subsequently carried out to obtain the final BC suitable for biomedicine applications (Cazón & Vázquez, 2021).

2.1. Cellulose nanofiber-based 3D wound dressings

CNFs are nanosized fibers with a width ranging from 5 to 50 nm. These nanofibers possess exceptional properties to be used as biomaterials, including a high specific surface area, remarkable mechanical

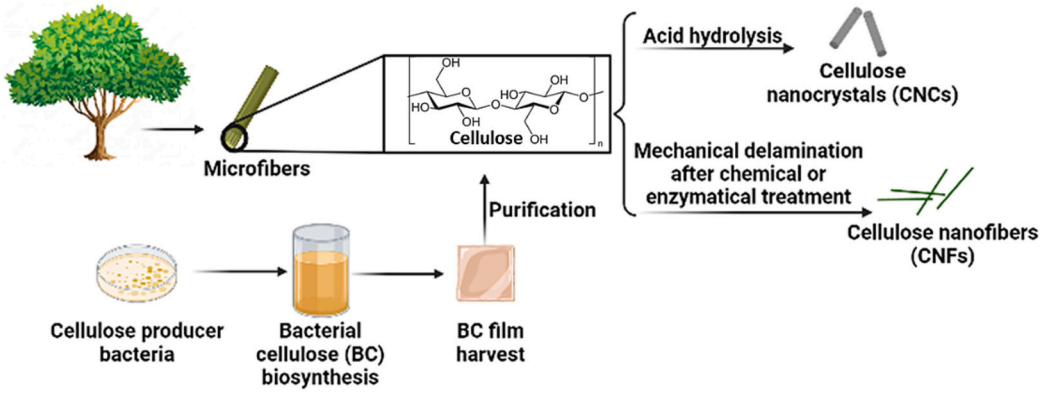

Fig. 2. Sources and procedures to obtain cellulose nanocrystals and nanofibers (*Partially designed with BioRender*).

strength, reactive surface chemistry, non-toxicity, and cost-effectiveness (Trache et al., 2020). These attributes have positioned CNFs as highly relevant materials in various biomedical fields, including drug delivery, wound healing dressings, and skin tissue engineering. The isolation of CNFs typically involves the mechanical delamination of cellulose fibers, which are pre-treated chemically or enzymatically through processes like milling, acid hydrolysis, or bleaching (Sanchez-Salvador et al., 2022). CNFs can be combined with different polymers or bioactive molecules to create hydrogels or dressings for chronic wound therapies or other tissue engineering applications, such as addressing cartilage defects (Pandey, 2021). The incorporation of CNFs to hydrogel films or scaffolds improves the mechanical properties in terms of printability, shape fidelity of the printed scaffolds and flexibility.

CNFs have been successfully employed in the fabrication of scaffolds using 3D printing techniques. Among them, extrusion printing has gained widespread adoption due to its ease of use, ability to achieve precise printing of complex geometries using computer-aided design (CAD), and availability of multiple crosslinking methods. Moreover, extrusion printing offers high resolution at the micron scale, enabling the creation of customized shapes and sizes for applications in tissue engineering and regenerative medicine (Derakhshanfar et al., 2018; Jiang et al., 2020). A recent study evaluated the effect of CNF carboxylation on the printability and mechanical properties of printed scaffolds (Table 1, entry #1). CNFs with distinct carboxylation degrees (1, 1.3, and 1.6 mmol $g^{-1}_{cellulose}$) were obtained by TEMPO-mediated oxidation with sodium hypochlorite and further used as single components of hydrogel inks (0.6 w/v%). The increase in the degree of nanofibrillation of CNFs led to higher viscosities and enhanced printability. Interestingly, 3D printed CNF dressings presented an anisotropic orientation of the porous structure, likely due to the preferential alignment of the nanofibrils during the extrusion process. Then, *in vitro* studies using fibroblasts demonstrated that the oxidation degree and anisotropic alignment of carbon nanofibers (CNFs) exerted a considerable influence on cell proliferation. Specifically, these factors were found to upregulate pro-regenerative markers while concurrently suppressing inflammation-related markers (Rosendahl, Zarna, Håkansson, & Chinga-Carrasco, 2023).

The abundant hydroxyl groups present in the cellulose structure, along with the aldehyde and carboxylate groups incorporated on the surface of CNFs *via* TEMPO oxidation, provide a wide range of crosslinking alternatives. Recently, a hydrogel containing 1 w/v% TEMPO-oxidized CNFs was used as ink to develop 3D printed dressings with tunable mechanical strength in the 3–8 KPa range. The printed structures were strengthened by the addition of concentrated $CaCl_2$ solution during the printing process, which led to ionic interactions between Ca^{2+} and a pair of carboxylate groups present in the CNF (Table 1, entry #2). The dressings underwent post-crosslinking with 1,4-butanediol diglycidyl ether (BDDE), employing two different BDDE:CNFs weight ratios; namely, 0.01 for low crosslinking and 0.25 for high crosslinking. Cytocompatibility tests were conducted on the printed scaffolds, demonstrating their ability to promote fibroblast growth and wound closure *in vitro*. A positive correlation was observed between scaffold rigidity, as defined by the content of CNFs and the degree of crosslinking, and cell proliferation, indicating that higher rigidity leads to increased viability (Xu et al., 2018).

Another strategy is the incorporation of other biopolymers, such as gelatin, alginate, or modified carbohydrates, to enhance the mechanical stability of the printed constructs. A recent study (Table 1, entry #3) used gelatin methacryloyl (GelMA) in low concentrations (up to 1 w/v %) in combination with CNFs to 3D print wound dressings followed by double crosslinking methods. The crosslinking of CNF by Ca^{2+} during the printing process provided immediate shape fidelity, while the subsequent UV crosslinking of GelMA by irradiation of the freshly printed dressing ensured permanent shape fidelity even when Ca^{2+} ions were leaked to the medium (Xu et al., 2019). Ionic interactions between CNF and GelMA successfully prevented phase separation phenomena in the ink and facilitated the UV crosslinking of GelMA, compared to inks prepared without CNFs. The implementation of double crosslinking steps resulted in the formation of three-dimensional structures with adjustable mechanical properties, which could be fine-tuned by varying the CNF:GelMA ratio. Relevantly, the UV crosslinking of GelMA enhanced the Young's modulus of the dressing up to values of 2.5–5 KPa,

Table 1
Some relevant examples of wound dressings based on CNFs and tested *in vitro*.

Entry	Composition	Crosslinking agent	Results	Reference
#1	CNF (*Carboxylic acid content* (*CAC*): *1.036–1.593 mmol* g^{-1})	–	Higher CNF nanofibrillation improved 3D printing performance. *In vitro* cell assays showed down-regulation of proliferation marker genes correlated with vascularisation markers.	(Rosendahl et al., 2023)
#2	1 w% CNF (*CAC*: *1.14 mmol* g^{-1})	$CaCl_2$ and BDDE	Adjustable mechanical resistance (3–8 kPa) improved cell proliferation.	(Xu et al., 2018)
#3	CNF/GelMA (<1 w/v%) (*CAC*: *1.14 mmol* g^{-1})	MA groups	Low-concentration TEMPO showed improved rheological properties and UV cross-linking ability. Scaffolds with suitable mechanical integrity, stability, and *in vitro* biocompatibility	(Xu et al., 2019)
#4	CNFs/pectin/fibroblasts (*CAC*: *2.13 mmol* g^{-1})	$CaCl_2$	Multicomponent inks enable precision and shape fidelity. Embedded fibroblasts were viable after printing.	(Pitton et al., 2021)
#5	2 w% CNF/20 w/w_{CNF}% alginate (*CNF 3.7 nm width* *CAC*: *1.040 mmol* g^{-1})	$CaCl_2$	Bagasse CNF inks showed potential for cost-effective, cytocompatible 3D printing of tailor-made wound dressings.	(Chinga-Carrasco et al., 2019)
#6	CNF/(10–40 w/w_{CNF}%) alginate (*CAC*: *0.982 mmol* g^{-1})	$CaCl_2$ (50 or 100 mM)	The CNF/Alginate/$CaCl_2$ combination enables the manufacture of reinforced absorbent dressings.	(Espinosa et al., 2019)
#7	CNF/alginate (*CNF 6–7 nm*)	$CaCl_2$	Alginate/CNF hybrid inks obtained from algae demonstrated good printability, mechanical properties, and biocompatibility.	(Berglund et al., 2020)
#8	CNF/alginate/colloidal lignin particles (CLP)	$CaCl_2$	Increasing the concentration of CLP resulted in increased antioxidant activity and improved water uptake, without sacrificing cytocompatibility in *in vitro* assays with HepG2.	(Zhang et al., 2020)
#9	CNF-casein (*CAC*: *1.64 mmol* g^{-1})	Chitosan	Composite dressings showed enhanced blood clotting, adhesion, thrombin generation, and absorption abilities compared to commercial dressings.	(Biranje et al., 2022)
#10	CNF/alginate/glycerin/ nChiAvd (*CAC*: *1.1 mmol* g^{-1})	$CaCl_2$	CNFs were used as structural reinforcement of the dressings and improved the printability of the ink. Functionalization with avidin allowed the incorporation of bioactive molecules.	(Leppiniemi et al., 2017)

previously identified as the most adequate for fibroblast spreading and thus wound healing (Yeung et al., 2005). All printed dressings exhibited excellent adhesion and viability of 3T3 fibroblasts (Fig. 3A, B), but the fibroblasts proliferated remarkably more inside dressings containing low-to-medium mass ratios of GelMA compared to CNF solely dressing (Fig. 3C, D). These finding evidenced the relevance of a fine adjustment of the crosslinking density, which may affect not only to the stiffness of the scaffold but also to other surface features such as pore size and topography that also determine cell adhesion and proliferation (Xu et al., 2019).

CNFs have also been investigated as critical constituent of cell-laden bioinks to address the existing limitations in scaffold manufacturing by enhancing shape fidelity and printability. As an example, CNFs incorporated into cell-laden pectin bioinks notably improved their printability and stability (Table 1, entry #4). Only inks containing adequate CNFs concentrations rendered 3D structures with high shape fidelity comparable to the .stl model. Bioinks encapsulating L292 fibroblasts were printed using Dulbecco's Modified Eagle Medium (DMEM) as bioink solvent in order to better regulate the pH. The control of the pH facilitated an environment more conducive to cell viability. Through careful evaluation, the optimal composition was determined to be a combination of 2.5 w/v% pectin and 1 w/v% CNFs, revealing a satisfactory balance between printing accuracy and maintaining adequate fibroblasts viability (Pitton et al., 2021).

Among the various sources for obtaining CNFs, bagasse is an attractive alternative due to its cost-effectiveness and contribution to the circular economy (Chinga-Carrasco et al., 2019). Other naturally obtained polymers, such as alginate, have been combined with CNFs to prepare inks with improved printability and versatility (Table 1, entry #5). The most extended approach for CNFs printing is their blending with alginate by mechanical stirring to prepare homogenous inks that are crosslinked using $CaCl_2$ immediately after printing (Berglund, Rakar, Junker, Forsberg, & Oksman, 2020; Espinosa, Filgueira, Rodriguez, & Chinga-Carrasco, 2019; Leppiniemi et al., 2017; Zhang et al., 2020). These composite materials have been extensively studied to determine the optimal concentrations of alginate and $CaCl_2$ to achieve desirable properties (Table 1, entry #6). The incorporation of 1 w/v% CNFs in the inks significantly improved their printability. On the contrary, increasing the alginate concentration (from 0 to 40 w%) negatively affected printability and shape fidelity. The printing of alginate-CNFs inks led to porous dressings endorsed with consolidated structures and controlled swelling, adequate for the adaptation to the wound site. The presence of CNFs in the composition significantly improved the moisture absorption of the printed dressings while maintaining a moisturized environment without excess exudates (Espinosa et al., 2019).

Recycling of waste products is gaining increasing attention in the preparation of sustainable wound dressings. In a recent study, brown seaweed was used as a natural, cost-efficient source of alginate and CNF for the development of 3D printing inks (Table 1, entry #7). Instead of using distinct methods to extract and combine alginate and CNF as ink components, the proposed method enabled a streamlined process to obtain hybrid alginate/CNF inks that exhibited remarkable printability, ascribable to the shear-thinning properties of CNFs and the fast-crosslinking capabilities of alginate. The resulting dressings showed open porous structure and supported fibroblast proliferation *in vitro* (Berglund et al., 2020).

CNF-based hydrogels have also demonstrated their potential for incorporating bioactive molecules, including antibiotics, antioxidants and anti-inflammatory drugs. Indeed, the high affinity of CNFs for antibiotics has been demonstrated when used as a powder loaded with gentamicin (Lochman, Plodr, Páral, & Smejkal, 2010) or as electrospun nanofibers loaded with ciprofloxacin (Yazdanbakhsh, Rashidi, Rahimi, Khajavi, & Shafaroodi, 2018) directly applied to acute wound infections in animal models. This capability to uptake and sustainedly release bioactive substances may enable the development of 3D printed scaffolds that can effectively contribute to the wound healing process by addressing various challenges. For instance, by incorporating colloidal lignin particles into a CNF-alginate hydrogel, a dressing with antioxidant activity was obtained (Table 1, entry #8). This dressing maintained its shape and hydration for up to 7 days, ascribable to the hydrogen bonds between water and polar groups such as hydroxyl and carboxyl present in the structure of CNFs, which facilitated the normal healing progression (Zhang et al., 2020). Furthermore, the inclusion of bioactive molecules like casein in 3D printed dressings has also been explored to control bleeding in more severe wounds and accelerate wound healing (Table 1, entry #9) (Biranje et al., 2022). The process involved the covalent bonding of casein to the CNFs using N-(3-dimethylaminopropyl)-N′-ethyl carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). Subsequently, 3D printed dressings exhibited *in vitro* hemostatic activity, making them suitable for managing non-controlled hemorrhagic wounds. Also recently, CNFs have been combined with Cu_2O and silver nanoparticles in resins for 3D printed vat photopolymerization showing biocidal capabilities in preliminary tests (Vidakis et al., 2022).

The biofunctionalization of CNFs is also a promising alternative to tune their mechanical and biological properties. CNFs have been functionalized with thermostable chimeric avidin (nChiAvd) to further immobilizing proteins, such as biotin, within the hydrogel structure (Table 1, entry #10). The incorporation of functionalized CNFs in

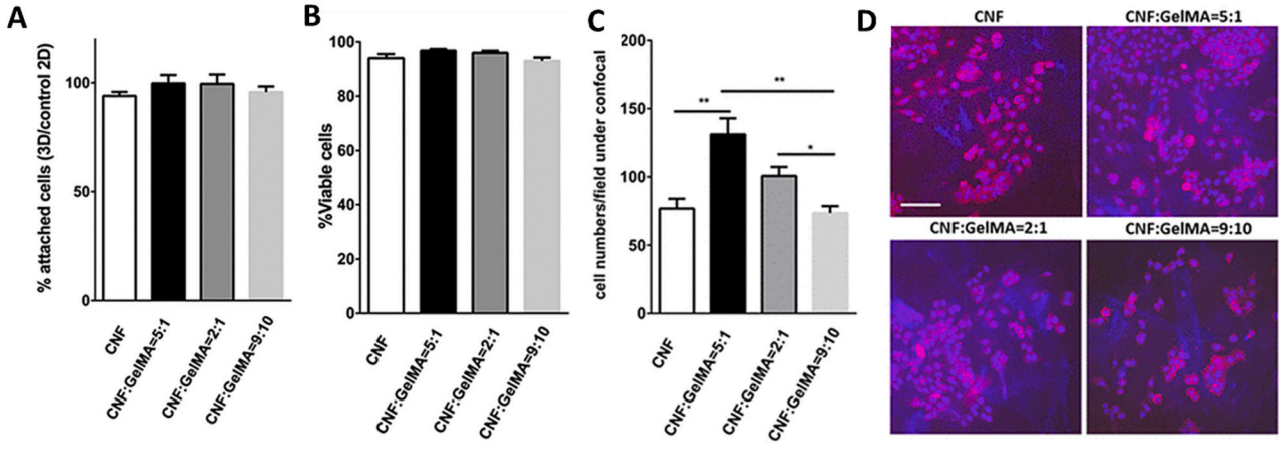

Fig. 3. Cell compatibility studies of CNF (1 w/v%) scaffolds prepared with increasing mass ratios of GelMA. (A) 3T3 fibroblasts cell adhesion after 12 h incubation evaluated with a crystal violet assay; (B) viability after 24 h direct contact; and (C, D) cell proliferation inside the scaffolds after 3 days of incubation evaluated by confocal microscopy. Scale bar on Fig. D: 50 μm.
Reproduced from Xu et al. (2019) under Creative Commons public use license CC-BY.

alginate-based inks not only significantly improved the mechanical stability of the printed dressings but also allowed for tailoring with specific therapeutic molecules. The resulting 3D printed wound dressings showed excellent water absorption in moist conditions. Moreover, the potential for biofunctionalization was demonstrated by attaching biotinylated fluorescent proteins and small molecules to the hydrogel *via* avidin, thus enabling the incorporation of bioactive agents to promote wound healing. This strategy provides a versatile platform for the development of wound dressings functionalized with biotinylated molecules, including cell adhesion motifs and other bioactive molecules (Leppiniemi et al., 2017).

In summary, the use of CNFs in 3D printing enables the fabrication of dressings with adjustable mechanical properties, controlled swelling, and anisotropic porous structures. Combining CNFs with other biopolymers or bioactive molecules allows enhancing the printability, stiffness and cell adhesion and proliferation. However, challenges remain in optimizing the selection of cellulose derivatives, their concentrations, and crosslinking methods to achieve the desired properties for wound dressing applications.

2.2. Cellulose nanocrystal-based 3D wound dressings

Cellulose nanocrystals (CNCs) are nano-sized cellulosic derivatives with a rod-like structure, which can be obtained from cellulose microfibers through mechanical, chemical, and enzymatic treatments (George & Sabapathi, 2015). The size of CNCs can vary depending on the source of cellulose microfibers and the extraction method, typically ranging between 3 and 50 nm width (George & Sabapathi, 2015). CNCs have attracted much attention as components of inks for 3D printing applications due to their high Young's modulus and strength, low density, and ecological sustainability (Li, Dunn, Zhang, Deng, & Qi, 2017). For instance, a 3D-printed hydrogel based on CNC and chitosan methacrylamide (Chi-MA) was used to develop customizable wound dressings with antibacterial properties (Table 2, entry #1). The hydrogel was prepared by homogeneous mixing of an aqueous CNC suspension and a Chit-MA dispersion. The targeted delivery of therapeutic agents, including small molecules, metal nanoparticles, and proteins, was accomplished using a multimaterial printing platform. This methodology allowed for precise control over the localization and release of the bioactive agents (Fig. 4A). The printed structure was crosslinked with UV light. As active agents, silver nanoparticles and endothelial growth factor were included in the composition to match specific wound healing requirements. The incorporation of CNCs led to nanofibrillar structures with controlled pore sizes and improved swelling properties. *In vitro* studies demonstrated a controlled release of the incorporated active agents and a significant antibacterial activity against gram-positive *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa*, commonly responsible for infections of chronic wounds. Moreover, the effectiveness of this composite on wound healing was evaluated *in vivo* using a mouse model (Fig. 4B). The hydrogel dressings promoted granulation tissue formation and vascularization compared to control groups. The study highlighted the potential of 3D-printed tunable dressings based on CNCs to enable personalized wound healing by controlling the release of biologically active agents and adapting the delivery profile over time (Alizadehgiashi et al., 2021).

Furthermore, the decoration of CNCs with C-dots, which are carbon-based nanoparticles, has shown promising antibacterial activity by sequestering iron ions and limiting the proliferation of various microorganisms, including antibiotic-resistant bacteria (Chekini et al., 2020) (Table 2, entry #2). Dressings prepared using C-dot/CNC hybrid nanoparticles and gelatin showed a high absorption capacity for Fe^{3+} ions, while maintaining adequate swelling properties due to CNCs, as needed for wound regeneration. Compared to conventional wound dressings that rely on the release of antibacterial agents, the developed dressings exhibited the ability to suppress the growth of various bacteria, including antibiotic-resistant strains, by effectively removing Fe^{3+} ions from the surrounding medium. The findings of this study demonstrated the effectiveness of iron sequestration against *Escherichia coli*, antibiotic resistant *P. aeruginosa*, and *S. aureus*. Therefore, the antibacterial performance of the dressings was achieved without the need for antibacterial surface agents or antibiotics, which is key to overcome antibiotic resistance.

Blends of CNCs and CNFs were also evaluated as main components of inks with tunable viscoelastic properties. Incorporation of CNCs (0.5 w/v%) in CNFs inks (4 w/v%) led to a significant reduction in the complex viscosity of the inks, which could be detrimental for cell encapsulation (Table 2, entry #3). By increasing the concentration of CNCs, the inks showed a significant decrease in their shape fidelity and structural maintenance. In order to avoid structure collapse during printing, alginate was incorporated in the composition, enabling fast crosslinking using $Ca^{2+}$and thus, significantly enhancing the geometrical retention of printed dressings (Heggset et al., 2018).

The effect of the incorporation of CNCs and CNFs in the composition of inks on the mechanical properties and biodegradation of 3D printed

Table 2
Reports on CNC and CNC/CNF combination wound dressing.

Entry	Composition	Crosslinking agent	Targeted microorganism	Results	Reference
#1	CNC/Chit-MA/silver nanoparticles/ gentamicin sulfate/bovine serum albumin (BSA) or endothelial growth factor (VEGF) (*CNC*: *aqueous dispersion 12.2 w%*)	MA groups	*S. aureus*, *P. aeruginosa*	BSA and VEGF loading in the dressings improved formation of granulation tissue compared to the control group. Silver nanoparticles and gentamicin prevented infection and promoted chronic wound healing *in vivo* (mouse).	(Alizadehgiashi et al., 2021)
#2	CNCs/C-dots/gelatin (*CNC aqueous dispersion 12.2 w%*)	–	*E. coli*, antibiotic resistant *P. aeruginosa*, and *S. aureus*	Antibacterial effect by sequestering Fe^{3+} ions.	(Chekini et al., 2020)
#3	• CNF (*3–4.5 w%*) • CNF/CNC • CNF/alginate (*0.5–1.5 w%*) (*CNF*: *solid content of 10 w%* *CNC*: *sulphate charge density*: *0.3 mmol* g^{-1})	$CaCl_2$	–	CNC/CNF ratio determined viscoelastic properties of the ink and, in turn, printing accuracy and mechanical properties of the printed dressings.	(Heggset et al., 2018)
#4	• CNC/alginate (*4/2 w/v%*) • CNFs/alginate (*1/4 w/v%*) (*CNFs*: *1 w/v% solid in water*, *width*: *20–50 nm*; *length*: *0.5–80* μm *surface group*: *carboxyl hydrophilic*)	$CaCl_2$	–	CNF improved mechanical properties, structural stability and cell viability compared to CNC.	(Temirel et al., 2021)
#5	• CNC/NIPAM/Ɛ-PL • CNC/CNF/NIPAM/functionalized Ɛ-PL	Ethylene glycol dimethacrylate (EGDMA)	*S. aureus*, *S. arlettae*, *E. coli* and *P. fluorescens*	Anisotropic swelling behavior and efficient antimicrobial properties.	(Fourmann et al., 2021)

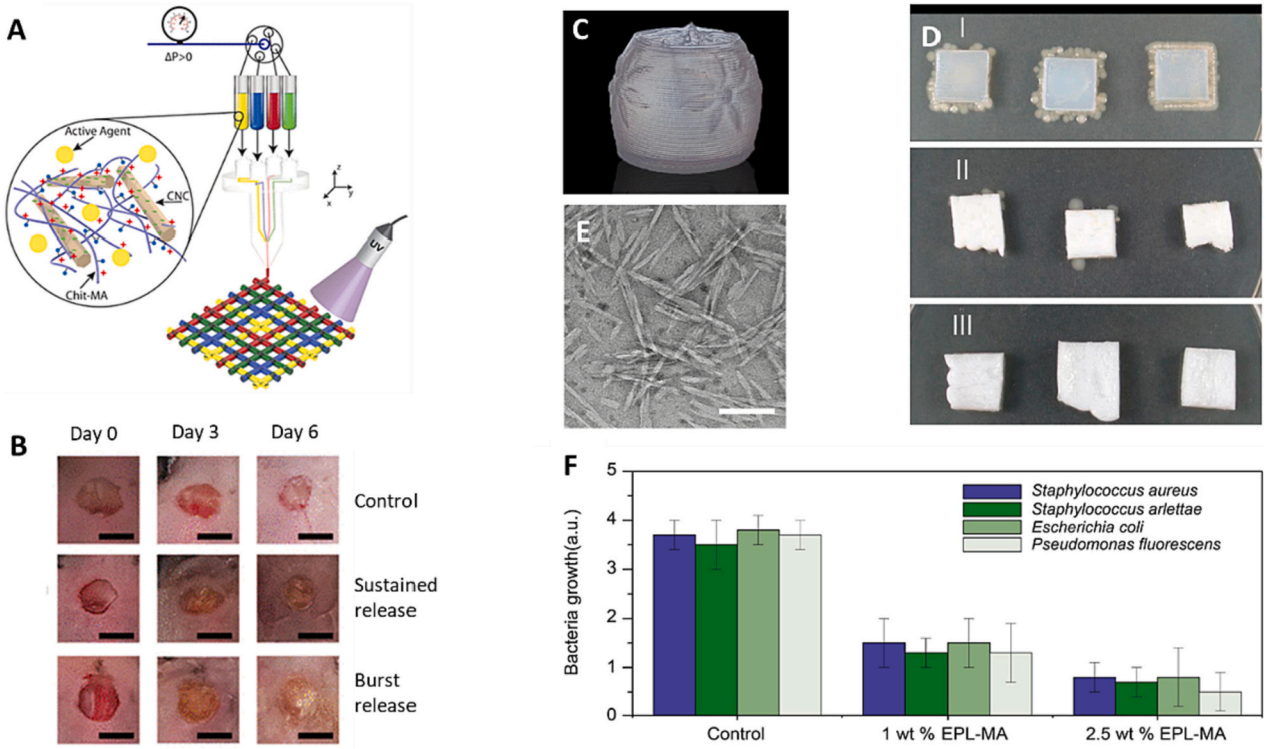

Fig. 4. (A) Procedure for co-printing multicomponent CNC-based dressings loaded with distinct bioactive molecules; electrostatic interactions between CNCs and chitosan methacrylamide (Chit-MA) rendered shear-thinning inks with adequate printability. (B) Results of full-thickness mouse wounds treated with VEGF-loaded Chit-MA/CNCs dressings with different VEGF-release rates. Adapted with permission from (Alizadehgiashi et al., 2021). Copyright (2021) American Chemical Society. (C) Sophisticated design printed using 20 w/v% CNC-NIPAM inks; (D) antibacterial activity test of (I) CNC-NIPAM dressings, and CNC-NIPAM dressings functionalized with ε-polylysine (EPL-MA) at (II) 1 w% or (III) 3.5 w%; (E) transmission electron image (TEM) of the CNCs used in the dressings; and (F) antibacterial effectiveness of the CNC-NIPAM dressings non-functionalized (control) and functionalized with ε-polylysine.
Adapted with permission from (Fourmann et al., 2021) under a Creative Commons license CC BY 4.0.

dressings has also been examined (Table 2, entry #4). Hybrid inks containing alginate and CNFs or CNCs were used to prepare personalized wound patches. While CNFs and CNCs incorporation in the inks showed a reinforcing effect compared to pure alginate, the compressive and tensile strength of the patches was significantly higher on CNFs inks. Patches printed with CNFs degraded slowly over a 30-day period without losing their mechanical integrity. Besides, *in vitro* studies demonstrated a significantly increased viability of NIH3T3 cells when cultured on CNFs wound patches, compared to CNCs. The balance between mechanical stability and biocompatibility is crucial for effective wound healing, as the dressings should provide support for tissue regeneration while gradually breaking down to accommodate the healing process (Temirel, Hawxhurst, & Tasoglu, 2021).

The high aspect ratio of CNFs and CNCs usually leads to anisotropic microstructures that can potentially compromise the mechanical strength of the printed dressings (Shi et al., 2022). However, these anisotropic microstructures can also facilitate cell guidance, proliferation, and differentiation. In a recent study (Table 2, entry 5), CNC and CNF inks were used to develop 3D printed wound dressings with intricated geometries (Fig. 4C). The mechanical properties of the printed structures were precisely tuned in specific directions by precisely adjusting the content and morphology of CNCs and CNFs in the ink (Fig. 4E). Specifically, CNCs (up to 35 w/v%) were included in the inks as reinforcement, while CNFs at low concentrations (up to 1 w/v%) significantly improved printability and shape retention of the printed dressings. The functionalization of the dressings with ε-polylysine *via* chemical modification provided them with antibacterial activity against multiple bacterial strains, *i.e.*, *S. aureus*, *E. coli*, *Staphylococcus arlettae*, and *Pseudomonas fluorescens* (Fig. 4D and F) (Fourmann et al., 2021).

In summary, although the utilization of CNCs as components of 3D printed wound dressings is quite recent, CNCs have already shown good performance as structural reinforcements of other polysaccharide inks and as efficient adsorbents of therapeutic substances and of ions that are vital for bacteria. Nevertheless, further investigation on the effects of the concentration of CNCs on shape accuracy, mechanical strength and biodegradation is needed to unveil their role in 3D printed wound dressings.

2.3. Bacterial cellulose-based 3D wound dressings

Bacterial cellulose (BC), as opposite to plant cellulose, is synthetized by various bacteria, such as *Gluconacetobacter xylinus* or *Komagataeibacter medellinensis*, which are common sources of BC for biomedical applications. Native BC consists of a nanocellulose network containing a high density of hydroxyl surface groups, that provide hydrophilicity, biodegradability, and chemical modification potential (Carvalho, Guedes, Sousa, Freire, & Santos, 2019). Compared to plant cellulose, the bacterial synthesis approach enables the production of highly pure BC, without other components such as hemicellulose or lignin (Moniri et al., 2017). The synthesis of BC initiates at the cell membrane, where cellulose synthase catalyzes the polymerization of activated glucose units. The resulting cellulose macromolecules arrange themselves in a nanofibrous pattern, interconnected by van der Waals forces and hydrogen bonds (Carvalho et al., 2019). The obtained BC presents distinct properties, such as ECM-like nanofiber network and high water absorption capacity, crystallinity, and biocompatibility. Owing to this, BC is largely considered an attractive biomaterial for wound tissue engineering (Oprea & Voicu, 2020). Specifically, the porous structure of BC fosters an environment favorable to moisture retention, facilitating gas exchange with the environment and allowing for drug incorporation

(Carvalho et al., 2019).

Recently, BC-based dressings for treatment on severe burns or ongoing wounds have entered the market. Biofill® or Xcell® are examples of commercialized BC wound dressings that have shown higher efficacy to promote wound healing, compared to traditional dressings (gauzes and synthetic polymers). Biofill® is commonly used in chronic wound and burns, while Xcell® has the ability to actively manage wound fluid, providing either hydration or moisture absorption and it is employed in venous ulcers (Kucińska-Lipka, Gubanska, & Janik, 2015). Promising advancements have been made in the development of 3D printed dressings based on BC for wound healing. The incorporation of BC nanofibers to alginate inks (4 w/v%) containing copper nanoparticles significantly improve their printability and shape fidelity, as well as the mechanical properties of the resulting 3D printed dressings. Two methods were employed in the preparation of the inks. One method entailed calcium cross-linking followed by an ion exchange with copper ions, whereas the second method consisted in the direct crosslinking of alginate with copper ions using a reducing agent (sodium borohydride) to obtain *in situ* alginate beads. Then, the incorporation of BC nanofibers in the composition (BC:alginate 70:30) was key to improve the printability of the inks and the mechanical stability and structural integrity of the printed dressings. The resulting BC/alginate/copper dressings performed as contact-killing materials against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Gutierrez et al., 2019). The presence of BC hampered the diffusion of copper ions and particles from the scaffold due to the affinity of BC for metal ions, and therefore a high concentration of bacteria-killing components remained within the scaffold while surrounding human cells could be protected from their deleterious effects.

The combination of BC and thermoplastic polymers, such as poly-ε-caprolactone (PCL) and polylactic acid (PLA), has also been investigated (Gregory et al., 2021). One notable example is the development of antibiotic dressings using BC/PCL inks containing broad spectrum antibiotics (ampicillin, amoxicillin, or kanamycin). Electrohydrodynamic 3D printing (EHD-3D) method, a technique that uses an electric field to control the deposition of materials and create intricate structures, was used to print the BC/PCL inks. The printed wound dressings presented a fibrillar structure and tunable pore sizes, offering improved biocompatibility and increased drug loading capacity, compared to pure PCL scaffolds. The single-step EHD-3D printing method enabled the rapid fabrication of personalized dressings using minimal excipients and endorsed with controlled drug release capabilities. Thus, antibiotics such as amoxicillin, ampicillin and kanamycin, exhibiting antimicrobial activity against *S. aureus* and *E. coli*, were released at a controlled rate above the minimal inhibitory concentration over a 14-day period. The release profiles showed nearly constant release rate of these antibiotics from the dressings, ensuring a prolonged presence of the antimicrobial agents at the wound site (Altun et al., 2021). The hydrophilicity of BC favored a moisture environment for an adequate healing process. BC has also been combined with PLA to prepare composite dressings using 3D printing. While the macroporous structure was controlled by the layer-by-layer deposition of the ink, the presence of BC directly impacted the hydrophilicity and microporous structure of the composite dressings. The study also investigated the effect of different surface topologies, including circular pore-like and stripe-like structures, on cell growth, resulting in higher proliferation rates (over 80%) on micron-sized pore structures compared to smooth surfaces (Wu, Wang, Wang, Huang, & He, 2022).

BC can also be modified to modulate its printability and biological properties. For instance, a recent study reported the carboxymethylation of BC to obtain carboxymethylated BC (CMBC) (Fig. 5A). The surface charge of anionic CMBC (fiber length: 20, 90 and 800 μm) was modified by introducing chitosan to form cationic surface-functionalized CMBC (fiber length: 1000 μm). Anionic and cationic CMBC inks were individually loaded in separate cartridges and extruded using coaxial nozzle. The blending of both inks resulted in rapid crosslinking *via* ionic bonding between the chitosan and carboxyl groups on the CMBC surface, thus improving shape fidelity and the mechanical integrity and stability of the printed dressings. The combination of cationic and anionic CMBC in different ratios led to dressings with tunable structural and mechanical properties, which was also influenced by the pH and charge stoichiometry (Fig. 5B). The dressings were evaluated *in vitro* to determine the cell viability using rat heart microvessel endothelial cells, resulting in high cytocompatibility (>80%) (Hospodiuk-Karwowski et al., 2022).

Overall, the feasibility of obtaining highly pure BC at low cost while preserving the high surface area and reactivity is prompting the use of BC in wound dressings and their translation to the clinic. However, compatibility with other polymers to achieve the desired properties for 3D printing still requires further assessment.

3. Cellulose ether-based 3D printed wound dressings

Cellulose ethers are widely used in the biomedical field. Compared to other cellulose derivatives, cellulose ethers derivatives are characterized by a high molecular weight and, in most cases, aqueous solubility. The etherification of cellulose molecules is typically achieved by nucleophilic reaction of cellulose hydroxyl groups with electrophiles, *i.e.* alkyl halides and epoxides, to the desired degree of substitution (Fig. 6) (You et al., 2022). Cellulose ethers can be tuned with distinct properties, including water absorption and retention, shear-thinning behavior, and thermoplastic film capacity, by changing the alkyl, hydroxyalkyl, or carboxyalkyl groups. Through their unique role in controlling viscosity and interfacial interactions, cellulose ethers facilitate the development of printable inks and bioinks with optimal rheological properties and reproducibility (Dai et al., 2019; Wan Jusoh et al., 2022).

3.1. Carboxymethyl cellulose-based 3D wound dressings

Sodium carboxymethyl cellulose (CMC), also known as carmellose, is a derivative of cellulose with carboxyl groups replacing some of the hydroxyl groups (Fig. 6). CMC is the most used cellulose ether in the pharmaceutical industry, mainly as an excipient in pharmaceutical formulations but also in tissue engineering scaffolds and wound dressings. CMC-based products are characterized by excellent biocompatibility with mucosa and skin, water absorption capacity, water vapor permeability, and hydrogel-like behavior. These properties make CMC an attractive material for wound healing applications due to its non-immunogenicity, exudate-sorption capability, and cost-effectiveness (Zennifer, Senthilvelan, Sethuraman, & Sundaramurthi, 2021). Moreover, CMC-based hydrogels can be combined with bioactive molecules for the development of active dressings with specific functionalities (Table 3). In fact, there are several clinically available CMC-based dressings containing silver nanoparticles as antibacterial agent (Minsart, Van Vlierberghe, Dubruel, & Mignon, 2022).

Recently, 3D printing technology has introduced CMC as a promising material for the development of personalized wound dressings. One notable example is the incorporation of ε-polylysine into CMC-based hydrogels (CMC viscosity: 800–1200 mPa·s) to confer antibacterial activity against *E. coli* and *S. aureus* (Table 3, entry #1). The hydrogel modification involved the introduction of glycidyl methacrylate (GMA) through UV light polymerization. The synthesis of ε-polylysine-GMA and CMC-GMA was achieved through ring-opening reactions under acidic conditions with GMA. Dressings fabricated from this ink perfectly fit for large, irregularly shaped wounds with significant levels of exudation. Moreover, the dressings effectively prevented *E. coli*, and *S. aureus* growth and mitigated the negative effect of reactive oxygen species (ROS) on fibroblast proliferation *in vitro*. A rat model with a full-thickness infected defect was employed to assess the *in vivo* potential of the 3D printed dressing. The findings revealed that the composition and antibacterial properties of the dressings played a crucial role in effectively preventing wound infection. Moreover, the dressing showed

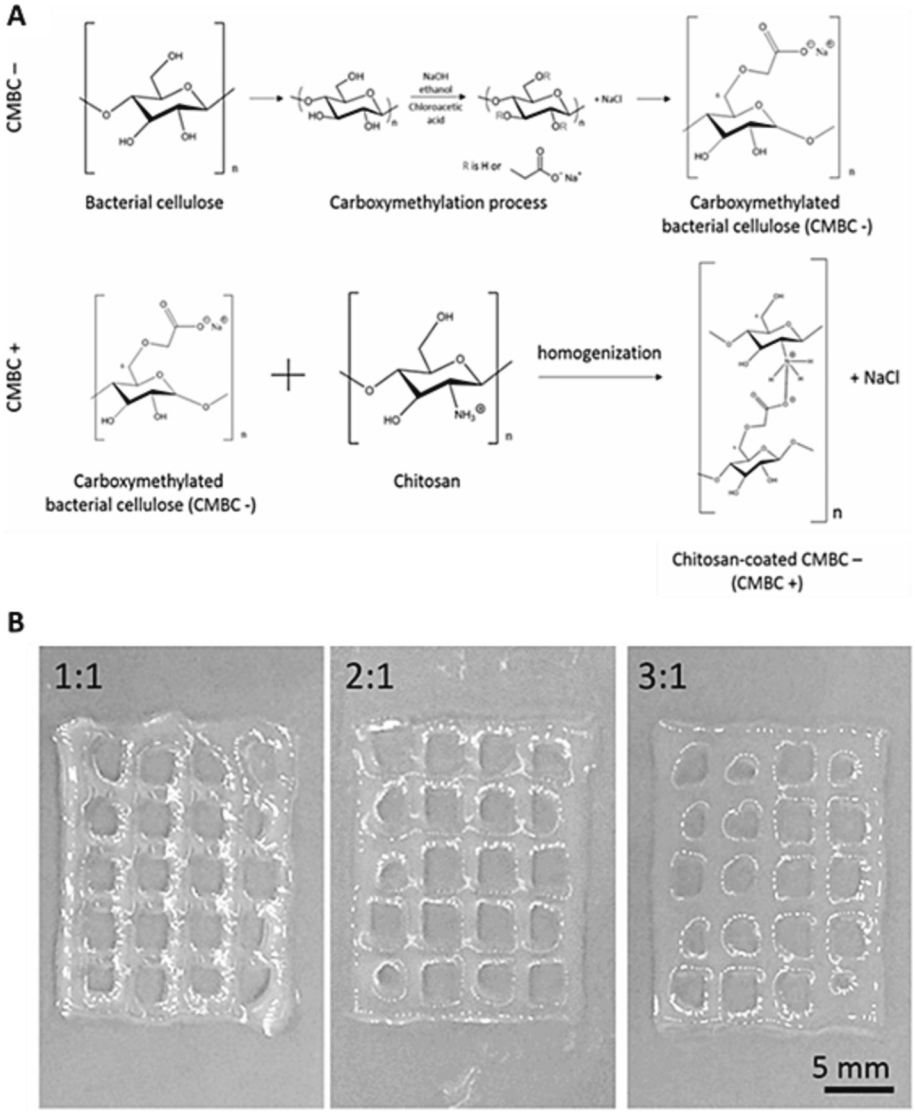

Fig. 5. (A) The functionalization of BC *via* carboxymethylation resulted in the formation of carboxymethylated BC (CMBC(−)) that was further mixed with chitosan to generate positively charged CMBC (CMBC(+)). (B) Representative images of a printed dressings using CMBC(+):CMBC(−) 1:1, 2:1 and 3:1 ratios. The highest printability and shape fidelity was achieved using inks prepared with a 1:1 ratio.
Adapted from Hospodiuk-Karwowski et al. (2022) with permission from Elsevier.

significant wound healing by promoting collagen deposition, revascularization and reepithelialization, compared to commercial dressings (Wang et al., 2021). Similarly, a hydrogel composed of CeO_2/N-halamine nanoparticles, CMC (Mw: 700 kDa, degree of substitution (DS): 0.9, viscosity: 2500–4500 mPa·s), xanthan gum, and GelMA exhibited wide spectrum antibacterial properties, effectively preventing infections during the wound healing process (Table 3, entry #2). The inclusion of CeO_2/N-halamine nanoparticles provided additional antimicrobial activity to the CMC-based hydrogel, enhancing its efficacy in wound management (Yang, Ren, & Liu, 2021).

In addition to antimicrobials, CMC dressings have also been investigated for their potential to incorporate other therapeutic agents, including anti-inflammatories and anesthetics, to address pain and inflammation in wound healing. For instance, dressings prepared using alginate/CMC inks were loaded with diclofenac sodium or lidocaine with the purpose of relieving patient discomfort associated with wound pain (Table 3, entry #3). The combination of alginate and CMC (Mw: 90 kDa, DS: 0.7) gave inks with high printability and reproducibility. The dressings exhibited a precise controlled drug release over a 2-days period, starting within the first 30 min. This drug release pattern enabled a quick relief and alleviation of pain in wounds (Maver et al., 2018).

Growth factors have also been loaded into CMC-based hydrogels for wound healing applications (Diaz-Gomez et al., 2022). A recent example involved the loading of platelet-rich plasma (PRP) in CMC dressings to promote angiogenesis and wound healing (Table 3, entry #4). The inks were prepared by dispersing CMC (Mw: 395 kDa, DS: 0.9, viscosity: 2 % NF 2960 mPa·s, 8.8 % Na) in sterile water, followed by direct extrusion printing to fabricate dressings. To sterilize and crosslink the printed dressings, a single-step procedure using citric acid as a crosslinker was employed. Subsequently, the dressings were incubated in PRP, allowing for the incorporation of growth factors. *In ovo* and *in vivo* studies were conducted to evaluate the effect of the composition and growth factors release on cell migration (Fig. 7A, B) and proliferation (Fig. 7C). Angiogenesis and wound closure tests in a diabetic wound model in rat evidenced the advantages of combining both CMC and PRP (Fig. 7D, E) in the detrimental environment of diabetic ulcers. CMC was crucial to maintain a moisture environment and control the release rate of the growth factors present in the PRP (Diaz-Gomez et al., 2022).

Tissue-engineered skin grafts have been proposed as effective tools to

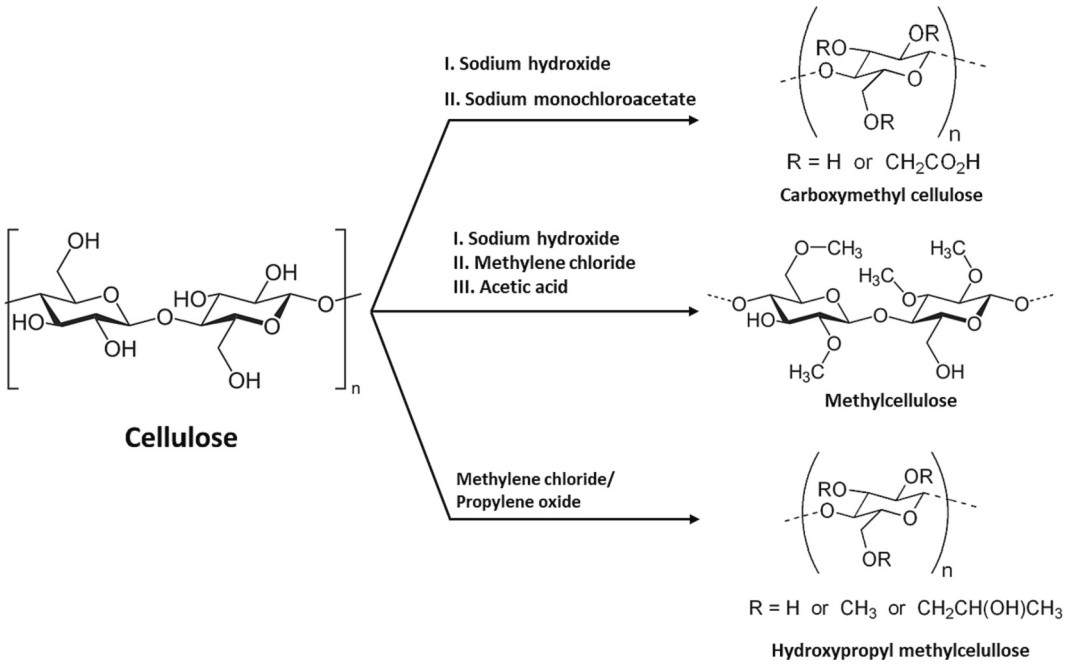

Fig. 6. General methods for the preparation of cellulose ethers.

Table 3
3D printed scaffolds made of CMC for wound dressing.

Entry	Composition	Crosslinking agent	Targeted microorganism	Results	Reference
#1	CMC/Ɛ-polylysine (*CMC*: *viscosity*: *800–1200 mPa·s*)	Glycydyl methacrylate (GMA) *via* UV light	*E. coli* and *S. aureus*	Dressings with antioxidant and antibacterial properties enhanced the expression of VEGF and CD31, accelerating tissue granulation and regeneration.	(Wang et al., 2021)
#2	CeO2/N-halamine nanoparticles/CMC/xanthan gum/GelMA (*CMC*: *Mw*: *700 kDa*, *Degree of substitution* (*DS*): *0.9*, *Viscosity*: *2500-4500 mPa·s*)	MA groups	*S. aureus* and *E. coli*	The scaffolds prevented wound infection and possessed good biocompatibility and blood clotting capacity.	(Yang et al., 2021)
#3	Alginate/CMC/diclofenac sodium (DCS)/lidocaine (LID) (*CMC*: *Mw*: *90 kDa, DS*: *0.7*)	–	–	Dressings showed good stability, porosity, and sustained drug release for up to 2 days. Combination of CMC with LID and DCS showed promising activity for managing painful wounds.	(Maver et al., 2018)
#4	NaCMC/PRP (*CMC*: *Mw*: *395 kDa*, *DS 0.9*, *viscosity*: *2% NF 2960 mPa·s*, *8.8% Na*)	Citric acid	–	Dressings promoted angiogenesis and re-epithelialization in diabetic wound models.	(Diaz-Gomez et al., 2022)
#5	SA/NaCMC (*CMC*: *Mw*: *265 kDa*)	$CaCl_2$	–	Dressings showed improved stability, durability, mechanical strength, and prolonged degradation kinetics.	(Zhang et al., 2021)
#6	CMC/tyramine (TYR) (*CMC*: *Mw*: *90 kDa* *DS*: *0.7*)	Metal-complex tris (2,2′-bipyridyl) dichlororuthenium (II) hexahydrate ($[RuII(bpy)_3]^{2+}$)	–	Incorporating CMC-Tyr as a precursor allowed for the control of porosity in the composition of the dressings.	(Al-Abboodi et al., 2019)
#7	Alginic acid/CMC (*CMC*: *Mw*: *700 kDa*, *DS*: *0.9*)	$CaCl_2$	–	The scaffolds were biocompatible and guided the wound healing process.	(Milojevic et al., 2021)

regenerate large wound defects. However, there is a lack of suitable materials as components for bioink development in wound regeneration that could recapitulate the composition and structure of skin ECM. A study proposed sodium alginate (SA)/CMC blends as a bioink for 3D printing artificial skin (Table 3, entry #5). Different compositions of SA/CMC hydrogels were printed and characterized, revealing the distinct biocompatibility and degradation rates of the resulting dressings. The incorporation of CMC (Mw: 265 kDa) in the composition significantly improved the mechanical strength and cytocompatibility of the dressings. The potential of the dressings to promote wound regeneration were evaluated in large skin defects using a rabbit model. The results showed that the 3D printed dressings promoted wound regeneration similar to autologous skin grafting (Zhang et al., 2021).

Recently, a novel approach for manufacturing *in situ* tissue dressings using visible light to control the crosslinking of 3D printable hydrogels was reported (Table 3, entry #6). Gelatin-hydroxyphenylpropionic acid conjugate (Gtn-HPA) and CMC (Mw: 90 kDa, DS: 0.7)-tyramine (CMC-Tyr) were blended to prepare *in situ* printable inks. The rapid photo-crosslinking and non-cytotoxicity of the inks, and the excellent mechanical properties of the printed dressings make them excellent candidates for wound dressing applications. CMC-Tyr led to a significant porous microstructure within the printed dressings, enabling high

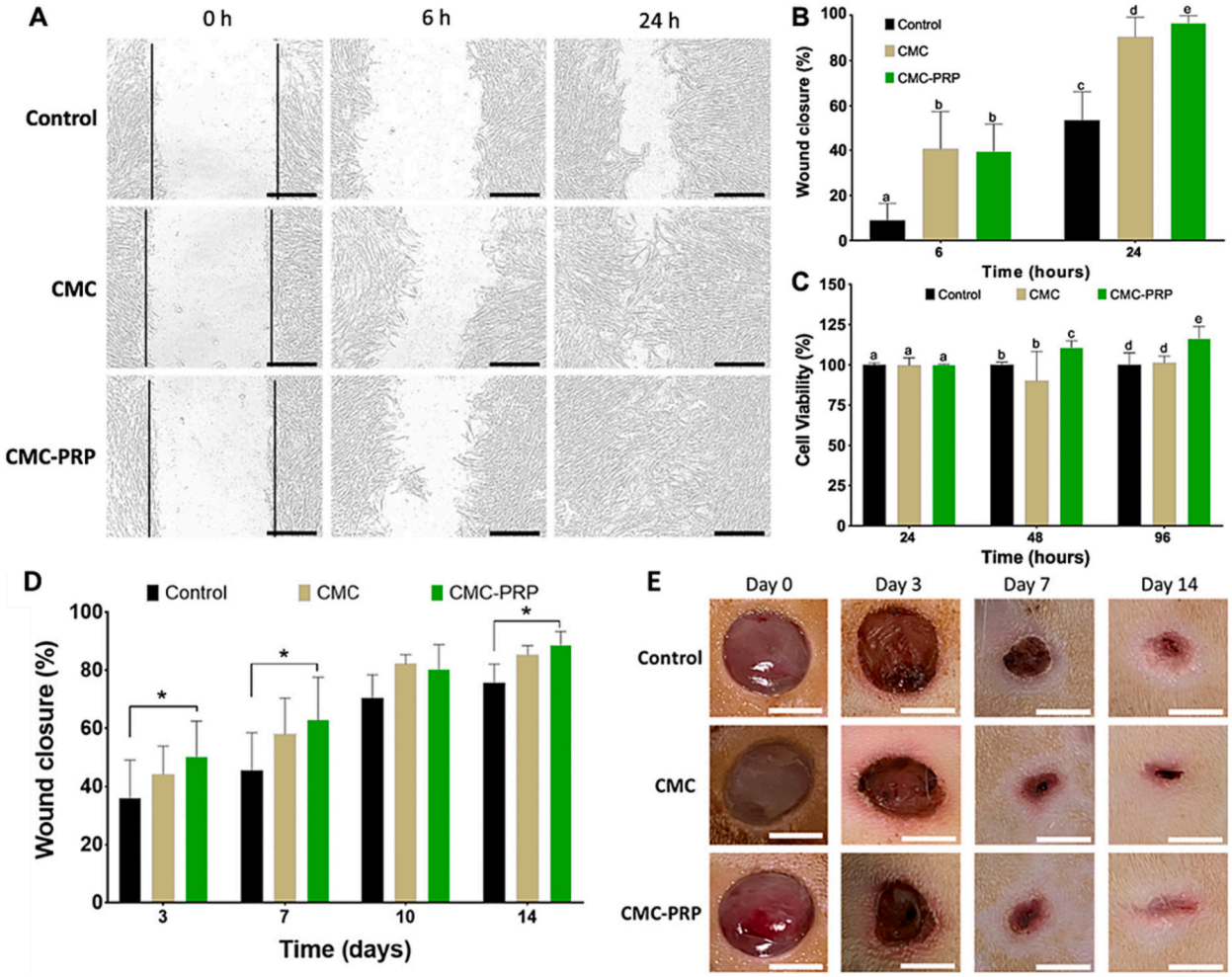

Fig. 7. The effect of CMC and CMC-PRP dressings on the migration and proliferation of mesenchymal stem cells. (A, B) Scratch assay showing gap reduction after 6 and 24 h of culture in release medium from CMC and CMC-PRP dressings. (C) Proliferation of mesenchymal stem cells cultured with CMC or CMC-PRP release medium. (D) Quantification of the *in vivo* wound healing process in a full thickness diabetic rat wound model assessed by wound contraction expressed as a percentage of the initial area at different days post-wound infliction, and (E) representative images depicting wound healing in both treated and untreated groups. Adapted from Diaz-Gomez et al. (2022) with permission from Elsevier.

swelling degrees and the ability to release encapsulated bioactive molecules. Additionally, the straight-forward *in situ* extrusion printing enables the incorporation of labile bioactive agents, such as growth factors, expanding their use as active wound dressings (Al-Abboodi et al., 2019).

A recent development in hybrid extrusion-based bioprinting enables the simultaneous printing of stiff thermoplastic polymers and softer hydrogels at different temperatures (Table 3, entry #7). This strategy renders hybrid hydrogel-thermoplastic polymer scaffolds with tunable structural and chemical properties for tissue engineering and regenerative medicine applications. By employing a layer-by-layer deposition approach, a soft hydrogel matrix based on alginate and CMC (Mw: 700 kDa, DS: 0.9) was integrated into a stiffer synthetic framework made of PCL. The hybrid fabrication approach allowed for the precise control of filament thickness, pore size, macro, and micro-porosity, as well as functional properties such as swelling capacity, degradation rate, and wettability. The resulting scaffolds exhibited a range of properties between pure CMC and pure thermoplastic polymers, including controlled degradability, permeability, and mechanical strength. The hybrid scaffolds also showed good biocompatibility and the potential for controlled drug release. This methodology led to mechanically superior scaffolds by combining soft hydrogels with stiff polymers, opening new strategies for tailoring scaffolds to mimic the native microenvironment and promote the wound healing process (Milojevic et al., 2021).

In summary, the incorporation of bioactive molecules such as ε-polylysine, antimicrobial nanoparticles, anti-inflammatories, anesthetics, and growth factors into CMC-based hydrogels has demonstrated the potential to enhance wound healing. Main difficulties for 3D printing are related to the concentration-dependent viscoelasticity of the CMC inks, which demands further studies in the identification of the adequate ratio and the adequate adjuvants.

3.2. *Methylcellulose-based 3D printed wound dressings*

Methyl cellulose (MC) is widely used in the pharmaceutical industry as an emulsifying excipient. The production of MC involves mercerization, etherification, and purification steps (Fig. 6). Cellulose is treated with 50 w/v% of NaOH for 24 h. Then, the solution is filtered, and the residue is exposed to methylene chloride. Finally, the solution is neutralized with acetic acid, filtered, and dried in an oven at low temperature (Vieira et al., 2012). Recently, MC has gained significance in regenerative medicine due to its inverse thermal gelling properties and thixotropy, which favor the printability and stability of hydrogels. Moreover, the gelation temperature of MC can be fine-tuned by the addition of salts to match skin temperature, so the wound dressing rapidly undergoes gelation after placing at the wound site. A recent study optimized gelling properties of a MC (6 w/v%) ink by incorporating sodium citrate and sulfate salts (0.5–0.75 w/v%). The inks were also loaded with distinct drugs for burns treatment, *i.e.*, lidocaine,

ibuprofen, amoxicillin, or silver sulphadiazine (Table 4, entry #1). Moreover, a computational fluid dynamics model was used to investigate the relationships between the structure of the printed hydrogel and the printing parameter (Teoh, Abdul Shakoor, & Wang, 2022).

MC can also be blended with other biodegradable polymers for developing personalized, 3D printed wound dressings. In a recent study MC-alginate (MC viscosity: 15 mPa·s) inks were prepared incorporating bioactive agents, *i.e.*, Manuka honey, *aloe vera* gel, and eucalyptus essential oil (Table 4, entry #2). The printability and printing accuracy of the inks was significantly improved in inks with higher MC:alginate ratios, also exhibiting adequate swelling properties under moist conditions and effective antimicrobial and antibiofilm activity against a variety of bacteria. The dressings showed excellent biocompatibility with human dermal fibroblasts *in vitro*. Moreover, the wound scratch assay evidenced that the inks significantly increased cell migration, as compared with untreated controls (Karavasili et al., 2020).

Moreover, MC hydrogels have been used as bioinks for incorporating fibroblasts. Alginate-MC (DS: 1.50–1.90, viscosity: 1500 mPa·s at 2 w% in water) inks containing gallium nitrate as a crosslinker and antibiofilm agent were recently reported. The inks showed good printability due to the shear thinning and self-healing properties of MC, which are crucial for high-resolution 3D printing (Fig. 8). The incorporation of gallium nitrate ensured effective antibacterial activity against *S. aureus* and *P. aeruginosa* (Table 4, entry #3). Optimization of gallium nitrate concentration ensured antibacterial activity without compromising cell viability. This research exploited the potential of gallium nitrate as a crosslinker to enhance the antibacterial properties of the dressing while maintaining biocompatibility (Rastin et al., 2021).

3.3. Hydroxypropyl methylcellulose-based 3D printed wound dressings

Hydroxypropyl methylcellulose or hypromellose (HPMC) is a semi-synthetic derivative of cellulose that has found applications in various biomedical devices, including wound dressings. HPMC is synthetized by partially replacing the hydroxyl groups of cellulose with hydroxypropyl and methoxyl groups (Brady, Dürig, Lee, & Li, 2017). This modification is achieved through a reaction involving alkyl cellulose exposed to a mixture of methylene chloride and propylene oxide (Fig. 6). HPMC has been widely used in several biomedical devices, such as contact lenses and, recently, in the field of wound dressings (Tudoroiu et al., 2021). HPMC exhibits remarkable water absorption capacity, enabling the maintenance of an optimal moisture level to facilitate faster wound healing (Yin, Fang, Xu, & Ahmed, 2021). In addition, HPMC is biodegradable and biocompatible, reducing the damage caused by dressing changes during the healing process. In many cases, the dressing does not need to be removed, minimizing patient discomfort. HPMC is also frequently used as thickening agent to optimize the rheological properties of inks, thereby enhancing their printability and enabling the development of patient-specific wound dressings (Cheng, Shi, Jiang, Wang, & Qin, 2020; Polamaplly et al., 2019).

One innovative application of HPMC in wound dressings involves the preparation of superporous dressings. A recent study used a combination of HPMC (33 w/w%) and polyacrylamide (PAM), incorporating silver nanoparticles (AgNPs) as personalized wound dressings (Table 4, entry #4). The printing and further crosslinking of the inks resulted in semi-interpenetrating polymer network dressings with tunable structural and swelling properties. The incorporation of HPMC in the formulation was critical for achieving dressings with an open, interconnected porosity. The polymeric network controlled the release of AgNPs, ensuring a balance between cytocompatibility and antibacterial activity in the dressings. The highly porous architecture of the dressings facilitated rapid water uptake, while the macropores created by the 3D printed templates buffered the swelling and reduced the risk of detachment from wounds. The crosslinked dressings also promoted faster wound healing *in vivo*, leading to reduced scar formation and enhanced new tissue, compared to non-loaded AgNP controls (Wu & Hong, 2019).

Overall, the reports on HPMC-containing inks for 3D printed wound dressing are still just a few and more information is needed to fully elucidate the advantages of this polysaccharide.

4. Conclusions and future perspectives

The combination of cellulose derivatives and 3D printing technology holds immense potential to develop wound dressings with precise geometries, customizable designs, and patient-specific features. As outlined in this review, researchers can now design wound dressings that perfectly conform to the wound site, optimizing contact and promoting accelerated healing. Traditional cellulose-based films, although widely used, have limitations in treating complex diabetes wounds as they lack patterning for cell guide and porosity for regulation of fluids and gases exchange. Differently, 3D printing allows for the creation of intricate structures and the incorporation of a wide range of bioactive functionalities, such as precise localization of drugs and growth factors and their controlled release, thereby facilitating personalized wound therapies. The rapid on-demand manufacturing of wound dressings not only reduces production time and costs but also enhances accessibility and patient comfort.

Compared to synthetic polymers, cellulose, as the most abundant natural polymer on Earth, offers a sustainable and renewable resource derived from diverse biomass sources, including agricultural waste and dedicated crops. Cellulose derivatives stand out due to their unique properties that make them highly suitable for wound dressings and 3D printing inks. Their inherent biocompatibility and biodegradability minimize the risk of adverse reactions and seamlessly integrate with the human body. Additionally, cellulose derivatives communicate

Table 4
3D printed scaffolds made of MC and HPMC for wound dressing.

Entry	Composition	Crosslinking agent	Targeted microorganism	Results	References
#1	MC (*6 w/v%*)/drug (*0.5–0.75 w/v %*) Lidocaine hydrochloride, Ibuprofen, Amoxicillin, Silver sulfadiazine	Trisodium citrate dihydrate and sodium sulfate	–	3D printed dressings provided sustained drug release.	(Teoh et al., 2022)
#2	Alginate/MC/bioactive molecules Manuka honey, *aloe vera* gel, eucalyptus essential oil (*MC*: *viscosity 15 mPa·s*)	$CaCl_2$	*S. aureus* and *E. coli*	The dressings demonstrated anti-biofilm capacity, maintained a moist wound environment and effectively absorbed excess exudate.	(Karavasili et al., 2020)
#3	Alginate/MC/gallium (Ga^{3+}) (*MC*: *DS*: *1.50–1.90*, *viscosity*: *1500 mPa·s at 2 w% in water*)	Gallium nitrate	*S. aureus* and *P. aeruginosa*	Dressings exhibited antibiofilm activity due to gallium that acted as a crosslinker and antibacterial agent.	(Rastin et al., 2021)
#4	HPMC (*33 w/w%*)/PAM/silver nanoparticles	–	*S. aureus* and *E. coli*	Customized porosity dressings ensured faster healing compared to commercial dressings.	(Wu & Hong, 2019)

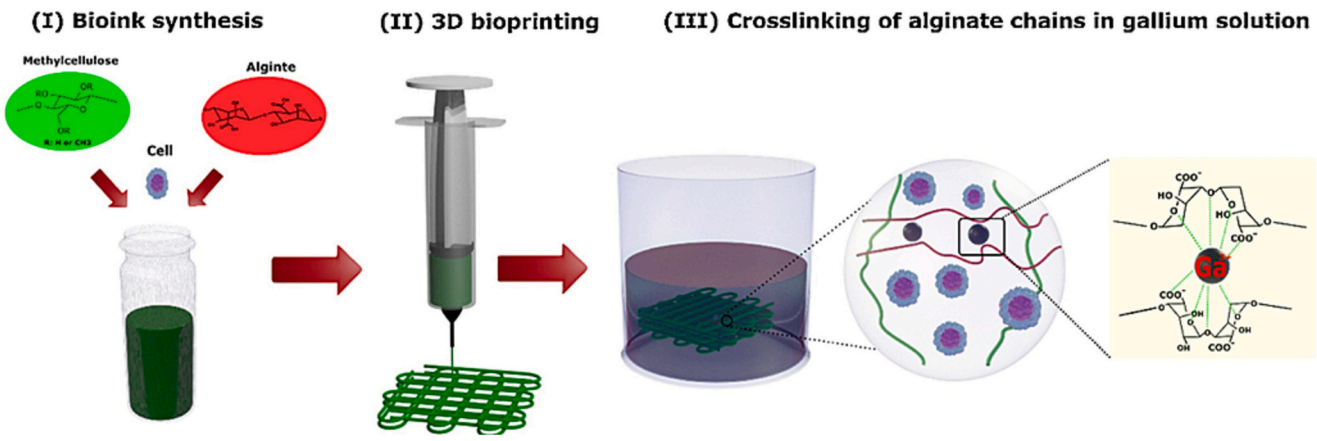

Fig. 8. (I) Preparation of a cell-laden antibacterial bioink based on MC/alginate hydrogels suitable for (II) the creation of multi-layered dressings with high printing resolution. After printing, (III) the crosslinking of alginate chains with gallium cations leads to mechanically robust structures. Moreover, gallium endorses the dressings with effective antibacterial activity against both gram-negative and gram-positive bacteria.
Reproduced from Rastin et al. (2021) with permission from Elsevier.

exceptional mechanical properties, including high strength, flexibility, and porosity. This review highlighted the potential of incorporating cellulose derivatives into inks as an effective strategy to improve control over ink rheology during the printing process. The shear thinning behavior exhibited by cellulose derivatives enables the printing of objects with exceptionally high resolution. This property allows for precise deposition of ink during printing, allowing on-demand manufacturing of intricate and detailed structures. Relevantly, the capability of celluloses to act as traps of ions that are vital for bacteria and to release killer metal ions may help addressing the multidrug resistance in wound healing applications. Nevertheless, as any other material of natural origin, precise characterization, and identification of critical variables (*e.g.*, degree of substitution, molecular weight, particle size) that determine the quality of the final product is needed for product standardization. Although quality-by-design (QbD) protocols and more preclinical studies are needed, the so far obtained *in vitro* and *in vivo* results evidenced that cellulose-based dressings offer multifunctional properties, including antibacterial activity, antioxidant effects, and controlled release of bioactive factors that can be tailored to address the specific needs of diabetic wound treatment.

It is foreseeable that the recent but intense research on cellulose-based 3D printed wound dressings will soon be reflected in its translation to the clinic. As of today, the search on the web https://clinicaltrials.gov/ for "3D printing AND cellulose" does not reflect any clinical trial, unlike "cellulose wound dressing" with 24 outcomes or "3D printing AND wound" with 71 outcomes. However, it should be mentioned that this later group of outcomes includes materials for tissue regeneration other than skin tissue and also orthosis. In parallel with improving preparation techniques, cellulose-based 3D-printed dressings need to meet safety and efficacy requirements set forth in medical device regulations. When active substances are incorporated, the line that separates a medical device from a medicine becomes increasingly narrow. The regulatory process as medical device will depend on whether this active substance is considered only ancillary, with the cellulose framework assuming the primary mode of action in wound healing. On the contrary, if it is the drug that plays the fundamental role in the wound healing, the product will be considered a medicine and should follow the regulation for drugs.

Regulatory authorities play a vital role in setting standards and guidelines to ensure quality control throughout the manufacturing process. For example, the standard ISO/TC 261 aims to streamline biofabrication approaches to enable consistent and reliable development of medical devices and products obtained applying additive manufacturing. Regarding safety, the FDA has addressed regulatory considerations associated with 3D printing of medical devices by recognizing the unique features of biofabrication and providing technical considerations for additive manufactured medical devices. The guidance emphasizes the importance of ensuring safety, quality, and effectiveness in the manufacturing process and final product. The FDA also recommends conducting biocompatibility testing, following the guidance provided in the ISO 10993-1 standard, to assess the potential biological risks of the final device. On the contrary, other countries including UK and EU have no specific regulations on 3D printed biomaterials intended for regenerative medicine, thus they are regulated by guidelines for medical devices, biologics, and cell therapy. Added to these regulatory difficulties is the consideration of some cellulosic products as nanomaterials (one dimension no larger than one hundred nanometers), especially in the case of CNFs, CNCs, and BC particles. Nanomaterials are subject to specific risk assessment rules, both by general chemicals legislation (REACH) and by sectoral legislation addressing their use in certain products, including medical devices (EuropeanCommission, 2022; FDA, 2022). Therefore, updated and refined regulations and guidelines are needed to ensure the safety and efficacy of 3D printed medical devices and combination products, including wound dressings.

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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

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

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