Review

Are Mechanically Adjusted Cellulose Nanocrystal (CNC)-based Bio-targeted Hydrogels Satisfying the Requirements of Biologically based Applications?

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Abstract: Cellulose nanocrystals (CNCs) are a kind of nano-inclusions that have experienced tremendous expansion in the material industry due to their outstanding mechanical qualities, sizable surface area, and capacity for functional tuning. Due to their vast potential, however, present practises fall short of fully using them. For example, employing CNCs in various matrices with various surface chemistries can be challenging; this problem tends to get worse if the focus is furthered on bio-based applications. This paper reviews the use of CNCs as fillers in natural and man-made polymers; we have explored in depth the production, characterisation, and of CNCs from various sources and their inclusion into various matrices. Surface alterations and the introduction of CNCs in biodegradable polymer can have a significant impact on several industrial behemoths such as tissue engineering and biomedical applications; therefore, pursue of current manuscript is extremely warranted. Throughout the manuscript various assembly techniques that involves alteration and adjustment of polymer network in building up a hydrogel with higher fracture energy and mechanical properties are also included. From rheological perspective the hydrogel processing is also discussed with some models routinely used in the literature to describe these hydrogels. Finally, bio-based hydrogels mechanically reinforced with CNCs such as Xanthan Gum, Alginate, protein, and polysaccharides were discussed.

Keywords: cellulose; hydrogel; nanocrystals; 3-D printing

1. Introduction

The most abundant polymer on earth capable of being naturally produced in plant and animal kingdom is cellulose [1-5]. Cellulose is better however to be processed into nanomaterials such as cellulose nanocrystals (CNCs) to boost their potential. First in 1940s and 1950s CNCs a by product of cellulose produced through acid hydrolysis process was ushered into material world [6-8]. CNC can be sourced from multiple resources; in fact CNC can be found abundantly in nature; however the most quoted and preferred platform for nanocellulose extraction to meet the twin aim of waste valorization and environmental conservation is through lignocellulosic biomass[9]. These ecologically safe and noncytotoxic[10] nanoparticles exhibit unusual qualities such as extremely large surface area (150-300 $m^2.g^{-1}$)[11-12], high tensile strength (7.5-7.7 GPa)[13], high elastic modulus (110-220 GPa)[11], and fascinating optical and electrical properties[14]. These properties make them appealing for a wide range of applications, including reinforcing of ceramic and polymeric matrices, medication delivery, foam stabilization [15], supercapacitor creation[16], and rheological property alteration, to name a few. One aspect of CNC usage is to use them as filler inside polymers; due to CNC elongated shape, high surface area and high mechanical properties creating a lightweight and cost effective sustainable hydrogel should be an easy task but in practice researchers face many challenges [17-18]. The issue surrounding this facet of product manufacturing out of CNC will be explored later on in detail.

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One of the challenges stems from mixing CNC suspension with water soluble polymers dope. Suspension of acid hydrolyzed CNCs suspension due to their negative charges are Brownian, i.e., they move around suspension with thermal motion of media molecules. Therefore, CNC charges are very important when mixing with water soluble polymer and dispersion and distribution of CNCs are important for ensuring CNCs are well distributed across the matrix of polymer for best reinforcement[19]. The creation of hydrogen bonds is encouraged by the prevalence of hydroxyl groups on CNC's surface[14, 20]. Their innate hydrophilicity can lead to poor wetting and insufficient dispersion, especially when combined with weak polar or nonpolar polymers[21-22]. The final thermo-physical and structural characteristics of the nanocomposite are reduced as a result of these two factors combining to form nanocrystal aggregations[23-24]. One of the most well-known and successful methods to avoid aggregation in hydrophilic polymeric matrix and to enhance their interfacial contact is potentially face by adding moiety to the CNC surface[25-26]. This challenge of surface modification of CNC to meet bio-based applications aims will be also explored later in the manuscript.

In case of poor mixing with the host polymers, several approaches have been suggested, with chemical alterations of CNCs being the most popular. Heux et al. [27-28] formed a coated layer around the CNCs using phosphoric esters as a surfactant and observed good dispersion in hydrophobic molecules polymer. Cellulose oxidation caused by the 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO) has also been found to increase cellulose solubility in organic solvents and has been claimed to have notable impacts on nano-fibrillation of CNF as well [29-30]; TEMPO-oxidation also provide better thermal stability as opposed to bare CNCs. In fact, this route of chemical treatment is deservedly regarded as the most effective method of performing surface oxidation of cellulosic materials and isolating nanofibrils from fibres[31]; the method also produces more consistency in term of properties and maintaining them as negatively charged carboxyl groups are less labile than sulphate half-ester groups[31]. Furthermore, TEMPO's toxicity and recycling are noteworthy mentions[32].

There are other methods that can produce less polar CNCs. In fact, boosting hydrophobicity can also equally effectively be implemented using esterification[33] and silylation[34]. Solvent exchange[35-36] and other techniques even though able to boost the dispersion of CNCs have several drawbacks. The surfactants around CNCs, for instance, serving as a plasticizer, softening composites[37], and other chemical changes and solvent exchange may compromise the integrity of celluloses and need difficult processing techniques[38-39].

Another criterion to consider is geometry of CNCs. CNC size and shape play a deterministic influence on its mechanical properties and in case of mixed with polymers in general, these polymer can be polylactic acid (PLA)[36, 40], starch [41-42] and poly (vinyl acetate) (PVAc)[43] among others. Due to hydrophilic nature of CNCs; however, unsatisfying improvements brought on by cellulose nanoparticles' poor dispersion thermodynamically driven, though, were observed in some cases in refs [40, 44], and clearly in ref [45], resulting in an attempt among academic researchers to address the shortcomings. Size and shape of CNCs are determined by generally how aggressive acid hydrolysis is allowed to wash away impurities and amorphous regions around CNCs, type of processing route employed, and sources of CNCs. Acid hydrolysis can shorten length of CNFs to CNCs and later with further modification even spherical CNCs are producible.

A variety of polymer groups have been chosen to be combined with CNCs; these polymers already fulfil the material requirements of the goods they are used in; CNC are only used in these situations to enhance or modify their features. Acrylate-based matrix and polyacrylamide constitute another major group of polymers to make added value products with CNCs. Due to innate functional groups possessed; copolymerization and cross-linking are relatively easy to implement [46]. One of the most notable example is thermoresponsive poly N-isopropylacrylamide (PNIPAM) polymer matrices, showing temperature dependent swelling that interactively changes mechanical properties [47-48]. For implementation of tweak inside mechanical properties of these hydrogels reinforced with CNCs it is a usual practice to use small molecule cross-linkers such as methylene

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bisacrylamide (MBA) [48-50]. These hydrogels are permeable enough to let CNC be exposed to the flow of material within, as Zhou et al. [51] demonstrated the ability of physical polyacrylamide gel packed with CNC to absorb methylene blue.

In case of deciding not to use CNCs inside a polymeric host matrix; one can make 3-D spanning matrix of CNCs using self-assembly process. Self-assembly is a wonderful and widespread process in nature and in organisms [52]. It refers to the spontaneous organising of molecules into more prominent and organised patterns. Self-assembly events occur on a wide range of sizes, from small cells to the massive human body. Significantly, self-assembly processes are crucial for "bottom-up" nano - structured formation, which is predominantly driven by thermodynamics. Nevertheless, kinetics play an important role in structural regulation and functional integration[53]. Here, self-assembly refers to CNC joining together to form 3-D spanning hydrogels, either by auto aggregation due to the large concentration of these rods or by adding salt to screen their charge so they are not impacted by electrostatic repulsion.

Finally, Over the past few years, researchers have studied CNC-based nanocomposites (functionalized or not). These studies have been reported in several review evaluations, such as those by Dufresne[54-55], Eichhorn et al.[17], Habibi et al. [56-57]and others [58-61] and although these assessments are of high quality, they are unable to cover a subject that develops so swiftly and sees the publication of hundreds of publications annually; therefore addition of current manuscript to the literature to update the reports is warranted. The current review may provide additional and useful information for researchers. Particularly emphasised is the recent use of functionalized CNC as fillers in nanocomposites. In this work, the mechanical properties of CNC pure gels, CNC-nanocomposite hydrogels with bio-based and synthetic polymers, and certain bio-based applications based on CNC integration are explored.

2. Hydrogel Characteristics

Mechanical properties of CNC based hydrogels

For some colloidal particles including CNCs, the colloid crossover (sol-gel transition), and isotropic-anisotropic transitions (liquid crystal formation), determines the final physical characteristics and structural characteristics of CNC suspensions. To define sol-gel transition criteria, systems with solid-like qualities known as colloidal gel networks must fulfil two conditions: (i) the formation of 3-D spanning networks, and (ii) local kinetic arresting of particles [62]. Increasing the loading concentration [63-64]; mixing with macromolecules [65-67]; mixing with organic coagulants [65, 68-69], mixing with surfactants [70-71], hydrothermal methods[72], or using routine freeze-thaw cycling [73] are just a few of the ways that CNC suspension can be made to gel. To obtain a CNC based gel one has to gelify CNC this can be done through various routes as comprehensively covered in our previous manuscript [53]. In all of these methods; Brownian colloids become viscoelastic and are kept together by attraction forces that are strong enough to withstand thermal motion of water molecules [74]. The slowing of particle diffusion, solid-like viscoelasticity, and other nonlinearities (such as yielding, shear thinning, and divergence from the Cox-Merz rule) are all associated with this transition if one assess these transition with lens of rheology[75-76]. As practical application, several industrial sectors, including food and 3-D printed items, have adopted nanoparticle or CNC gels [77-79]. A revolutionary 3-D direct-write building method for porous scaffolds, photonic crystals, and microfluidic devices are some of the most sophisticated applications [80].

CNCs works well as a filler in hydrogel nanocomposites due to their stiff character (emanating from high crystallinity); however, CNC in pure gel offer poor mechanical properties due to weak connection they posses with surrounding CNCs contrary to other nanofillers that due to their elongated shape; ability to engage in entanglements with their co-fillers. In the work by Ureña-Benavides et al[81]. gel formation occurred at concentration range of 14.5 and 17.3 vol % CNCs without addition of any coagulant[82-85]; additionally, gels were thixotropic and self healing similar to results of other researchers [85-86].

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However, there are two major issues in this scenario. One concentration of CNC is quite high when compared to similarly manufactured gels with much lower CNC concentrations; second, mechanical qualities are poor since there is no mechanism for transferring load between CNCs.

There are measure to be taken to remedies issues such as mixing CNCs with other beneficial ingredient such as polysaccharides[65] that dually gel and strengthen CNC gel. Addition of hydroxyethyl cellulose (HEC), hydroxypropyl guar (HPG), or locust bean gum (LBG) to CNC dispersions induced the gelation of dilute CNC dispersions. In their experiment by adding 3wt% (roughly 2vol%) CNC and 0.2wt% polysaccharide improved storage modulus in linear regime by nearly 3-fold raising storage modulus from 32 to 100 Pa. When these figures are compared to the report of ref[81], it is clear that the storage modulus increases if a mechanism of stress transfer is introduced into the CNC network, such as encapsulating the CNC with soft jelly polymers, as in this case that facilitates stress transfer. Researcher mix CNC and arabinoxylans for food applications as an added bonus they also gelify CNCs[87]. Ways of boosting stress transmission, such as the examples discussed here, can enhance gel strength even further, other areas of improvements are tuning the flexibility of the polymer structure, increasing molecular weight, and so on.

Another venues of gel formation is to screen charges with addition of salt; to provide figures, in one study gel strength grew 100 fold from 10-1000 Pa when salt quantity varied from 1.72-172 mM [88]. Whether this product is applicable to real world practices however is debatable due to high salt content; for instance, when cells are placed in a saline solution with an osmolality higher than the intracellular fluid, the flow rate out of the cells surpasses the rate of flow into the cell. In these cases, cells shrink and deform to the point of non-functioning. This problem can to some degree be remedied using lesser quantity of higher valence salts [89]. For instance, in case of 4wt% (2.54vol%) CNC suspension, the storage modulus rose to 1390 Pa with $CaCl_2$ as compared to 443 Pa with NaCl. At 6wt% gel strength with Calcium gelified system was 2-fold higher than sodium aggregated system. Stronger effect of calcium ions on CNCs compared to sodium was also reported in previous studies[90].

There is dearth research regarding effect of salinity of CNC gel on cells viability. For instance, Bertch et al. [91] also produced hydrogels with the help of CNCs and salt; the biocompatibility of effluence was validated with a HeLa cell assay. The key conclusion from the study was to keep drugs in the stomach before pH-triggered release in the duodenum by using CNC hydrogels as pH-responsive delivery systems. Other methods, as previously mentioned, can produce CNC salt-free pure gels, such as freeze-thaw cycles or hydrothermal routes; however, the economic feasibility of these methods is questionable due to the amount of heating and freezing required, particularly in large-scale operations, impeding the sustainability of these hydrogels.

3. CNC surface modified

As previously stated, good interaction between filler and matrix is required for achieving a matrix that is reinforced with filler; frequently, the matrix used in which CNC acts as a reinforcer is of polymeric nature; most of these polymers are non-polar in nature, so there is little interaction between hydrophilic CNC and hydrophobic matrix; therefore, surface modification of CNCs is required if reinforcement is required.

Despite significant progress, several hurdles remain that prevent large-scale application and commercial usage of CNC hydrophobic modification. These approaches are often plagued by one or more of the following issues: (1) the requirement for successive stages (e.g., solvent exchange and activator connection), (2) the consumption of huge quantities of organic solvents in which CNCs are not colloidally stable, resulting in nonhomogeneous goods, (3) the utilization of costly and nonsustainable reactants, (4) low produces of altered CNCs, and (5) modifications in CNC morphology and degree of aggregation. Yoo et al. [92] successfully avoided a lot of such difficulties by esterifying CNCs in water using bioderived lactic acid and fatty acids, improving the dispersion of modified CNCs in

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organic solvents such as acetone and toluene. However, their reaction needed high temperatures, vacuum, and hazardous catalysts, that did not completely match "green" standards (Sustainability), and the modified CNCs' surface tension was not documented.

Placing a polymer on top of CNC is one notable method of inducing surface modification on CNCs. Larger surface changes, such as connecting polymer chains using a grafting onto CNC technique, have been demonstrated with polypropylene, polytetrahydrofuran, polycaprolactone, polyethylene glycol, and others to enhance contact angle and compatibility with polymer matrixes. However, steric hindrance restricts the graft densities that may be achieved[37, 93-94]. Grafting from a CNC method was therefore employed to achieve larger graft densities. The most popular technique for the synthesis of CNCs with grafted polyesters such as biobased polylactic acid is catalytic ring-opening polymerization from CNCs, which uses the surface hydroxyl groups as starting sites[95-97]. Furthermore, atom transfer radical polymerization has been intensively investigated for the synthesis of well-defined polymers on CNC surfaces[98-101]. These grafting techniques have produced highly compatible CNCs with contact angles ranging from 62 to 94°; however, the preattachment of initiator, polymerization processes, and workup/separation are time-consuming. We performed free radical grafting of vinyl polymers from CNCs in water to bypass the necessity for initiator attachment and these organic solvent-based reactions (using ceric initiation). The process, however, creates a substantial quantity of homopolymer, making separation difficult, and the graft densities achieved were low, resulting in CNC contact angles of only 35-47° [102].

Polymer grafting onto the surfaces of CNCs can be dually achieved using both approaches dubbed as "grafting-to" and "grafting-from" [103]. “Grafting to” refers to attaching pre-synthesized (and described) polymerization to the surface’s hydroxyl or sulphate ester groups on CNCs. “Grafting from” refers to studies in which in situ polymerization is emanated initiators that are generally immobilized on CNCs surface thus creating grafted polymer through traditional polymerization route including ionic[104], radical[105], or more sophisticated ring-opening polymer processes[97, 106]. Some instances of grafting from technique were examined in previous paragraph. Araki and Kloser et al. [29, 107] investigated epoxy-terminated polyethylene glycol chain onto CNCs and authors in ref [108] with DNA oligonucleotides employing peptide binding on carboxylated CNCs. In the first approach, sterically stabilized CNCs were created but in the second approach complimentary DNA strands were attached to distinct population of CNCs.

Literature has also dabbled with "Grafting from" approaches as well. One notable example grafting PNIPAM onto CNCs[109-112]. Using free radical polymerization authors crated grafted CNCS that generated dually responsive smart system adaptable to Pickering emulsions[113-114]. Random copolymer grafts of Random copolymer grafts of dimethyl aminoethyl methacrylate and napthyl-functionalized methacrylate were attached to the surfaces of nanoparticles and merged with PVA strands to create rigid and self-repairing hydrogels[115]. Different polymer grafting strategies have been undertaken[57, 106, 116].

Grafts implanted on top of CNCS can have many functionalities for instance making CNC-hydrogel macromolecule free. This approach does not entail additional step of polymerization. Literature calls the generated system one component CNCs[117] . These systems were additionally making the CNCs melt processable. Chang et al. [118] also reported within similar theme of action synthesis of CNCs-g-poly(ethynylene fluorene); however mechanical properties of the materials generated were not examined. Obviously, these system beside chemically modifying CNCs for various aims; have some side benefits as well such as enhancement of thermal stability due to arguably presence of polymer grafted chain as reported here through extension of processability to melt state and others in the literature [119]. Another notable feature of grafting is to check whether self-assembly into liquid crystal formation of CNCs is lost with these systems; a suspension with grafted CNC displayed birefringence at room temperature however after heating birefringence disappeared due to possible steric natured interactions between modified CNCs[120].

Another notable issue with regards to surface modification of CNCs is to have the modification done with a route and chemical that are sustainable for continual cycle of

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CNC extraction from nature, modification of it and its usage within products. This problem somewhat can be addressed through more careful adjustment of CNCs surface; such management can be carried out through grafting stimuli-sensitive polymers that confers steric stability and responsive characteristic invokable with temperature, acidity, or light to derivatize CNCs in diverse matrices[121-122]. For illustrate, following adsorption in poly (ethylene oxide), the CNCs dispersed more readily in (hydrophobic) low-density polyethylene, and the resultant matrix composites could be formed into homogenous structures through melt extrusion. There are reports in the literature on the alteration of cellulosic and wood products with acid anhydride using iodine as a catalyst[123-124].

Other routes for CNC compatibilization includes functionalization using small molecules[57]. Most studies have focused on usage of surfactant or covalently linked less polar compounds to break hydrogen bond stemmed interactions between CNCs[14, 125]. Non-covalent modification can use CNCs' anionic charge density to interact with cationic modifier; thus coating CNCs and allowing their hydrophobic tails to function at the interface of CNCs[126-127]; this way of modification is similar to layer by layer assembly route frequently mentioned in the literature[128]. Substantial dispersion of CNCs/poly(lactic acid) and CNCs/atactic poly(propylene) has been recorded in chloroform[129] and toluene[37], respectively, thus endorsing potency of these techniques. In these cases chemical modifier is in nature an adsorbed polymer onto CNC[130]. The interaction with surfactant can also form functional groups on the surface of the CNC molecule, increasing its response readiness[25]. CNC surfaces have also been modified using quaternary ammonium salts[126]. The primary problem of utilising a surfactant to modify the surface of CNC is that huge volumes are required due to the high specific area of CNC, prohibiting this technology from being used for composite treatment in solvents[131]. Through introduction of low molecular weight additive; it is possible to lower threshold for self-assembly gelation of CNCs through surface modification with octyl-CNCs; thus providing a route to CNCs to glue to one another through their associative hydrophobic contacts[132]; however hydrogel produced this way has been made through physical associations between CNCs.

Another key issue for chemical modification of CNCs is to carry out the procedure in such a way that surface modification mission is accomplished without compromising CNC crystal structure and strength; original shape or polymorphic transformation occurs within single CNCs[25]. For instance, crystallinity of CNCs that might differ based on source and way of development can lead to mechanical properties to suffer. Organic dicarboxylic acids have been used to hydrolyze cellulose under pressure at high temperatures (>200 °C)[133-134]. After 3 hours of interactions with -cellulose at 105 °C, oxalic acid (pKa = 1.25) and maleic acid (pKa = 1.9) exhibited negligible cellulose degradation[134]. Because the majority of the preceding experiments were conducted with the purpose of creating glucose and other biofuels rather than modified CNCs, the influence of dicarboxylic acid on overall morphology and crystallinity is unclear.

Surface-initiated atom transfer radical polymerization was used in **Figure 1a** to convert CNCs into hydrophobic poly (butyl acrylate). The result was a core of polymer latex particles with suitable CNCs that were successfully implanted. Later, the grafted polymer was mixed with poly (methyl methacrylate) monomer phase and polymerized as seen in **Figure 1b**. As outlined earlier, CNCs are frequently utilised as fillers in polymers to provide reinforcement. It might be challenging to avoid the accumulation of CNCs with insufficient reinforcing. This issue can be resolved by new nanocomposites made of nanoparticles with polymer graft as shown **Figure 1c**. In this study[135], cotton sourced CNC were surface modified using benzophenone moieties that later using UV photopolymerization their methyl or hexyl methacrylate components became polymerized (See **Figure 1d**). Another route of surface modification was introduced in ref[136] as shown in **Figure 1e**. Methyl(triphenyl)phosphonium exchanged CNC were utilised to generate well-dispersed epoxy composites without the need of a solvent and also well dispersed in polystyrene nanocomposites at elevated temperatures. They absorbed 30% less water than Na-CNCs and retained less water after desorption. With the aim of producing mechanically reinforced composites; high performance nanocomposites were created using generated

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acetalized CNCs, which was shown to be a successful reinforcing agent in hydrophobic polymer matrices. Acetalized CNCs showed outstanding reinforcing potential and efficient stress transfer behaviour when incorporated at a very low weight percentage (0.5 percent) in an epoxy matrix, resulting in a 73 percent improvement in tensile strength and a 98 percent increase in modulus[137]. A picture depicting the process is sketched in **Figure 1f**.

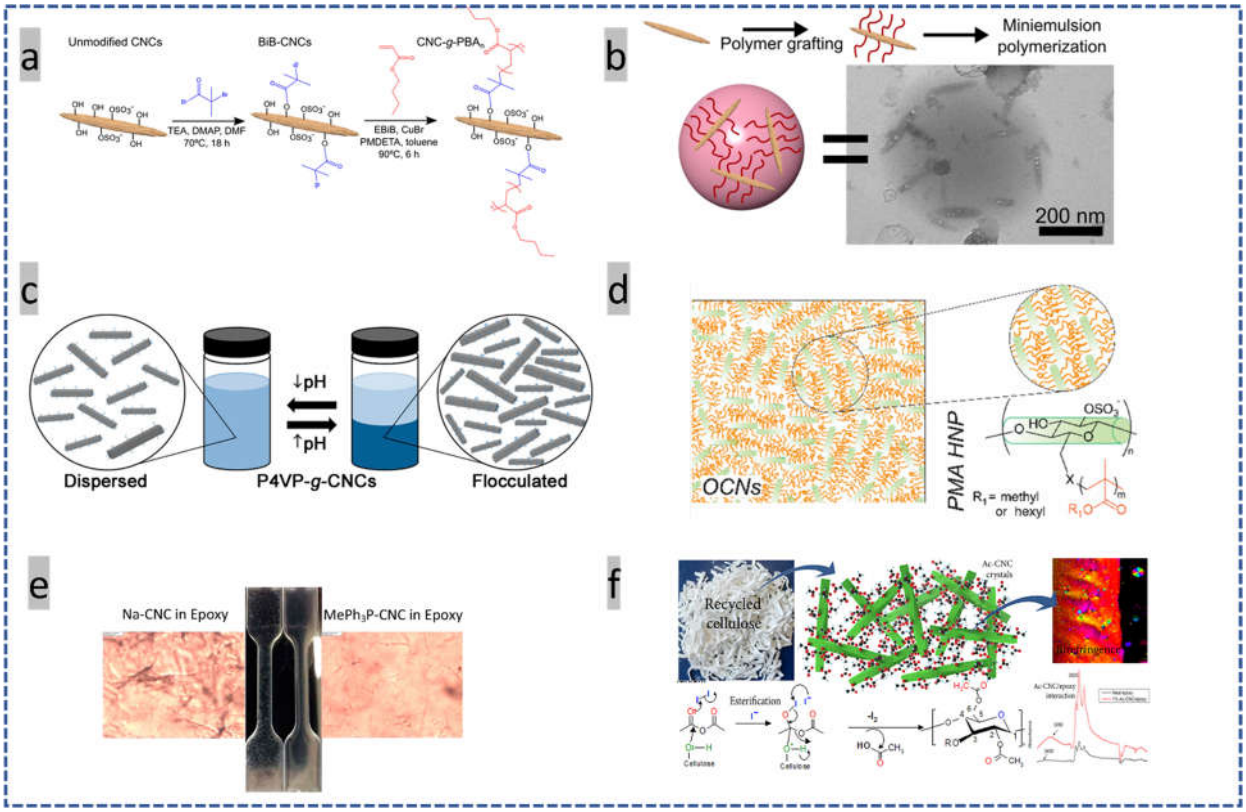

Figure 1. Various routes to surface modification of CNCs encompassing esterification and sophisticated grafting routes. **(a-b)** Diagram illustrating how unmodified CNCs may be changed with ATRP initiator groups to create BiB-CNCs and how BA can be modified with SI-ATRP to create CNC-g-PBAn, where n is the desired degree of polymerization. Surface modification adapted with permission from ref. [138]. **(c)** P4VP grafted CNC under changeable acidity flocculated versus suspended schematic. adapted with permission from ref [102]. **(d)** One component nanocomposite hairy or grafted CNC with ability to form composite without addition of polymer. Adapted with permission from ref[135]. **(e)** Surface modification of CNC and its impact visually and in tensile stress tests. Adapted with permission from ref [136]. **(f)** Esterification of CNC and its impact on network formation within polymer matrix. Adapted with permission from ref [137].

In addition to technicalities of surface modification of CNCs; their actual influence on mechanical properties is worthy of most attention. Poly lactic acid (PLA)/surface-acetylated CNC matrix composites created in one reference, displaying much higher tensile strength and modulus than plain PLA, with a loading of 10% acetylated CNCs achieving a 150 percent increase in tensile modulus [139]; thus signifying a better inherent stress transfer within polymeric matrix. Novel sustainable thermoplastic materials containing poly(glycerol succinate-co-maleate) (PGSMA) films and CNC were tested, results implied incremental increase of 20-40 percent in tensile strength and Young's modulus[140]. Quaternization of CNCs also leads to stronger interface in cross-linked poly (acrylic acid) (PAA) networks[141] . In another work from same group [142]; Quaternization of CNCs were added poly(acrylic acid-*co*-acrylamide) matrix with similar chemical make up as PAA. Glycidyl methacrylate (GMA) was grafted onto CNCS to render it crosslink-able in presence acrylamide monomers[143]. To produce stronger interfaces; authors[144] also chemically modified CNCs with pre-grafted CNCs with polymerizable groups have been used as

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nano crosslinking joints and nano reinforcements to synthesize highly elastic hydrogels. Composite made this way shared similarity with one component CNCs explored earlier[117].

The unmodified CNCs due to poor interaction with host polymers can cause weak boosting to the mechanical strength of CNC-gel thus undercutting their full potential. Some studies demonstrate CNCs with carefully modulated surface changes can act both as a filler and physical crosslinker to strength hydrogel systems. The amine group on gelatin (natural host matrix) can interact with the aldehyde groups on the CNCs developed in ref [145]; however the prospect of these hydrogel as injectable was poor due to necessity to dry them out for long period of time. CNC can also operate as a multifunctional crosslinker to cross link polyacrylamide through in situ polymerization of monomer on CNC thus providing compatibilization and polymer synthesis in one box[51]. To provide a better reinforcement; covalent bonding alkene modified CNC to poly(acrylic acid) hydrogels by co – polymerization[146]; however in both of these technique due to toxic environment needed for polymerization (involving KPA) and a heating step needed for gelificaiton; has completely ruined their prospect for usage in most therapeutic systems.

4. CNC reinforced hydrogels

Mechanical property reinforcement is dependent on stress transmission inside the hydrogel; any factor that facilitates and make the process of stress localization more even can produce a better enhancement in mechanical properties. In case of pure suspension of CNCs with high load of particles; higher aspect ratio leads to higher contact points among CNCs thus improved mechanical properties; however, extent of addition of CNCs inside the nanocomposites in most cases not high enough that can rely on direct stress transfer between CNCs. CNC clearly due to being rigid (higher persistence length) than cellulose nanofibrils (CNF), find it harder to bend and wrap around neighboring CNFs even if the concentration higher to make stress transfer through entanglement; entanglements are beneficial to stress transfer in cases that entanglement is in opposite direction of tensile load thus resisting expansion of CNC network beyond a certain strain. Visually, the difference between CNCs and CNFs is that CNCs are a pile of short sticks and CNFs are a pile of broken yarns; broken yarns, depending on their length, can lead to permanent physical entanglement, as seen with ordinary items such as shoelaces. Similar visualization are perceived in loadings above overlapping concentration in polymer as chance of entanglement severely rises a situation parallel to CNF; experimentally and theoretically leads increased reinforcement effect on CNT woven yarns[147] and increase in geometrical percolation thresholds[148]. Logically with increase in bendability of particles, probability of intersection within arbitrary units of particles decreases[148].

5.1. Physical or chemical network

Favier et al. [149-150] innovated the use of CNCs as a reinforcing agent in a polymeric matrix, seeing an increase in the mechanical properties of poly(styrene-cobutyl acrylate) when a solution of this polymers was coupled with CNCs isolated from tunicates and processed into films. CNC imposes 4 fundamental problems in polymer; however, these are; (i) excessive effects of water uptake, (ii) reduced temperature stabilities, (iii) poor dispersibility with nonpolar polymers, and (iv) permanent agglomeration of the dried CNCs are the four essential barriers impeding practical realisation of the empirical application of nanocellulose/polymer hybrids. **Figure 2a-d** depicts the process of injecting a hydrogel with chemical and physical cross links; differences in physical cross links are noted in yellow highlight portions.

While sulphate esters promote electrostatic attraction that aid in aqueous dispersion, they render colloidal stability very susceptible to ionic strength, prohibit dispersions in nonpolar environments, and result in disordered gel-like formations at high concentrations. The formation aggregate causes stress concentration and crack formation; however, there are mechanism that can mitigate these issues. One of the most promising reinforcing

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mechanisms is the use of "sacrificial" bonds as explored earlier, in which dynamic and reversible interactions reinforce structural stability upstream of a spreading fracture [151-152]. This is accomplished using different temporary connections like as hydrogen bonds, ionic contacts, or hydrophobic groups, allowing for a considerable amount of energy dissipation when stretching. Structural reinforcing in thermoplastic nano - composites is widely assumed to occur via a hydrogen bonded CNC net that trickles down inside the polymer matrix, allowing for efficient stress transmission[149-150]. A average reinforcing obtained is well anticipated by a percolation paradigm, which assumes that the CNC percolate net is created over a specific filler loading (i.e., percolation model criterion)[153-154]. However, in many cases, the actual reinforcement does not match with the prediction of this model[155-156]. One probable explanation is that it does not account for CNC aggregation, which we have recently demonstrated to be crucial[157].

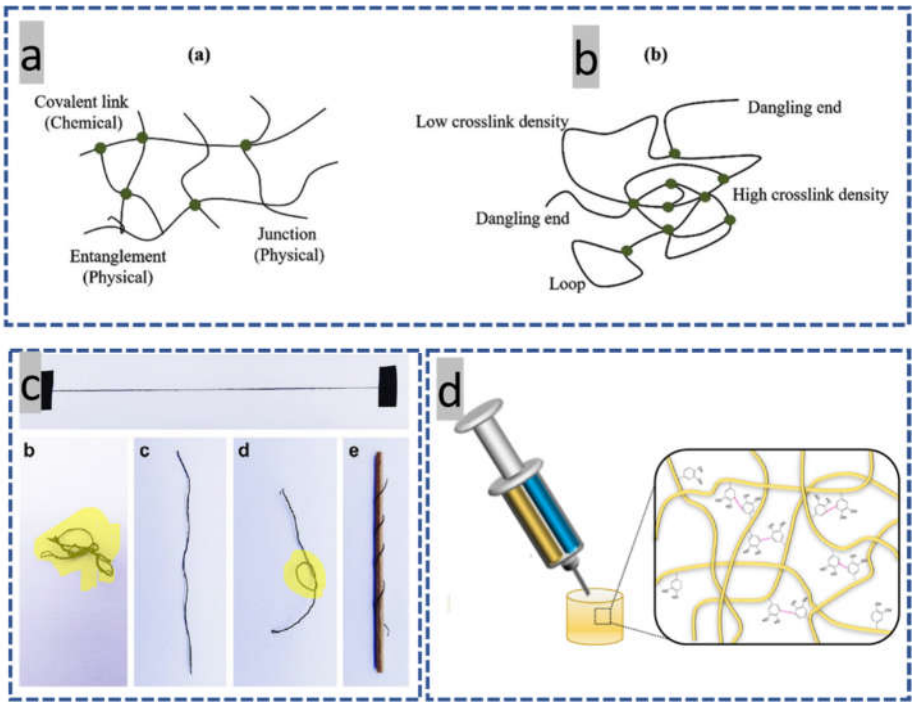

Figure 2. Differentiation between physical and chemical cross links. **(a)** Diagram of physical and **(b)** chemical bonding in a hydrogel along with some of the flaw. Adapted with permission from ref[158]. **(c)** Schematic representation of physical entanglements in carbon nanotubes Yellow highlighted parts shows physical entanglements. Adapted with permission from ref.[159]. **(d)** After creation of physical or chemical cross links now the hydrogel must be injected. Adapted with permission from ref. [160].

As stated earlier, CNCs are generated from plants with hierarchical cellulosic structure thus serving as skeleton for fabrication of composites having physiochemical qualities from each ingredient. Structures tried out in aerogel or hydrogel format are thus trying to mimic this nature cork-like rigid hierarchy. Ironically, the most well known issue within hydrogel network is standard covalently linked structure are often not tough and break apart under stress[152]. The even distribution of chain length among crosslinks due to inhomogeneous reaction, weak connection between chains in hydrogel due to chain being far away from another, and lack of proper mechanism embedded for proper energy dissipation leads to hydrogel poor toughness[161-162]. Various sophisticated attempts to remedy this issue have been introduced and pioneered by Gong et al. [163] and Haraguchi's nanocomposite hydrogels [164]. For instance, the double network hydrogel made up of two interconnected porous channel one made with short chain and later of that which are crosslinked by covalent bonds is a notable example. While double network is comprised of integrated network to toughen up the hydrogel; during dynamic loading irreparable damage occurs[165]. To remedy this issue, certain noncovalent bonds are included in the network

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to enable the structure to heal[166-167]. A variety of polyampholytes with diverse ionic combinations can acts as sacrificial bonds to enhance not only mechanical properties but also create a mechanism deep within hydrogel for energy dissipation [168].

Interestingly, Suo [169] and Zhao et al. [152] simulated a hybrid double network gel with high extension through combining Ca^{2+} crosslinked alginate gel deep embedded within polyacrylamide network. Like one component CNC gel; here Clay surface was grafted with PNIPAM. Here when gel is under stress; the small dendrite between clay surfaces also expand to minimise tensile stress [164]. Similarly same theme of mechanical load transference were extended to Carbon nanotubes [170], graphene sheets[171], hematite[172], and macromolecular microsphere composites [173]. Many nanostructure based on (such as chitosan, hyaluronic acid, and alginate) and their derivatives have recently been developed; electrostatic contacts, hydrophilic association and hydrogen bonds were embedded to act as sacrificial bonds to increase mechanical energy dissipation within these hydrogels[174-175]; nature of these contacts were reversible. Authors in refs[176-177] to make supramolecular hydrogel used electrostatic and hydrogen bonds with ability to dissipate energy efficiently. Other bio friendly host matrices such as hyaluronic acid and cyclodextrin can also equally benefit from double network hydrogel architecture[178]. Chitosan microcrystalline formation and chain entanglement can also turn matrix of chitosan-polyacrylamide more energy dissipative[179]; crystal formation similar to plastic deformation in polymers can dissipate energy through shear induced crystallization. Agar another eco-friendly hydrogel building block based on reported data in ref[180] can generated recoverable double network.

Reversible network can become tough if a mechanism become embedded inside the gel capable of dissipation of energy[152]. In one study, hydrogen bond was used between cellulose nanofibrils as energy dissipators while main network of polyacrylamide granted elasticity and network stability. In one report[176], chitosan was chemically crosslinked with epichlorohydrin and physically bridged with hydrophobic interactions. Likewise, Wu et al. [179] reasoned that in the mix of chitosan-polyacrylamide network, similar to previous network, one component impart elasticity and main stress bearing capability while the second network preserve the network through dissipative mechanism engineered.

Figure 3 displays a more extensive collection of potential reinforcing pattern for all type of hydrogel not necessarily restricted to CNCs based ones **Figure 2**. These design are thermoplastic reinforcement, reinforcement based on inclusion of nanoparticles; reinforcement through energy dissipative networks such as supramolecular hydrogels, interpenetrating network or IPNs, Functionalization\dual crosslinking, supramolecular IPN, sliding crosslinking, functionalization nanocomposites, Nanocomposites IPNs, crosslinked microgels[181].

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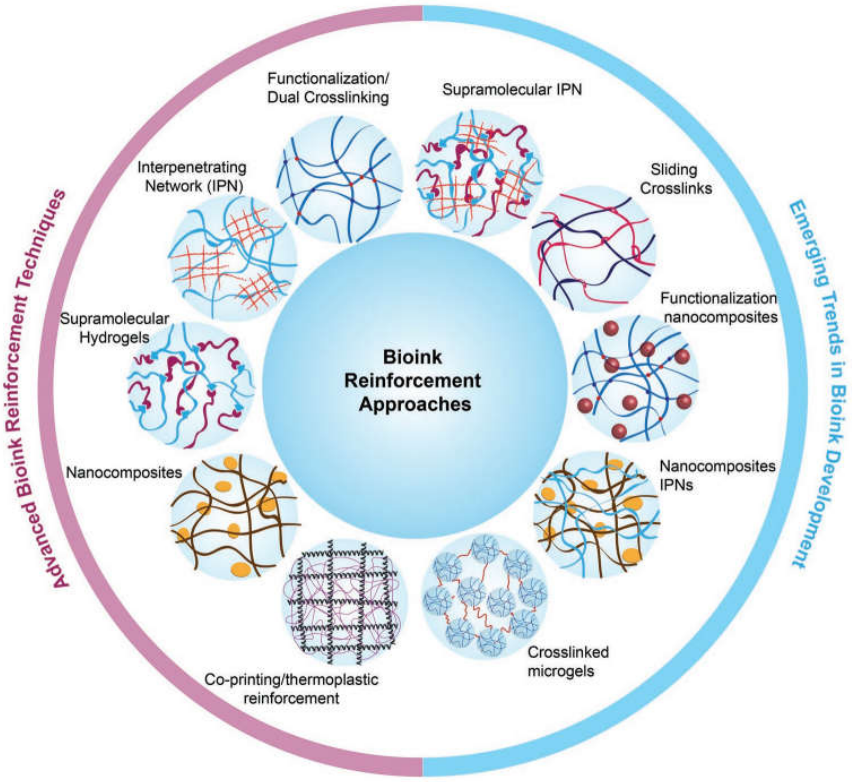

Figure 3. Classification of reinforcement methods adaptable to all hydrogels. Co-printing\thermoplastic reinforcement, nanocomposite reinforcement, supramolecular hydrogels, interpenetrating network (IPN), Functionalization\dual crosslinking, supramolecular IPN, sliding crosslinking, functionalization nanocomposites, Nanocomposites IPNs, crosslinked microgels. Adapted with permission from ref [181].

Example of usage of these networks in shape of hydrogels are CNC\PEG as nanocomposite hydrogel [175], semi-IPN CNC/PEG/PDMAA hydrogel as IPN [182-183], PVA/CNC-UPy supramolecular nanocomposite hydrogel [184], hydroxypropyl-modified α-cyclodextrin (Hy-α-CD) and Acrylamide-PEG20000-Acrylamide as sliding movable crosslink supramolecular hydrogels [185] to just name a few. In randomly crosslinked hydrogels stress is concentrated; increasing crosslinking density leads to doubly crosslinked networks as the crosslinking links now acts as the second network. Supramolecular hydrogels are another unique class of hydrogel that entail having reversible crosslinking that function as energy dissipators. In case of nanocomposite hydrogel, main polymer making up the hydrogel can act as a component that maintain elasticity while nanoparticles interacting with one another, or the host polymer can act as energy dissipation through possible hydrogen bonds or reversible electrostatic crosslinkers. A sliding hydrogel contain many crosslinks that is developed within hydrogel. The sliding hydrogel's molecular mobility allows stem cells to rearrange surrounding ligands and modify their shape in 3D. Sliding hydrogels promote effective stem cell development toward numerous lineages, including adipogenesis, chondrogenesis, and osteogenesis, without affecting matrix stiffness[179].

These engineered hydrogels increase mechanical properties in variety of modalities, including the formation of crosslink sturdier, evenest stress distribution, and the dissipation of mechanical energy by the incorporation of sacrificial bonds. In response to stress, the sliding cross link structure depicted in **Figure 3** can move about and impede stress localisation.

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5.1.1. Different networks definition

Here very briefly each of the networks introduced earlier will be defined: (i) dual crosslinked networks (ii) supramolecular hydrogels (iii) interpenetrating networked hydrogels (iv) nanocomposite hydrogels

5.1.1.1 Dual crosslinked networks

Hydrogel printing has evolved because of polymer modifications with novel chemical functionalities. Hydrogel functionalization provides the benefit of infusing the injectable gel with motif and biological activity. Natural polymers by nature physically crosslink through structure and physical interactions whereas man made hydrogel are reinforced mostly through covalently crosslinking process. Even though physical crosslinks are easier to break they can provide a dissipation mechanism and are easily tunable if factors such as temperature, acidity, and ionic concentrations are tuned. While dual crosslinked network definition is a broad term extendable to coexisting of various type of network such as ionically crosslink or chemical crosslink; cross link nonetheless are often necessary to impart further reinforcement within natural hydrogels. For cell carrying applications, functionalization can offer locations in the polymer network that are sensitive to breakdown as well as cell adhesion [186]. The chemical functionalization of natural polymers like as gelatin and collagen may be found in refs[187-188]; these changes can further unlock the potential of these hydrogels for bio related applications.

5.1.1.2 Supramolecular hydrogels

Generally, links that are sacrificial enhance fracture energy of hydrogel through amplifying network dissipative capability when this action is granted repeatability; the network can cyclically deformed many times before irreparable damage can occurs this has generated interest within research community to look into hydrogel with these capabilities such as supramolecular hydrogels. Supramolecular hydrogels are generally made with short length polymers with capabilities generated at the end of the these “sticks” to establish non-covalent interactions. Upon passing a certain threshold of stress, these non-covalent bonds can reorganize to accommodate the stress applied therefore they are able to reshape internally and dissipate energy. The flow properties of these hydrogel is elastic below yielding points but viscose liquid past yielding point as molecules are very short to impart viscoelasticity by themselves.

5.1.1.3 Interpenetrating Networks hydrogels

Interpenetrating networks or IPN can improve fracture energy and modulus through enjoyment of having two independent networks that are either crosslinked to one another also know as double networks or there is a physical association between two networks thus dubbed as ionic-covalent networks. Due to reaping benefit of both network these networks are elastic till 40-50 percent deformation and environmental factors examined earlier has no effect on their properties as at least one component is non-responsive to environmental factors. The first network can give in when cyclic stress causes the network to fatigue, but the second cycle is more resilient due to its sacrificial bonds[189-190]. These networks can further be mixed up with other type of networks shown earlier to provide more hybrid versions of networks.

5.1.1.4 Nanocomposite hydrogels

Depending on the shape and aspect ratio of the filler, nanoparticles operate as reversible crosslinks crossing numerous polymeric chains to reinforce the hydrogel. This allows stress to be efficiently disseminated across the network via probable disruption of nanoparticle-polymer cross links; this mechanism enhances hydrogel modulus and extension at break, as well as fracture energy[191-192].

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5.2. Examination of network

5.2.1. Polymer network

Classical polymer networks are mostly created by crosslinking a monomeric solution or linear or branched polymer chains[193]. Both methods build a randomly crosslinked heterogeneous network with joints that are dense in some regions and sparse in others. After loading the network, this heterogeneity causes localised stress concentration points that break faster than anyplace else in the network, hence crosslink distribution is critical to mechanical qualities. Because of their shorter length and lower entropy, network linkages with a higher degree of crosslinking are less elastic. Even while crosslinks are essential to polymer structure, increasing crosslinking results in a random heterogeneous network with rapidly declining extensibility and brittleness[193-194].

5.2.2. Fracture energy

Current hydrogel mechanical reinforcement reports include mechanical characteristics such as fracture, strain, and stress, as well as compressive, tensile, and shear moduli. Despite the commonplace of these metric to represent mechanical properties of hydrogel they fall short to provide ample descriptive unifying picture of hydrogels. On other hand fracture energy another not as commonly reported metric is a valuable measure for assessing the applicability of different hydrogels. This metric is the amount of energy required to propagate fracture across the 3-D structure of hydrogel; it is defined as the energy required to create unit of crack growth as J.m-2 [152, 189]; this is an innate material property that is independent of geometry and method of testing; therefore valuable. For instance, bone fracture energy is reported to be 12.7±3.2 J.m-2 in one report[195]; however this is changeable depending on collagen orientation within the bone [196].

This energy can be divided to (i) energy required to break through polymer chains that depends on density of chain in plane of crack path, number of bonds in those chains and strength of those bonds. Therefore hydrogels have lower energy threshold in hydrogel form as opposed to dry concentrated format of polymers 10 $J.m^{-2}$ vs 50-100 $J.m^{-2}$ [152, 193]. (ii) Viscoelastic energy dissipated to surrounding network; therefore, like viscoelastic response defined in the literature is a property that is velocity (frequency) and temperature depending; therefore, type of loading static and dynamic affect the energy dissipation. Moreover, crack velocity and energy are correlated through a power law dependency; that is if crack is assumed to be propagating fast within the hydrogel it also requires higher amount of energy. Mechanism of dissipation in some polymers with dense polymer population is to dissipate energy through movement and rearrangement of polymer chains that is energy demanding due to close interactions between chains; however in hydrogel polymer is used in dilute form so polymers have very low number of neighbors thus energy dissipation is absent through polymer-polymer interactions thus fracture energy is low and hydrogel is not tough [152, 193].

In ref[175], CNC-PEG nanocomposite hydrogels outperformed plain PEG hydrogels in terms of Young's modulus (12-31 kPa), fracture strength (99-375 kPa), and fracture strain (650-1300%). The high modulus was correlated to the large cross-linking density (described later in this study), where the CNC had multifunctional physical cross-links at the interface with the PEG matrix. The high flexibility and fracture stress were a result of effective dissipation of energy: when a crack arises at deformations, the dynamic rearranging of CNC/PEG morphologies disperse the energy and enhance crack propagation resistance, resulting in high hydrogel strength. Due to the creation of a continuous layer of clusters, the remarkable mechanical characteristics are well understood. The stiff network is mostly attributed to the strong contacts between CNC and polymer nanoparticles, and strength is well anticipated by a percolation process. In reality, the percolation of cellulose nanocrystals in composites has a well-documented function[150, 197], where the reinforcement's special qualities are due to the high mechanical characteristics resulting from the hydrogen bonds. CNCs have a significant propensity for self-association as a result of

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their strong interactions with surface hydroxyl groups, which is helpful for the creation of load-bearing percolating networks inside the host polymer matrix.

The damaged or broken contacts (hydrogen bonds) between CNC and PEG inside the covalently cross-linked networks are primarily responsible for the substantial improvement in the mechanical characteristics. Due to the uniform distribution of CNCs throughout the polymer matrix and the interplay of physical CNC/PEG hydrogen contacts with covalent cross-links from PEG diacrylate, CNC-PEG nanocomposite hydrogels exhibit greater mechanical toughness than plain PEG hydrogels (related to the viscoelastic properties). The mechanical characteristics of composites are thought to be influenced by interactions between polymer-polymer, polymer-filler, and filler-filler[198-199]. Fracture stress and modulus rose by 5.7-fold and 4.1-fold, respectively, when CNC volume fraction percentge increased from 0 to 1.4%. The presence of evenly dispersed CNC inside the polymer matrix and the high stiffness of the CNC itself are credited with the strengthening of mechanical characteristics. When CNC loading is increased further, negative impacts on mechanical characteristics are seen, and the fracture strength and modulus drop because of CNC aggregation. Additionally, the inclusion of CNC increased the extensibility of the hydrogels, which is attributed to the interference of the CNC/PEG interactions with the permanent cross-linking of PEG polymer chains. In order to strengthen the qualities of the polymeric matrix, CNCs are connected to the surface or pore within the polymers where the covalently cross-linked PEG polymer chains create the interconnected network.

Adding CNC increases the critical energy release rate for fracture propagation. Even though this result prevents us from determining the fracture velocity in the steady state, it shows that the addition of CNC considerably toughens the PEG hydrogels. The critical energy release rate increased from 48 to 143 J/m2 as CNC increased from 0.2% to 1.4%. The systematic loading-unloading tensile tests show the presence of sacrificial bonds in the nanocomposite. The behaviour of plain PEG hydrogels is similar to that of a perfect chemical gel, which can be thought of as elastic and entirely restores its original shape when the tension is removed[200] if the stress is low enough. Almost no hysteresis loop is noticed. In marked contradiction, the CNC-PEG nanocomposite hydrogels showed substantial looping on the cyclic tensile performance. The temporary CNC/PEG contacts are disrupted, and the network requires some time to return to its initial conformation. Low deformations (strain 200%) cause plastic deformation in the network, which may be seen as elastic and in which polymer chains change from their initial coiled conformation to a greatly extended state. The PEG surface absorbed CNCs start to dissociate at high deformations (strain >200%), and it is thought that this structural reorganization might release a significant amount of energy and hence limit the crack propagation.

The double network (DN) hydrogels are made up of two interpenetrating networks with distinct covalent cross-links between the first network (short chains) and the second network (long chains) in the first network. The DN structure uses an embedded brittle network to toughen the hydrogels, however following cyclic deformation, it is always permanently damaged and cannot be repaired[165]. Noncovalent bonds have been used in the DN to overcome this limitation and make the network recoverable in the event of an internal rupture[167, 201]. For instance, a series of polyampholytes were used to create hydrogels with high toughness and viscoelasticity. These polyampholytes had various ionic combinations that serve as sacrificial linkages[168]. Of particular note, Suo and Zhao et al. [169 202] created hybrid DN gels that had good stretchability by fusing Ca2+ cross-linked alginate gel with a covalent cross-linked polyacrylamide (PAAm) network. Poly(N-isopropylacrylamide) hydrophilic polymer chains were in situ grafted from a clay surface for NC gels. To prevent stress concentration, the chains dispersed among the nearby clays gradually lengthen when the NC gels are stretched[203].

Recently, a variety of distinct nanostructures based on polysaccharides (like chitosan, hyaluronic acid, and alginate) and their derivatives have been used to create tough and strong hydrogels. Dynamic interactions, such as electrostatic interactions, hydrophobic association, and hydrogen bonds, serve as sacrificial bonds to improve the hydrogels' ability to dissipate energy and to improve their mechanical properties[174-175, 204]. For instance,

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electrostatic interactions and hydrogen bonding were used by Cai[205] and Zhang et al. [177] to create supramolecular hydrogels based on chitosan.

In ref[206], according to uniaxial tensile tests, adding the CNC skeleton significantly improves the mechanical properties of composite hydrogels. For example, when the CNC fraction is increased from 0.2 to 1 vol%, the fracture stress and elongation at break remarkably rise from 73 to 151 kPa and 647% to 1388%, respectively. The composite hydrogels' fracture energy—a characteristic used to assess the resistance to crack propagation—scales with the volume fraction of the CNC skeleton with a power dependency with index of 1.3 and reaches a maximum value of 2.8 kJ/m2, emphasising the importance of reinforced in blunting and energy dissipation. When the CNC volume percentage is greater than 1.5%, the fracture stress only slightly increases to 173 kPa, while stretchability clearly decreases to 1247%. When compared to composite hydrogels, the maximal elongation and corresponding fracture stress for pristine PAAm gels are 450% and 35 kPa, respectively. Similar to this, the CNC2 and PAAm hydrogels' Young's moduli (E) are found to be 29.8 and 7.3 kPa, respectively, demonstrating the stiffening effect of the CNC skeleton.

Authors also created composite hydrogels with randomly dispersed CNC for comparison in order to show the effectiveness of a CNC skeleton as reinforcement in PAAm hydrogels. According to the results, hydrogels with CNC skeleton reinforcement have fracture strengths that are 1.04–1.26 times greater than hydrogels with randomly dispersed CNCs at the same filler fraction. This suggests that the continuous and well-structured network of CNC skeleton offers superior mechanical reinforcement. According to earlier research, the randomly distributed nanoparticles had several point-to-point connections, and more filler was often needed before the percolation network could develop[207].

The hysteresis energy, or the loop area, is strain-dependent and exhibits a self-toughening effect at high stresses[152]. The stress-strain curve for the composite hydrogels progressively returns to its initial form after about 60 minutes. The covalent character of the PAAm network elasticity and the recovery of transient hydrogen bonds during relaxation are balanced in this time-dependent elastic recovery behaviour, which requires a two-stage process[208]. While the elastic contraction becomes weak and inhibits the recovery of the chains to their equilibrium condition at small strain, it still dominates the rupture of reformed links at big strain.

The outstanding reinforcing function of the CNC skeleton was also demonstrated in the compression test, and the stiffness improves nonlinearly with increasing CNC percentage. For instance, the compressive strength of the composite hydrogels is more than an order of magnitude greater than that of pure PAAm hydrogels, ranging from 165 to 1017 kPa (92 kPa). It is possible that the anisotropy of the cellulose fibers, where nanofibrils match up along the surface via a hydrogen bonding arrangement with a special parallel conformation in native cellulose crystalline regions[175], is the cause of this remarkable asymmetry between tensile profiles and compressive profiles. The loading process can be seen to have three major stages: the initial elastic region, in which the stress varies linearly with the strain (25%); the plateau region, where the majority of the absorbed energy is dissipated (25%); and the final densification region, where the stress grows rapidly (> 70%), mimicking the behaviour of an open-cell structure of a natural cork under compression. In fact, at high loads, densification of the porous structure causes the CNCs to bend toward one another, preventing collapse and fibre slip[115].

By performing repeated loading-unloading experiments without a break in between , the fatigue resistance characteristics of the composite hydrogels are evaluated. The microscopic morphology shows that the networks after cyclic compression do not show significant damage compared to the original condition. The hydrogel pores progressively constrict and thicken during initial loading. The porous structure can return to its initial condition during the subsequent unloading procedure.

Whenever the compression is relaxed, the hydrogen bonds may be better rearranged thanks to the water molecules acting as plasticizers, which helps the network recover [209].The stress at 90% strain reduces from 647 kPa for the first loading to 512 and 358 kPa

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for the second and the third repetitions, respectively, with the exception of the first three cycles displaying substantial hysteresis . The stress-strain curves are almost constant after the fourth cycle, and the hydrogels continue to exhibit compression force of 265 kPa after 8 cycles.

Using dynamic processes to waste a sizable amount of energy during deformation and preserve the original configuration after release is the overall approach for the creation of tough gels. In this study, the covalent PAAm network provides for elasticity and upholds network stability, while the hydrogen bonds act as dynamic cross-links in a sacrificial manner by providing a reversible framework for nanofibril transition between association and dissociation. In fact, other hydrogels made from polysaccharides frequently use this hybrid cross-linking technique.

5.2.3. Flow of polymers past the nozzle

During extrusion of hydrogel, gels are generally un crosslinked or contain monomeric or oligomeric polymers to facilitate extrusion with low viscosity [210-212]. The viscosity in nature is the single most important characteristic that can shape flow behavior of hydrogel and it is the most quoted and reported value describing hydrogels specially in bio-aimed applications. Viscosity however has simultaneous positive and negative effect on extrusion of cell laden hydrogels; from one side having high viscosity signifies high gel strength and subsequent high print fidelity therefore printed scaffold is immune to sagging on the other hand higher viscosity during shearing in the nozzle and extrusion exposes cells to high shear stress that renders cell unviable and reduces surviving cells proliferation potential. Shear stresses have been demonstrated to induce morphological alterations (cell geometry), cytoskeleton remodelling, and the formation of invasive reactive oxygen. This is highly dependent on type of cells involved; shape of cells, the quantity and extent of exposure to shear (time and shear stress absolute value) [213].

Furthermore, it is expected that high yield stress (correlated with high viscosity) cause nozzle clogging; which adds to ink jetting inconsistency due to nozzle constriction during extrusion and deposition[214]. As outlined earlier in case of low viscosity hydrogel; shape fidelity suffers, and cell can be rearrangement in unsuitable position post extrusion and further cell sedimentation can clog hydrogel pore thus effectively blocking communication routes between cells. The concentration of hydrogel, the molecular weight of the polymer, the temperature, the form of the nanofiller, the kind of polymer, whether the hydrogel includes cells, and the type of cells all impact viscosity and viscosity versus shear behaviour[213, 215-216].

5.2.4. Models

Hydrogels that display consistent viscosity of the range of print conditions are often modeled as Newtonian fluid model. This behavior is mostly seen in hydrogel with low polymer concentration or very dilute suspension where there is no network established inside the suspension\solution that can resist flow; moreover thermal motion of solvent bombardment at very low shear prevent inclusions alignment[217]. In opposite to Newtonian behavior non-Newtonian behavior such as reduction in viscosity as shearing become intense has been seen in hydrogel mixes. Moreover, shear thinning qualities are advantageous to cell distribution via hydrogel because they combine the benefits of having good print fidelity high viscose hydrogels and low shear viscosity that causes minimum harm to cells while being transferred; due to gradual reduction in viscosity with shear it is still recommended to select an optimum yield stress as cell laden hydrogel still has to go through viscosity reduction phase. Shear thinning is more noticeable in settings when the network of polymer particles is already well established [218]. The simplest model to capture shear thinning to establish relationship between stress and shear rate with a power law equation.

Many hydrogels display a non-Newtonian viscoelastic behavior with a non-zero yield stress. Yield stress by definition is the minimum stress needed to initiate flow, until

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this yield stress is breached material behave similar to solids like ketchup[218]. Shape fidelity and self-reliance of hydrogel post printing state is directly influenced by this yield stress specially in cases that are there no crosslinking is involved post printing. Additionally yield stress material display slip behavior thus in nozzle they create plug flow in the center of flow profile this shearing is limited to the narrow region close to the wall this effectively shield encapsulated cells within the hydrogel [212, 219]. Even though high yield stress is a sign of mechanical integrity of a hydrogel; it causes the process of incorporation of vulnerable cells into the mix pretty challenging considering mixing in highly viscose material required intense mixing; thus, there should exist an optimum point for yield stress. Mathematically to incorporate yielding into flow equation of the hydrogel one can add an intercept into power law equation introduced earlier to produce Herschel-Bulkley model. Several hydrogel publications in the literature have used this paradigm[88, 220-221].

If rheological characterization needs to capture flow behavior at very low or high shear rates to differentiate between hydrogels one can resort to more sophisticated models such as Carreau equation. This model has also seen success in the literature[222-223]. It is notable, that these equations come with flaw such as inability to forecast recovery of the gels post extrusion; this recovery is a time dependent process that in cases can take from minutes to many hours to establish to prehearing point. In case of faster recovery one can lock in the structure and assist with shape fidelity post extrusion[224-225]. Thermos responsive or smart hydrogels (or the one based thermos-responsive surfactants) can help with pace of structural recovery though temperature change; this is needed when printing taller extrudate due to lower layers being exposed to weight of top filaments. Based on data in the literature, the three primary important rheological properties to produce hydrogel are viscosity, yield stress, and rate of recovery[85, 88, 220]. However, hydrogel design can grow more complicated, such as measurement of oscillatory shear response in dynamic loading when the hydrogel is in a region such as the knee area.

5.2.5. Bio-related mechanics

The mechanical property of hydrogel is vital to its performance on both the macro and micro scale. Macroscopically, the eventual goal of hydrogel carrying cell is to implant in the body which require a minimum level of mechanical sturdity to be achieved. For example, the implant is expected to have similar level of compression as surrounding tissue without becoming detached due to breaking down and fatigue or complete separation due to mechanical unharmony; even minute difference in mechanical fingerprint of hydrogel over long term can become huge. It is also expected from the hydrogel to be self-reliant thus withstand gravity and have no perceivable gel collapse that might clog pores of hydrogel. The self-supporting of the hydrogel depends heavily on mechanical properties.

Another major factor is communication with cells. Elastic modulus has been found to extensively influence differentiation of stem cells[226-227]. Other important factors, hydrogel stiffness plays a key role in tissue repair though guiding stems cells; viscoelasticity regulates cell behavior, for instance stress relaxation promotes myoblast proliferation; rapid stress relaxion increases proliferation and differentiation of stem cells[228-230].

5.3. Synthetic polymers

Many synthetic or man-made polymers have been coupled with CNC to create a versatile hydrogel; to display acceptable biocompatibility[231], biodegradability[232], and high elasticity, cheapness of PVA matrix has been combined with CNCs in literature is a notable example of these efforts [85]. PVA is an example of nanocomposite hydrogels as CNC the second network inside the PVA hydrogel is acting as an energy dissipative component. When compared to PVA-only hydrogels, Abitbol et al., Han et al., and Gonzalez et al. showed a modest increase in the mechanical characteristics of PVA-based hydrogels [127, 233-234] with McKee et al. [235] recording highest recorded CNC-PVA values. Although every item in their process of production was intricately cross-linked. Raising the

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concentration of CNC from a low level of 0.1 to 0.2 weight percent relative to the weight of the entire gel led to an increase in gel storage modulus values from 3.8 to 14.3 kPa; in a similar report [236] with just 1-2 wt percent CNC, tensile modulus increased by 38-49 percent for the PVA matrix. Crosslinking agents or methods such as glutardehyde [237], formaldehyde [238], freeze-thaw cycles (plateaued after 5 cycles) [239] [240], 1,2,3,4-butane tetracarboxylic acid (BTCA) [241], alcohol-borax[234] are another method for cross bridging CNCs. To enhance the dispersion of CNC in the PVAc matrix, borax crosslinking of PVAc and vinyl acetate *in situ* polymerization in the presence of CNC can be utilised simultaneously[43]. CNC also improves water retention in PVA[237] for spill sponge application, altering rheology of CNC-PVA gel[242] and can be a controlling agent in the developed morphology of aerogels [243].

Although electrospinning is not a practical approach for delivering cells for tissue engineering purposes, it can be useful for wound dressing[244]. Fully hydrolyzed PVA fibre mats at 15 wt percent CNC electro spun PVA/CNC nanofiber mats demonstrated a 3-fold improvement in storage modulus (from 15 to 57 MPa)[245-246]. Notably, process is not only abounded to wet spinning as nanocomposite fibres made of cellulose acetate and CNC can also be mass produced through dry spinning. One notable difference between electrospinning and conventional injection methodology is better alignment of CNCs and subsequent better axial mechanical properties of fibers in comparison to injectables. 34 weight percent CNC loading resulted in maximum gains of 137 percent in tensile strength (from 67.7 to 160.3 MPa) and 637 percent in elastic modulus (from 1.9 to 14.0 GPa)[246]. Gel spinning was used to produce polyacrylonitrile (PAN)/cellulose nanocrystal (CNC) fibres with varied CNC loadings[247]. Tensile modulus increased from 14.5 to 19.6 GPa and strength increased from 624 to 709 MPa as CNC load increased by up to 10 wt%. Wide-angle X-ray diffraction analysis showed that the presence of CNCs enhanced PAN chain alignment and boosted PAN crystalline nature. Because the filler is functioning as a heterogenous nucleator, an increase in PAN crystallinity is a sign of close contact between the polymer chain and filler.

Other notable mixture of polymer and CNC for mostly reinforcing purposes are: Mixing with random terpolymer [248], Thermo-responsive hydrogels[235, 249-250] poly(acrylic acid) (PAA) [146, 162, 251], polyacrylamide[49, 51, 252-255], poly(ethylene glycol) (PEG) [175, 256], poly(methyl vinyl ether-co-maleic acid) and polyethylene glycol[257], hyaluronic acid (HA) [258], poly(oligoethylene glycol methacrylate) [259] [90], poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) cationic polyelectrolyte and a poly(di(ethylene glycol) methyl ethermethacrylate) (PDEGMA) [260], xyloglucan (XG) [261-262], xylan[263], poly-ε-caprolactone/chitosan[264], poly(N,N-dimethylacrylamide) [265]. poly(2-hydroxyethyl methacrylate) [266], interpenetrating polymer network (IPN) [267], poly(acrylamide-co-acrylate) [268] poly(N-vinylcaprolactam), PNVCL[269], poly(2-hydroxyethylmethacrylate)[47] and P(AA-co-AAm-co-AMPS)-g-NC/PVA[270] . Sometimes gel self heal after printing and healing is chemically reversible as opposed to physical healing this was done with implantation of reversible boronic ester bond onto CNCs[271]. The form of a polymer-reinforced CNC can occasionally be quite complicated, such as in the case of an elastomeric hydrogel with a core made of rod CNC and a shell made of polyacrylamide [13]. Because xylans and glucomannans, which are pentose and hexose monomers linked by short or branched chains, make up the majority of hemicelluloses found in plant cell walls, the reintroduction of xylan-based polymers as an encompassing hydrogel encasing CNCs is justified in light of the earlier discussed nature mimicking objectives.

Because chains entangle with unbound chain during rest periods, **Figure 4a** demonstrates that polymer is de-adsorbed from CNC during periodic loading. In the event of reversibility, the de-adsorption of the polymer chain functions similarly to the sliding cross-link hydrogel network that was previously described. Therefore, it was rationally assumed that the network's favourable physical connections would increase hydrogel fracture strength by reversible adsorption-desorption interactions on the CNC surface[253]. Due to the need to replicate the structural and viscoelastic characteristics of soft tissue, such as cartilage, the structure of nanogels with elastic properties has attracted attention in biomedical applications. Pore alignment's effect on viscoelastic properties has received

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much too little attention. In this work, collagen and cellulose hydrogels with different pore orientations—such as randomly spaced holes, vertically aligned pores, and horizontally aligned pores—were combined to form a multilayered hydrogel with a multi-structure that resembles cartilage formation (See **Figure 4c**) [272]. An electrochemical technique was used to compress CNCs, and access aligned micron-sized CNC fibres. As shown in **Figure 4b**, the tensile properties of the aligned fibres are much better than those of collagen/CNC films with no alignment in the plane of the films. The mechanisms engaged within the hydrogel fabric that are capable of dissipating energy are necessary for improving the viscoelastic characteristics of the gel in the event of surface in loss modulus.

Compression tests were done to evaluate the effects of a double network and a single network; the findings show that the second network and the fibre had a synergistic effect on the reinforcing (See the development in **Figure 4d**). It was discovered that the rise that might be attributed to the effect of 0.6 wt % fibre loading increased by as much as 150 percent. This value is more than what has previously been noted in several nanofiller systems. As a result, the fibre may be used in other hydrogel systems with a similar mechanism as merely an environmentally friendly reinforcement.

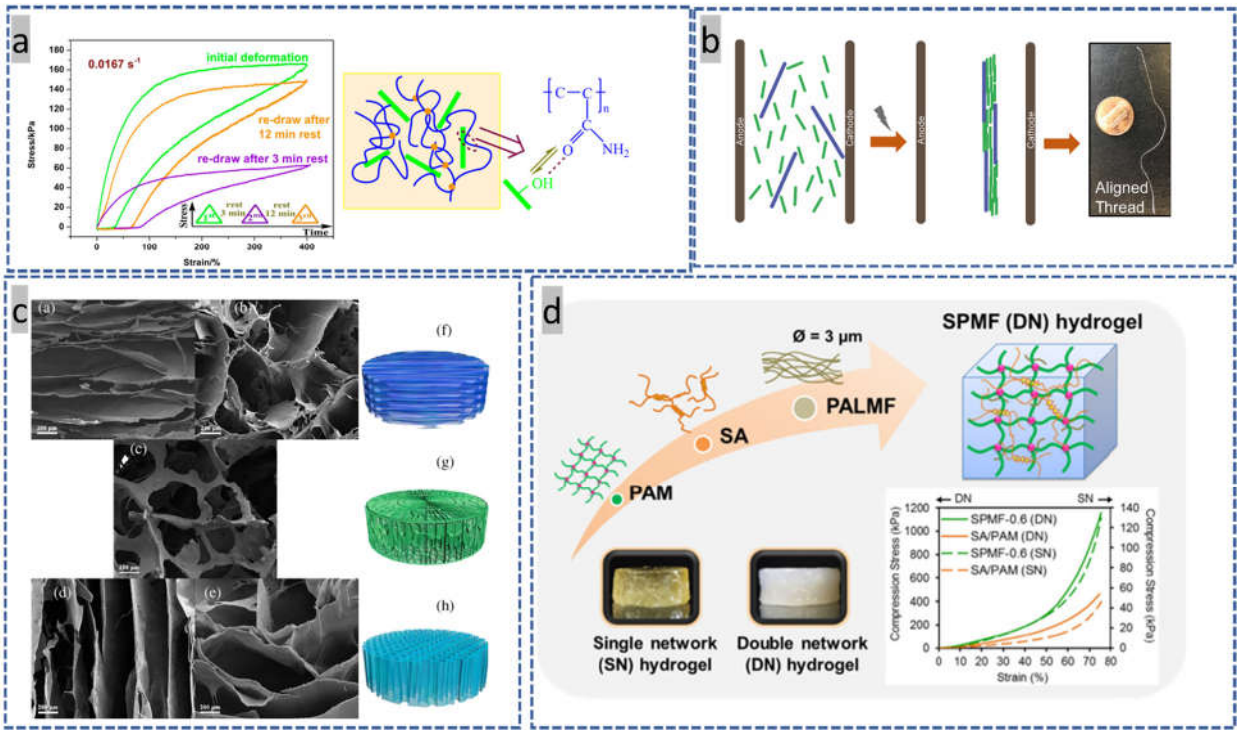

Figure 3. Reinforcement impact of CNC in hydrogel. **(a)** Mechanism of reinforcement in CNC leads to gel toughness[253]. **(b)** adapted with permission from ref. [273] **(c)** SEM images (a–e) and corresponding schematic diagrams (f–h) of hydrogels with different pore orientations. (a) and (b) are the front and left view of horizontal pore structure (f), (c) is the cross section of the random (g), and (d) and (e) are the front and left view of the vertical (h). Adapted with permission from ref. [272] **(d)** Adapted with permission from ref [274].

Pluronic F127 is a low-temperature soluble nontoxic triblock copolymer comprising poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO100-PPO65-PEO100). As temperature gets close to human body temperature, PPO sections (relatively hydrophobic) start to aggregate and form a dense core whereas the hydrophilic PEO blocks go through an extension. The resultant shape is a spherical micelle with different core and shell[275]. Upon growing of the microgel, hydrogel components start to interact after passage of certain temperature and form a gel; thus, gelation process is reversible if temperature tune the microstructure. Authors findings showed a previously unknown function for CNC network formation in the micellization and gelation behaviour of a triblock copolymer. Linear and nonlinear rheological analyses show that for low and

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intermediate nanocrystal loadings (1-3 percent by weight), the composite gel softens and deforms significantly more than the neat Pluronic F127 gels. The softening effect is caused by the rodlike CNCs disrupting the densely packed micelles. However, at high concentrations, the nanocrystals create their own network, trapping the micelles within the CNC meshes. Therefore, at 4 to 5 percent nanocrystal loading, the original (neat F127) hard-gel modulus is regained, but the composite gel is substantially more deformable (and harder) in the presence of the CNC network. The whole process schematic is shown in **Figure 5a**[275]. This hydrogel in an equivalent to PNIPAM hydrogel as temperature is seen to induce shifts in mechanical properties; interestingly for a quicker return to pre-shear state hydrogel developed here can be tweak with temperature to produce a quicker return thus impede sagging of the 3-D printed hydrogel. Other components such as its effect on cells and adhesion to adjacent cells at position of injection are other notable factors that require further deliberations.

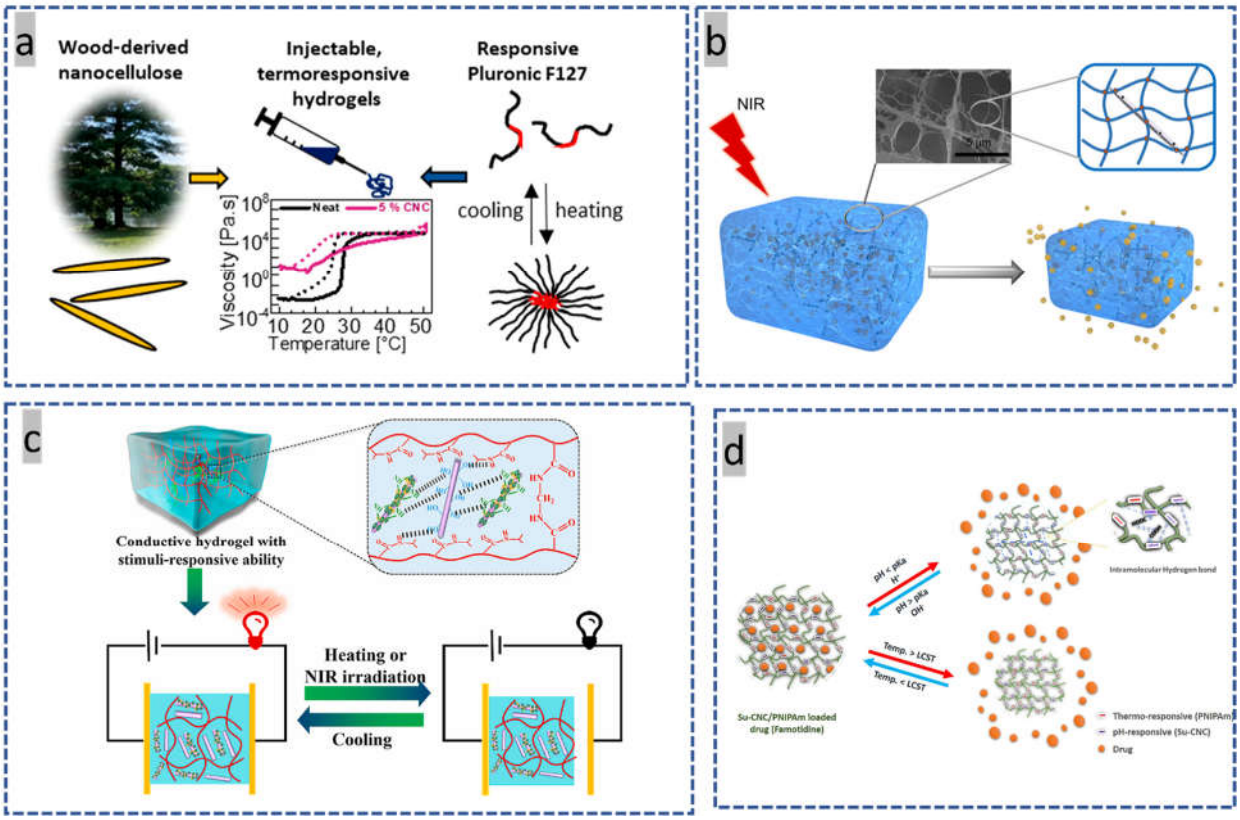

Figure 5. (a) Schematic of usage of surfactant gel and CNC to make thermo-responsive reinforced hydrogels. Adapted with permission from ref[275]. Thermo-responsive hydrogel reinforced with CNCs with PNIPAM. **(b)** A highly stretchy nanocomposite hydrogel built on PNIPAM was produced and reinforced with CNCs. Adapted with permission from ref [276]. **(c)** an approach for developing multi-stimulus full control hydrogels with high potential for use as wirelessly regulated switch devices in materials and tissue repair. Adapted with permission from ref [277]. **(d)** Design of an esterified cellulose nanocrystal-based dual pH and temperature sensitive hydrogel for potential drug release. Adapted with permission from ref. [278].

A highly stretchy nanocomposite hydrogel built on PNIPAM was produced, with a maximum elongation of 2200 percent. The hydrogels demonstrated NIR release of drug with drug loadings of up to 10.18 g/g. These strong, on-demand hydrogel can be used as good drug stores for wound contraction and healing. One barrier to the use of PNIPAM -based hydrogels is the inability to attain the high drug loading levels required for a single sustained release system. This issue becomes much more pressing when a certain medication dosage is required at a specific place for a lengthy period of time[279]. Nevertheless, the many -oh group (-OH) on the face of Nanocrystals have been shown to promote

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further formation of hydrogen bonds with hydrophilic medicines and PNIPAM chains, culminating in a notably high drug loading capacity and strong hydrogels[280] (See **Figure 5b**).

Several smart microgels with both conductivity and sensor characteristics have recently been developed into elastic semiconductor switches[281-282]. Temperature[283-284], pH[285-286], humidity [287-288], and specific ions/molecules [289-290]might all cause these hydrogels to react. Natural polymers, such as alginate, albumin, chitosan, and others, can also be pH sensitive. As explained earlier in CNC pure gels, for tissue engineering and medication delivery, injectable hydrogels made of biocompatible materials are in high demand. Cellulose nanoparticles might be used as pH-responsive medication delivery systems, preserving medicines in the stomach, and then releasing them in the duodenum. Alternatively, mechanical properties of CNC-hydrogel is also tunable using PNIPAM thermosensitive polymer [291]; to tune in mechanical properties of hydrogel before, during and after injection to mitigate structural damage to the hydrogel and cells in case of cell deliveries. For instance in one report, water content of microgel decreased from 90% at temperatures below 30 °C to approximately 50% at 45°C; this was conjugated with increase in mechanical properties in terms of modulus from 100 kPA to 600 kPa with increase in temperature[292].

Designs can get more advanced, such as changing channels with remote controls, releasing medications, and delivering cells with the push of a button. In Shi's study[121], for example, a as thermo-responsive switching made of PNIPAM and also in generated metallic polypyrrole (PPy) demonstrated good electrical conductivity (up to 0.8 S/m) and a quick thermo-responsive speed. Even so, whenever the switches were used in flexible bioelectronic devices that worked in dark in-vivo climates, the device needed to be endowed with remote wireless abilities [293-294], so that the 'ON/OFF' states of the switches might be governed seamlessly and comfortably with no immediate communication. By incorporating relatively low amounts of CNC@PPy (cellulose nanocrystal decorated with polypyrrole (PPy)) (0.6 wt percent) and CNC@PDA (cellulose nanocrystal decorated with polydopamine (PDA)) (1.0 wt percent) nanoparticles into thermo-responsive PNIPAM hydrogel networks, a dual thermo- and NIR light-responsive conductive hydrogel system was designed (See **Figure 5c**). To make hydrogels responsive to both pH and temperature; succinylated CNCs were incorporated inside a hydrogel made with PNIPAM as shown in **Figure 5d**[278]**.** Temperature release for patches implanted under skin is particularly interesting for these state-of-the-art applications.

Mechanical properties of PNIPAM is tunable 4-14 kPa as a function of crosslinking ratio and monomer concentrations [122]. Knowing roughly how much reinforcement can be extracted from hydrogel alone is useful for hydrogel design; as previously stated, PNIPAM is advantageous to other polymers due to its thermos-responsive behaviour, particularly around body temperature; this transition temperature can be tuned for cases where hydrogel implementation is designed to be implemented on animals. There are other alternatives to PNIPAM as a matrix hosting CNCs. Liu et al. [295] grafted methylcellulose with the synthetic PNIPAM, combining the thermogelling properties of both materials. It was possible to make rapid transiently thermogelling hydrogels by varying the proportions of the two components. They observed that as compared to PNIPAM, a low methylcellulose proportion (MC) reduces LCST, but a high MC ratio increases LCST. They also observed that addition MC to PNIPAM polymers boosts mechanical strength without producing syneresis. Therefore, this example of hydrogel can be adopted to animals depending on MC ration in the hydrogel for hydrogel destined for pet care applications. Bhattarai et al.[296] added poly(ethylene glycol) (PEG) into chitosan and were able to form a thermos-reversible hydrogel without the need of any extra copolymers. Furthermore, PEG grafting enhanced chitosan solubility in water, and gelation was discovered to be achievable at physiological pH levels. By reacting with maleic anhydride (MA), an altered progenitor of the proteolytic enzymes degradable dextran (Dex) was generated, and the Dex-MA polysaccharide was given thermoresponsive characteristics by photocrosslinking with PNIPAM. Because of the hydrophilic and reversible characteristics of Dex-MA, the

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resultant hydrogel was partly biodegradable and had a higher LCST than PNIPAM. Furthermore, the hydrogel's pH is sensitive due to the carboxylic end groups of Dex-MA[297].

A coinable term to address the sustainability of hydrogels is "sustainability index," which is defined here by the authors as the proportion of the material that can be replenished based on accessible material in nature. This value can range from 0 to 1 (unsustainable to totally sustainable). For example, cellulose can be claimed to have a sustainability index of 1, but crude oil products should have a number between 0 and 1; any substance in between can have a score between 0 and 1. Grafting PNIPAM on totally sustainable materials should result in materials with a sustainability between 0 and 1.

5.3.1. Cell-consideration

Electrospinning cannot be used for cell delivery as exposure of stem cells to electrical field causes them to perish [244]. The vitality of mesenchymal stem cells was lowered from 88 to 19.6 percent after electrospinning, while that of mononuclear cells was reduced from 99 to 8.38 percent.

5.4. Bio based hydrogels

CNC are mixable with other biopolymers such as alginate, protein, collagen and xanthan gum to provide long lasting solution for manufacturing CNC based hydrogels. Although injected in vivo crosslinking hydrogels hold promise for using stem cells for sake rebuilding cartilage; the encapsulating cells are subjected to huge stress during injection through cell survival and subsequent proliferation are affected. Surface modified CNCs are heralded as greener more biodegradable reinforcement agent for collagen hydrogels. A simple one-pot oxidation was used to create aldehyde-functionalized CNCs (a-CNCs).

Under pathophysiological conditions, a hybrid a-CNC/collagen gel cross-linked fast by fluid Schiff base linkages built on a-CNCs and collagen. When contrasted to CNC/collagen hydrogels lacking Schiff base connections, the a-CNC/collagen hydrogel demonstrated faster shear-thinning, self-healing, and better elastic modulus. After that, the a-CNC/collagen hydrogel was tested for mesenchymal stem cell (MSC) administration. In vitro, MSCs encapsulated in the a-CNC/collagen hydrogel demonstrated excellent cell survival following extrusion. MSCs packed in an a-CNC/collagen hydrogel were subcutaneously injected, resulting in better implant integrity and cell retention. The suggested self-healing collagen-based hydrogel would not only preserve cells after injection, but would also conform into the uneven cartilage defect, offering promise for delivering MSCs for cartilage tissue engineering via less invasive techniques. The procedure visually is shown in **Figure 6.** CNCs have been used to increase mechanical strength and improve viscoelastic behaviour in covalent cross-linked hydrogels because to reversible hydrogen bonding[145, 175].

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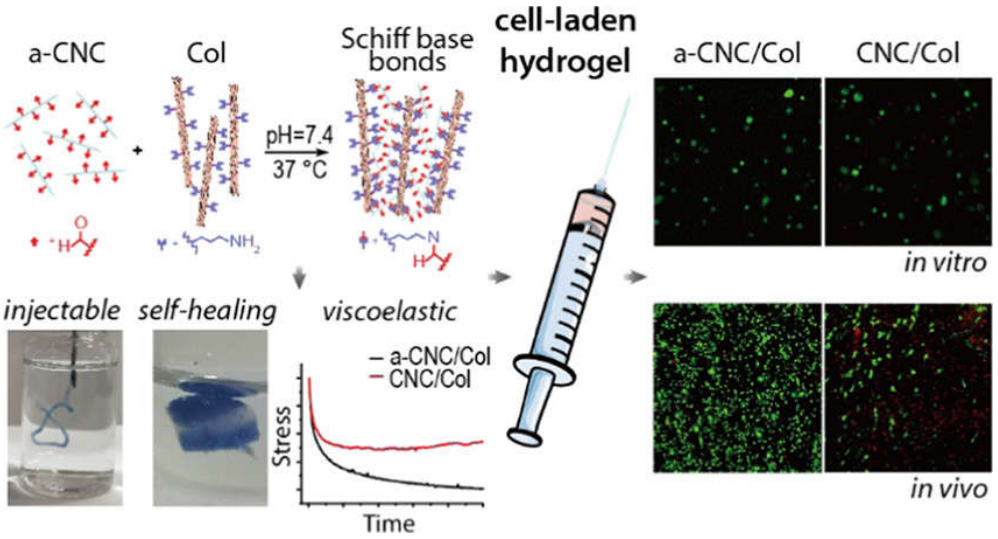

Figure 6. Schematic Representation of the Preparation of Aldehyde-Functionalized CNC/Collagen (a-CNC/Collagen) Hydrogel. Adapted with permission from ref [298].

Figure 7a depicts the authors' attempt to rheologically assess the self-healing property of a doubly network CNC-PVA hydrogel; clearly, the system shows self-healing property in the long term by gauging storage modulus as a function of time, as shown in the first figure, and then in the short term by analysing the gel's large amplitude cyclic response. The timing of these curves, i.e., when the time storage modulus hits specific thresholds, is critical for several applications as injected hydrogel *in vivo* should not let cells becomes rearranged as hydrogel is being implanted with injection; moreover in some applications hydrogel is under dynamic loading such as hydrogel implanted for cartilage repair around knee area while the patient is walking, jumping or running; in these scenario short term recovery shown by large amplitude oscillatory shear is advantageous to long term storage modulus recovery. Knowing the type of test or characterization to be implemented prior to hydrogel design is critical in smart design of hydrogels. **Figure 7c** shows a more advanced model of the hydrogel seen in **Figure 7a**, with CNC being surface modified and PVA being slightly crosslinked to increase the elasticity and rate of self-healing.

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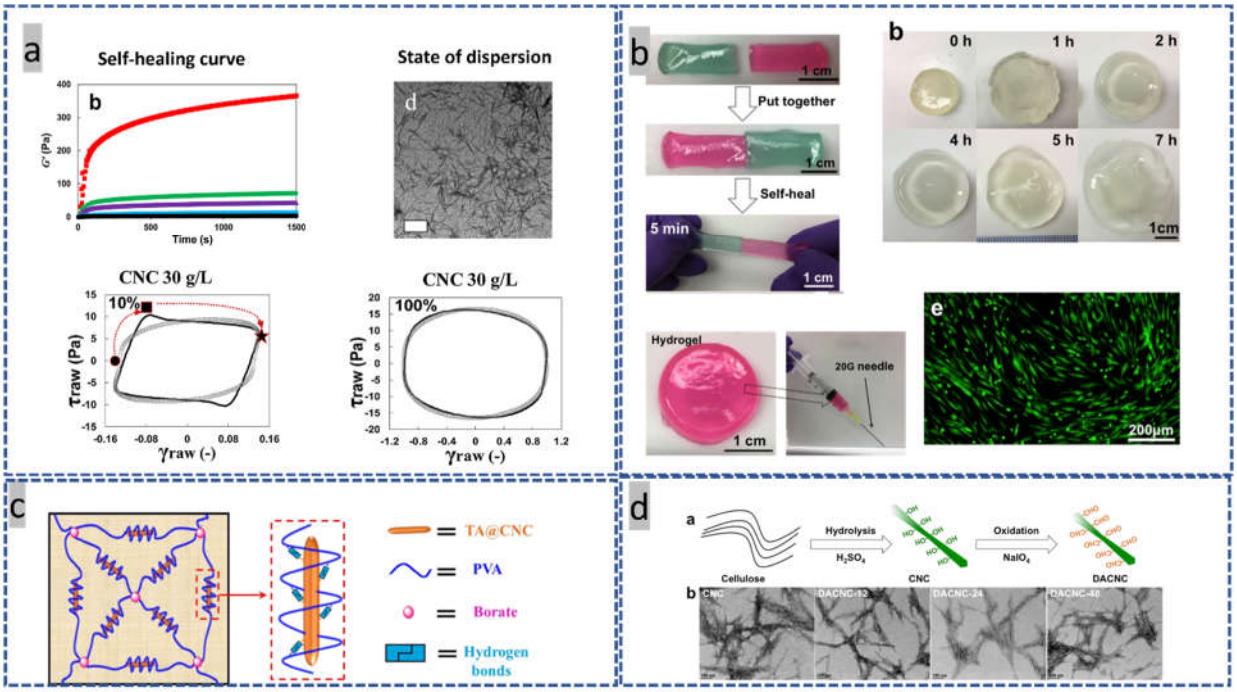

Figure 7. Self-heapability of CNC reinforced gel **(a)** CNC-PVA double network that is self-healable over time. Long term rheological response and short-term rheological response for static and dynamic shearing and assessment of self-healing **(b)** Dissolvable self-healing using carboxymethyl chitosan and CNC Hydrogel for biomedical applications. Adapted with permission from ref. [299] **(c)** Tough and self-healing cellulose nanocomposite hydrogels mimic the dynamic adhesiveness and strain-stiffening behaviour of biological tissues. Adapted with permission from ref [300] **(d)** Synthesis and structural characterizations of DACNC along with TEM images. Adapted with permission from ref. [299].

The mechanical strength of the CMC/DACNC (shown in **Figure 7b**) hydrogel might be customised by varying the aldehyde content of the DACNCs and the molarity of amines to aldehydes, implying useful uses for these CMC/DACNC hydrogels in tissue - engineered of soft tissues with elasticity scales ranging from 0.1 to 10 kPa[301-302]. How CNC change morphology is shown in **Figure 7d.** Later hydrogel has been attempted to culture normal adult human primary dermal fibroblasts; It has been frequently utilised to test material cytotoxicity and biocompatibility[303]. Here rheology is not needed as gel visually self heal; also, extent water uptake is promising for wound dressing application as shown with swollen light yellow color gels shown at the top right corner. **Figure 7c** depicts the creation of a dynamic but reversible crosslink with tannic acid coated CNC into PVA crosslinked with borax (excellent mechanical strength (104 kPa) and self-healing efficiency (98.2 percent)), resulting in an extraordinary soft gel with quick self-healing coupled with strain stiffening capabilities for soft tissue applications[300].

5.4.1. CNC reinforced xanthan gum

By using the freeze-drying procedure, xanthan gum/silica glass hybrid scaffolds reinforced with CNCs were created [304]. When combined with silica glass and CNCs, the resultant mixture displayed a highly porous structure with tunable and enhanced mechanical stability. Additionally, it was shown that excellent pre-osteoblast cytocompatibility improved over time and with gel stiffness. In the work by Rao et al[305], the mechanical performance of developed hydrogels was improved significantly as increased CNC content (from 2 to 10 wt%).

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5.4.2. CNC reinforced alginate

In one research, merging CNC with alginate[306] resulted in the creation of a bilayer membrane structure to tune drug release; in one report[306] mixing with cationic CNC slowed down the release. CNC refining polymers often need to be biocompatible as well. In the work by Al-Sabah et al[307]. CNCs were combined with sodium alginate and used to 3D print hydrogels for articular and nasal cartilage engineering and shows good integration, promising cartilage regeneration and mechanical stability over 60 days of implantation in mice. Like this[308], a scaffold that was 3-D printed using a CNC-alginate mixture had gradient pore structure and modulus in the range of 0.20-0.45 MPa, which meant that 3D scaffolds met the specifications needed for applications involving cartilage regeneration. While the outside membrane is comprised of ordinary alginate, the inside composite hydrogel of the double-membrane hydrogel is stabilised by electrostatic interactions between cationic CNC and anionic alginate. The thickness of the outer layer can be controlled by how long neat alginate adsorbs, and the architecture and dimensions of the double-membrane hydrogel can be directly influenced by the shape of the inner layer (microsphere, capsule, and filmlike shapes). In some reports beads of alginate are made mixed with CNC for water purification or dye removal [309-310]. In addition to being reinforced with CNC, sodium alginate may also be modified to produce self-healing gel that relies on chemical reversibility rather than physically stemmed processes[311]. Microspheres travel readily and do not offer adequate support for cells or tissues. A combination of microspheres and porous scaffolds might enable sequential or extended release of multiple bioactive substances, therefore meeting the local requirement for growth factors during tissue creation[312].

5.4.3. CNC reinforced protein

Using the gelatin-CNC method, hydrogels with exceptional sensitivity to pH fluctuation were created[313]. In the case of the 25% CNC sample, the effect of crystallinity on the modulus resulted in a shift in the storage modulus from 122 Pa to 468 Pa. The study of the swelling studies showed that CNC-gelatin hydrogels had a remarkable pH sensitivity. The ability of the CNC-gelatin hydrogel to respond differentially to different pH levels demonstrated its exceptional competency in drug delivery applications. With similar idea in mind[312], authors created biodegradable gelatin microspheres filled fibroblast growth factors that then shipped inside the porous composite of collagen\CNC for drug release applications.

Due to their elongated structure, CNCs aggregates tend to break following shear in most of these applications, such as 3-D printers, and then slowly realign themselves. As a result, they facilitate processing and produce superior mechanical qualities in the end. In the study by Dash et al[145]. the preparation of hydrogel with superior mechanical properties of a gelatin matrix that was cross-linked with CNCs explained. They discovered a relationship between the level of crosslinking of gelatin and CNCs and the rise in aldehyde content. The increased gelatin stiffness brought on by the presence of CNCs altered local chain dynamics as well as gel swelling behaviour[314].

5.4.4. CNC reinforced polysaccharides

Hynninen et al[315]. showed that composite hydrogels comprising methyl cellulose (MC) and cellulose nanocrystal (CNC) colloidal rods display a reversible and enhanced rheological storage modulus. The storage modulus of the composite gels was an order of magnitude higher at 60 °C compared to that at 20 °C. The tendency of the MC to form more persistent aggregates promotes the interactions between the CNC chiral aggregates towards enhanced storage modulus and birefringence. Thus, MC in combination with CNC hybrid networks offer materials with tunable rheological properties and access to liquid crystalline properties at low CNC concentrations.

In You et al [316]. injectable hydrogels made of polysaccharides provide several benefits when used in the biomedical field. However, these hydrogels' subpar mechanical qualities

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are the fundamental barrier to their use in therapeutic applications. This problem is solved by the authors through in situ gelling of stiff rod-like cationic CNCs and quaternized cellulose (QC)-based nanocomposite hydrogels. In every instance, a rise in temperature resulted in quick gelation, and the modified were dispersed uniformly throughout the hydrogels. Because of the strong association between CNCs and QC chains mediated by the cross-linking agent, the nanocomposite hydrogels displayed increasing orders-of-magnitude in the mechanical strength, high extension in degradation, and the sustained release time.

6. Conclusions

CNCs are a class of rapidly expanding nanomaterials that have excellent mechanical properties, large surface areas, and the capacity to display variable surface chemistry; however, it is very challenging to incorporate them into various matrices that contain various functional groups. In this paper, we examine the utilisation of CNCs as fillers in biodegradable polymer matrices. With an emphasis on their thermophysical and mechanical characteristics, we talk about the creation and characterisation of CNC-based nanocomposites. In-depth research is done on the characterisation, separation of nanocellulose from a range of raw material sources, and many surface modifications. Several industrial industries may be impacted by the incorporation of CNC in biodegradable polymers, as well as nanocellulose surface modification and environmental trends. Innovating new materials with a distinctive blend of toughness and strength is made possible by the creation of hybrid cross-linking hydrogels with superior mechanical characteristics and the basic load transfer design idea from combination microstructures. We provided examples of hydrogels with both natural and artificial host matrixes from the literature. We illustrated some advantages of employing these systems for medication delivery, 3-D printing, cell carrying, and design requirements that must be adhered to for precise use of these systems.

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