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Biomimetic synthesis of two different types of renewable cellulosic nanomaterials for scaffolding in tissue engineering

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Abstract: As a rapidly growing area in materials design, the biomimetic approach at the frontier between biology and materials science aims to introduce advanced materials with structural diversities and functional versatilities by mimicking remarkable systems available in nature. Inspired by the fascinating nanostructured assembly existing in the cell walls of different plant species, we designed two fully bio-based green nanomaterials reinforced with renewable polysaccharide nanoparticles in the form of cellulose nanowhiskers (CNWs). In our initial design, the CNWs were incorporated into a cellulose acetate propionate matrix to form a bionanocomposite film, while in the second design the CNWs were entangled within a network of a collagenous medium to introduce a bionanocomposite hydrogel. Tensile and rheological measurements were carried out to study the system's deformation as subjected to axial force or oscillatory shear. Biocompatibility was tested via incubation of human bone marrow-derived mesenchymal stem cells *in vitro*. Careful control of the processing conditions resulted in a three-dimensional rigid CNW network percolating within both biopolymer matrices, giving rise to an excellent performance at only a small fraction of CNWs at 3 wt.%. This study reveals that the fully bio-based green nanomaterials with enhanced mechanical percolation could construct a suitable platform for scaffolding in tissue engineering.

Keywords: biomimetic design; cellulosic nanomaterials; mechanical percolation; nanotechnology; tissue engineering.

1 Introduction

The increasing demand for the green, economically efficient, and environmentally sustainable materials has imposed an essential need to limit the consumption of petrochemical-based materials and substitute them with renewable ones. Biological systems available in nature offer numerous examples of the high performance green materials where their energy-efficient syntheses are facilitated at moderate temperature and low pressure [1]. Renewable materials that stem from these living organisms always integrate in a composite arrangement where their molecular interactions are optimized in a fashion to direct their stable functionalities [2]. Mimicking such remarkable composites has become a major source of inspiration to design advanced materials with superior properties and functional versatilities for diverse range of applications.

Recent advances in nanoscience and nanotechnology have further empowered material scientists to expand such biomimetic approaches and design capabilities in order to intimately mimic nature materials at micro- and nano-scales. As an example, the cell walls of different plant species represent a fascinating hierarchical nanostructured biomaterial where the mechanical stiffness, strength, and toughness are combined in a way to offer a lightweight system with unique properties despite a highly hydrated state surrounding the microenvironment [1, 3, 4]. To mimic such exceptional bionanomaterial assembly, different polysaccharide nanoparticles have been incorporated within different networks of host matrices to fabricate highly reinforced bionanocomposites. Nonetheless, natural polysaccharide nanoparticles, derived from cellulose, chitin, and starch, still offer explicit advantages when compared with inorganic nanoparticles mainly due to their availability, biocompatibility, biodegradability, relatively easy and cost-efficient processing, considerable mechanical performance, and reactive surface integrity [5]. The renewable nanoparticles are effectively prepared by digestion of the amorphous region of the biomass using their reaction to acid hydrolysis under controlled processing conditions while leaving the rigid crystalline regions intact. The extracted crystalline domain from cellulose

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and chitin forms in whisker-like nanoparticles, whereas that of from starch granules shapes in platelet-like nanoparticles [5].

In this work, we mainly focus on extracting whisker-like nanoparticles from microcrystalline cellulose (cellulose nanowhiskers, CNWs). The CNWs offer exceptional properties such as high aspect ratio, large interface area, significant mechanical integrity, controlled biodegradability, reasonable permeability, and susceptibility to aligned morphology [6]. The rigidity of the nanoparticles as well as their strong intermolecular hydrogen-bonding interactions constructs a three-dimensional rigid CNW network as percolates within a host matrix resulting in a substantial capacity to carry external loads when compared to other types of particulate reinforcing agents [7].

The native tissue microenvironment or extracellular matrix (ECM) is typically a porous bionanocomposite substrate with dynamic and hierarchically organized features offering biochemical signals as well as mechanical support to cells while regulating their essential activities [8, 9]. To closely mimic such microenvironment and to take advantage of the excellent reinforcing properties of the CNWs in exploiting their potential in ECM scaffolding applications, we have fabricated two different yet fully bio-based green nanomaterials in the forms of a film and a hydrogel in this work.

Based on previous studies in the literature, bio-ester cellulose such as cellulose acetate (CA) and cellulose acetate propionate (CAP) offers a viable scaffolding candidate with excellent properties such as insignificant foreign body and inflammatory response, controlled biodegradability, and nonacidic by-products in the form of glucose as opposed to that of standard synthetic-based scaffolds [10]. In particular, the glucopyranosyl residues available on the surface of the CAP polymer provide recognizable biomarkers to the carbohydrate molecules existing on the surface of cells, which ultimately promote biochemical signaling and subsequent cell adhesions [11]. Therefore, our first design centers the attention over an all-cellulose film nanomaterial where the CNWs are entangled within the biopolymer network of CAP. The dispersed filler phase forms a three-dimensional rigid CNW network percolating within the CAP host matrix, which imparts considerable mechanical strength to the entire bionanomaterial at only small CNW loadings. Nonetheless, the fabricated CAP-CNW nanocomposite film was too stiff and brittle and, hence, would most likely not be suitable for its intended scaffolding application.

To impart enhanced ductility with the desired flexibility to the bioscaffold material, and to better mimic the structural features displayed by protein and polysaccharide nanoparticles in natural ECM [8], another biopolymer matrix was required, and the most natural choice has been collagen (COL). As the most abundant biopolymer in major building blocks of connective tissues, COL has been aggressively pursued for biomedical applications due to its excellent biocompatibility, safe biodegradability, and very low antigenicity [12]. Conversely, scaffolds purely made with COL reveal disadvantages such as poor resistance to adequately absorbing water, fast structural breakdown, and lack of mechanical integration while deforming [13–15]. Thus, by incorporating the CNW nanoparticles into the collagenous network, we designed a COL-CNW nanocomposite hydrogel in which the CNWs would impart mechanical strength, toughness, and surface reactivity, while the COL host matrix would provide mechanical ductility delivering an effective viscoelastic behavior to the entire substrate. The resulting bionanomaterial exhibited an excellent enhancement in mechanical rigidity at a small fraction of nanofiller, thus indicating the continuous CNW integration and their maximal entanglement within the collagenous medium.

Therefore, the main focus of this work is to study the morphology, the tensile and rheology measurements, and the biocompatibility of the bionanomaterial systems under controlled processing conditions. By taking a fully green synthesis process without using toxic chemicals, by monitoring the dispersion of the CNW phase at low filler concentration, and by maintaining the level of porosity within the microstructure network, we attempt to optimize the mechanical rigidity without compromising the material's inherent biocompatibility. As such, the designed substrate in this study could potentially offer a promising fully bio-based green nanomaterial candidate for scaffolding in tissue engineering.

2 Materials and methods

2.1 Synthesis of cellulose nanowhiskers

Microcrystalline cellulose (MCC), a commercial precursor in the form of cotton linter, was purchased from Sigma-Aldrich, Milwaukee, WI, in order to initiate the extraction process of nanoparticles. A sulfuric acid hydrolysis (62 wt.%, Sigma-Aldrich, Milwaukee, WI, USA) treatment was followed to effectively disrupt and quickly digest the amorphous region of the MCC. Right after, through successive cycles of centrifugation, a nearly clear non-turbid solution with suspended CNWs was formed. Then, a dialysis exchange against distilled water was brought into place in order to remove the sulfuric acid residues as well as to obtain a neutral suspension of CNWs in the form of stable colloids. Finally, to minimize the level of agglomeration of CNWs from the intermolecular interactions of their surface hydroxyl groups and to obtain their uniform distribution within biopolymer matrices, their stable colloidal suspension was gently freeze-dried and delicately dispersed in spectroscopic grade acetone (VWR, Atlanta, GA, USA) prior to bionanocomposite fabrication.

2.2 Preparation of bionanocomposite film

CAP was purchased from Sigma-Aldrich, Milwaukee, WI. The powder-based CAP contained 2.5 wt.% acetyl and 46 wt.% propionyl in the ratio of 1:14 with molecular weight of 75,000 g/mol. To prepare a clear solution, the CAP powder was initially dissolved in acetone (5 wt.%) overnight under constant magnetic stirring. Then, to better maintain the uniform level of CNW dispersion within the CAP host matrix, the CNWs were first pre-dispersed in acetone then mixed with the prepared CAP solution using several minutes of sonication with additional 2 h of magnetic stirring. Finally, the CAP-CNW nanocomposite suspension with different range of CNW concentrations (1, 3, 6, and 9 wt.%) was cast into a polytetrafluoroethylene mold (VWR, Atlanta, GA, USA) and formed into a 200 μm film as settled in room temperature. This variation was intended to better assess the effect of nanowhisker concentrations on the uniformity and property improvement of the resulting bionanocomposites as well as to obtain the optimum CNW content for the best bionanocomposite performance.

2.3 Preparation of bionanocomposite hydrogel

Commercially available type I COL from bovine Achilles tendon in microfibrillar form was purchased from Sigma-Aldrich, Milwaukee, WI. First, homogenous diluted acetic acid (AA, 0.05 м, Sigma-Aldrich, Milwaukee, WI, USA) was prepared overnight under magnetic stirring at room temperature. Next, a collagenous medium was formed by dissolving the microfibrillar COL in the prepared AA suspension (5 mg/ml) as subjected to additional 2 h of magnetic stirring at room temperature. Similar to the CAP-CNW nanocomposite film preparation, the CNWs were initially pre-dispersed in acetone and varied in concentrations of 1, 3, 6, and 9 wt.% prior to mixing with the COL host matrix. Then, all hydrogels were filtered and de-aerated under vacuum to remove possible entrapped air bubbles introduced during the mixing process followed by cooling down to –10°C for 24 h in order to introduce porosity in their microstructures. Finally, the hydrogels were stored at 4°C to maintain the gel stability and to control the system hydration prior to the following characterizations.

2.4 Atomic force microscopy

The topography, morphology, and distribution of the synthesized CNWs were probed using an atomic force microscopy (AFM) (3100 Veeco MultiMode AFM, VEECO Probes Inc., Santa Barbara, CA, USA). The AFM scans were collected in tapping mode using the etched silicon tip (1.6 μm) with a nominal spring constant of 1–5 N/m and a resonance frequency of 70 kHz. A droplet of the aqueous CNW suspension taken directly from the dialysis tube was dried on a pre-cleaned glass slide at room temperature prior to imaging.

2.5 Scanning electron microscopy

The microstructure and profile features of the CAP-CNW nanocomposite films as well as the COL-CNW nanocomposite hydrogels were respectively imaged using a LEO/ZEISS scanning electron microscope (SEM, Portland, OR, USA) at accelerating voltage of 5 kV and a FEI Quanta 200 FEG environmental scanning electron microscope (ESEM, FEI Inc., Hillsboro, OR, USA) at accelerating voltage of 10 kV. The specimens of each bionanocomposite group were deposited on silicon wafers, which were initially cleaned with ethanol and piranha solution (a typical mixture of H_2SO_4 and H_2O_2 (VWR, Atlanta, GA, USA) in the ratio of 3:1). Prior to imaging, the film samples were sputter-coated with gold for less than a minute, while the hydrogel specimens were allowed to dry overnight at room temperature to evaporate the solvent content.

2.6 Tensile measurements

The mechanical behavior of the bionanocomposite films was investigated using a classical tensile MTS Insight II (MTS Systems Corp., Eden Prairie, MN, USA). The instrument was operated with a nominal gage length of 20 mm and a crosshead speed of 1.2 mm/min. The specimens were initially cut into a standard dog-bone shape using a laser machine (Full Spectrum Laser, Las Vegas, NV, USA) followed by mounting into the instrument where the analyses were monitored under loading of 100 N at a rate of 20 Hz and temperature of 37°C. To confirm the consistency of the probed measurements and to verify the homogeneity of the fabricated films, three samples from different regions of each set of film were tested under the same specified condition.

2.7 Rheology measurements

Oscillatory shear testing was carried out on the bionanocompsite hydrogels with different CNW concentrations in their hydrated state using a macroscopic rotational rheometer (a controlled strain advanced rheometric expansion system, ARES, Rheomtric Scientific Inc., Piscataway, NJ, USA). The hydrogel samples, which were kept at 4°C, were allowed to stabilize from their surface moisture at room temperature to minimize their further evaporation effect during experimentation. Then, the samples were loaded onto the rheometer using a 25 mm parallel plate geometry. A frequency sweep experiment was probed with an angular frequency (ω) ranging from 0.1 to 1000 rad/s at a specified γ_{max} to determine the dynamic shear moduli of the hydrogels. The maximum strain and angular frequency were, respectively, set below 10% and 0.1 rad/s in order to monitor the hydrogel deformation within their linear viscoelastic regimes. All rheological measurements were analyzed using a gap of 1 mm and tested at ambient temperature of 25°C. Three specimens in each group of hydrogels were tested in order to verify the accuracy of the measured data and to confirm the homogeneity of the prepared samples.

2.8 Cell invasion assay

Samples of the pure COL and the COL-CNW nanocomposite hydrogel were cut into slices to fit into 96-well plates (Thermo Fisher Scientific Inc., Waltham, MA, USA) and were sterilized in 70% alcohol (Thermo Fisher Scientific Inc., Waltham, MA, USA) under ultraviolet irradiation for an hour. The alcohol residues were then removed by rinsing the samples in distilled water for 30 min. Next, the samples were washed three times with phosphate-buffered saline (Thermo Fisher Scientific Inc., Waltham, MA, USA) for a 15-min cycle and followed by immersion in a standard culture medium (alpha minimum essential medium, Thermo Fisher Scientific Inc., Waltham, MA, USA). Right after, human bone marrow-derived mesenchymal stem cells (MSCs) in an aggregated morphology were placed onto the hydrogels, which was then trapped on both sides with a fibrin hydrogel in a modification of our 3D cell invasion assay discussed prior [16, 17].

Following the fibrin hydrogel polymerization, the entire gel was placed into 24-well plates (Thermo Fisher Scientific Inc., Waltham, MA, USA) with the standard culture medium and incubated under standard conditions out to 8 days. The invading MSCs were digitally photographed at selected time points using bright field and fluorescent imaging (Olympus IX51, Olympus America Inc., Melville, NY, USA). Likewise, to better visualize the cell invasion through the thick layers of the COL-CNW nanocomposite hydrogels, MSCs were counterstained on the frame of an image by DAPI, and the replicate samples were utilized for fluorescent labeling of MSCs with the cell membrane stain PKH-26 (Sigma Chemical Co, Saint Louis, MO, USA) as previously described [16, 18].

The experiments for the cell culture and the analyses were implemented based on the approved protocols under Dr. Brewster's supervision at the Emory University School of Medicine, Vascular Surgery Division.

3 Results and discussion

3.1 Morphology of the CNWs and microstructure of the bionanocomposites

CNWs are generally insoluble in common solvents, and they tend to form a colloidal suspension when placed in an aqueous medium. The stability of the suspension depends on the size, polydispersity, and surface charge of the dispersed CNW particles [19]. Researchers have found that the surface charge introduced during the acid treatment of the CNWs can directly control the whisker-whisker interactions, the suspension rheology, and the aggregate stability [20]. For example, in the H_2SO_4-treated cellulose, the formation of sulfonic acid groups (-HSO_3) on the surface of the CNW particles shown in Figure 1 results in a negatively charged surface, which gives rise to the electrostatic repulsions and colloid stability against the CNW agglomeration while in suspension during synthesis [20, 21].

This phenomenon introduces a non-thixotropic behavior in the CNW suspension and their surface charge properties, leading to their acceptable level of dispersion in aqueous as well as organic media [20, 21]. The SEM images in Figure 2A and B confirm the presence of stable colloids in the H_2SO_4-treated cellulose suspension in this study where the samples were collected directly from the

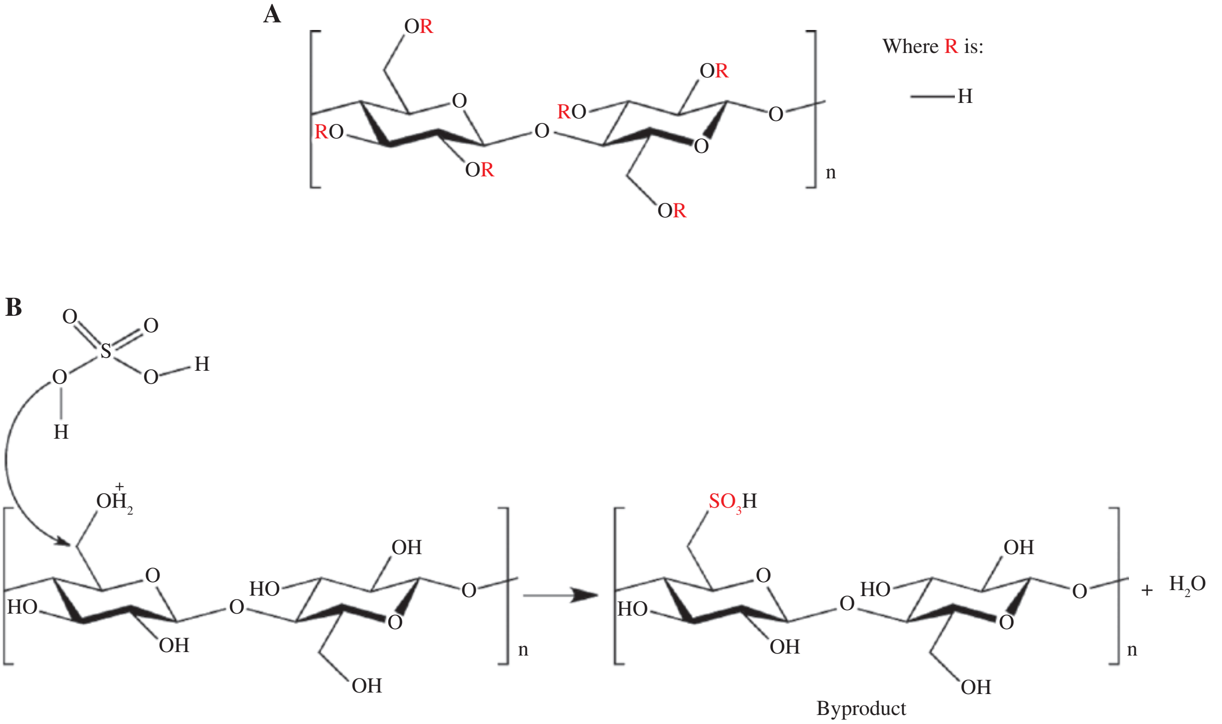

Figure 1: (A) The schematic of the linear chain of the cellulose macromolecule. (B) The esterification of the hydroxyl groups during the cellulose hydrolysis via sulfuric acid.

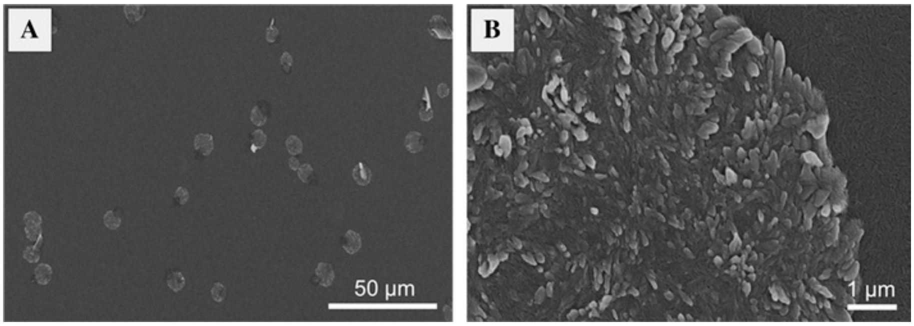

Figure 2: SEM micrographs of the aggregated CNWs in the form of stable colloidal suspension in an aqueous solution at different magnifications represented in A (×20) and B (×1000) (Reproduced with permission, April 2013, Elsevier, license number 3478340256244).

dialysis tubes. It is worth noting that the SEM microscopy was limited by the fact that the CNWs degraded at higher magnification due to their exposure to the high-energy electron beam hitting the sample surface.

Likewise, the AFM images in Figure 3A and B provide a closer look into the intimate features of the CNW stable colloids as well as their morphology and size distribution at higher magnifications. The average sizes of the CNW particles in their aggregated bundles are in the range of 500 nm to 1 µm in length and 10–50 nm in diameter. These values are in agreement with the dimensions reported in the literature using the same cellulosic source subjected to similar processing conditions, recognizing the fact that it is impossible to fabricate the monodispersed CNW sizes due to the diffusion-controlled nature of the acid hydrolysis [19, 22].

The successful extraction of CNW particles from renewable resources and their subsequent uniform dispersion within a host matrix do not always lead to fabricating a bionanocomposite with desired chemical and physical properties. Poor distribution of the CNW particles within a host matrix generally triggers the possibility of air bubble formation and gas entrapment while adversely increasing the formation of the CNW flocculation resulting in unfavorable bionanocomposite performance. This constitutes a major drawback in the mass production of CNW-based bionanocomposites, where the CNW agglomeration and difficulties associated with their surface interactions limit their extent in industrial practice [19]. As such, processing methods play a significant role in the filler-filler and the filler-matrix interactions during the fabrication of a CNW-based bionanocomposite, which was particularly observed in our previous work [23].

In this study, the processing methods such as treating the MCC with H_2SO_4 during the hydrolysis as well as the freeze drying and the pre-dispersing of the CNWs in the acetone suspension notably reduced the CNW intermolecular affinities, thus controlling their subsequent degree of agglomerations as they integrate within the host matrices (Figures 4 and 5). The leading role in the development of a fibrous porous microstructure in both nanocomposite systems is believed to be mainly due to the removal of

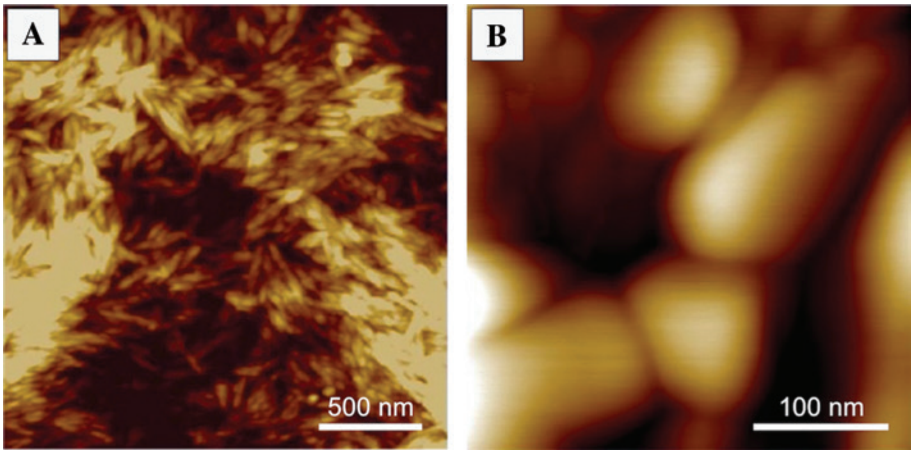

Figure 3: AFM images of the three-dimensional CNWs captured in an aqueous suspension at different magnifications represented in A (×20) and B (×100).

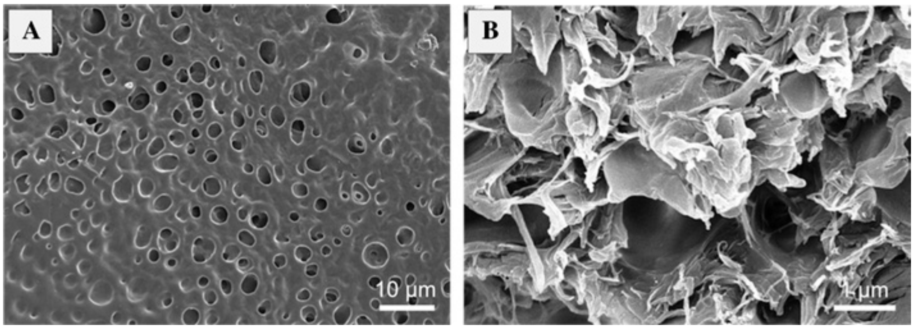

Figure 4: SEM micrographs showing the fibrous porous structure of the CAP-CNW nanocomposite film with the three-dimensional percolating network of CNWs at different magnifications represented in A (×100) and B (×1000).

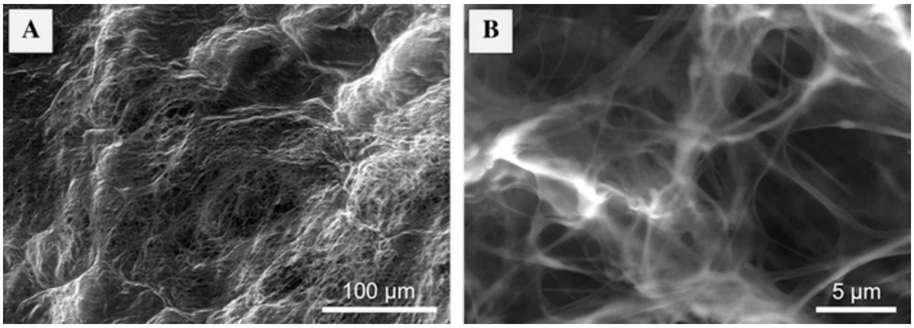

Figure 5: SEM micrographs showing the fibrous structure of the COL-CNW nanocomposite hydrogel with the three-dimensional percolating network of CNWs at different magnifications represented in A (×10) and B (×200).

the entrapped solvent water during the freeze-drying and the pre-dispersing of the CNWs prior to mixing with the biopolymer matrices.

3.2 Mechanical properties of the CAP-CNW nanocomposite film

In general, in nanocomposite science, parameters such as the nature of the host matrix, the filler/filler inter-connectivity, and the matrix/filler interactions can directly impact the optimum filler content while entangling within the host matrix resulting in the best nanocomposite performance. To accordingly analyze the mechanical behavior of the CAP-CNW nanocomposite films and to effectively obtain the optimum CNW content, the measurements were compared as summarized in Figure 6 while considering the pore distribution as an influencing parameter, which was discussed previously [7]. The results reveal a substantial increase in the tensile modulus as well as the ultimate tensile strength of the CAP-CNW nanocomposite film at 3 wt.% CNW. This unusual stiffening effect is believed to be due to the formation of a rigid CNW network percolating within the CAP host matrix below 3 wt.% and above their percolation threshold [7]. Nonetheless, a sharp decline in the tensile performance is evidenced in the samples with the higher CNW concentrations above 3 wt.%. The strong hydrogen-bonding interactions among nanowhiskers could increase the likelihood of unfavorable CNW agglomeration within the bionanocomposite microstructures beyond certain nanofiller contents. This was observed in our previous study where an increase in CNW concentration introduced a larger pore size within

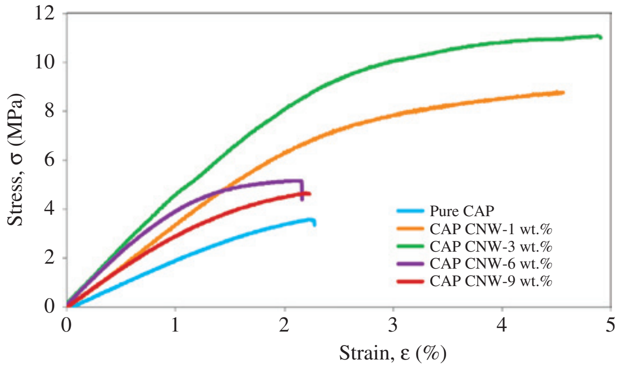

Figure 6: True stress vs. strain curve measurements of the pure CAP and the CAP-CNW nanocomposite films at different CNW concentrations.

the microstructure leading to an inferior tensile performance at 6 and 9 wt.% [23].

3.3 Mechanical properties of the COL-CNW nanocomposite hydrogel

Similarly, to obtain the optimum value of the CNW concentration and to monitor the response of the nanocomposite hydrogels subjected to increasing frequency, the oscillatory shear experiments are extended on samples having 1, 3, 6, and 9 wt.% of nanowhiskers. The elasticity of the pure COL and the COL-CNW nanocomposite hydrogels over increasing frequency is presented in Figure 7. It is evidenced that at any given CNW concentration, at lower frequency the hydrogel fibrils are more free to slide over one another, while at the higher frequency a pseudo-cross-link phenomenon is likely to occur due to the lack of enough time for molecular rearrangement and loosening, which gives rise to higher elasticity [24]. This is also in agreement with other rheological frequency sweep studies reported in the literature for collagenous media [25–27]. However, the higher rigidity observed in the pure COL in this work was due to the higher concentration of COL fibers diluted in the AA in contrast to the lower concentration used in the literature.

Furthermore, the results indicate that the higher the CNW concentrations, the larger the storage modulus is spotted up to about 3 wt.%. This is most likely attributed to the formation of a rigid network of CNWs percolating within the collagenous medium. Nonetheless, the elasticity declines at the higher CNW concentrations of 6 and 9 wt.%, similarly to what was previously observed in the CAP-CNW nanocomposite films. This could also be reminiscent of the nanowhisker flocculation due to their strong hydrogen-bonding interactions above the certain CNW contents.

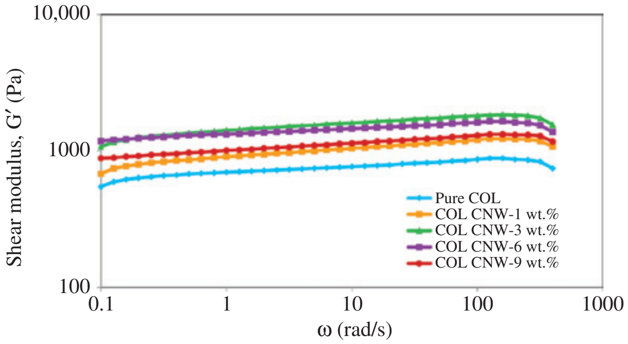

Figure 7: Oscillatory frequency sweep analyses of the pure COL and the COL-CNW nanocomposite hydrogels at different CNW concentrations.

Finally, while considerable mechanical improvement is evidenced in the CAP-CNW nanocomposite film at such low CNW content (3 wt.%), the high stiffness yet brittle nature of the bionanomaterial makes it a less attractive scaffolding candidate; thus, it would most likely not be suitable for its intended application. Alternatively, the enhanced mechanical ductility with the desired flexibility observed in the COL-CNW nanocomposite hydrogel at the similar range of CNW content (3 wt.%) could potentially offer a more suitable scaffolding candidate with good biocompatibility, as discussed next.

3.4 Biocompatibility of the COL-CNW nanocomposite hydrogel

The biocompatibility of the COL-CNW nanocomposite hydrogel is captured in Figure 8A and B, while the MSCs invade from the bionanomaterial into the surrounding fibrin hydrogel as is pronounced out to day 8. Similarly, Figure 8A–D illustrates the cell invasion through the thick layers of nanocomposite hydrogel where the replicate samples had MSCs' nuclei stained with DAPI (Figure 8A and B) or the MSCs' cell membrane stained with PKH-26 (Figure 8C and D).

Similar trends of the cell growth and proliferation surrounding the cellulose-based materials are also reported in the literature [10, 28–30]. Likewise, the biocompatibility of COL has been previously well established [12]. All these evidence together subsequently verify that the constituent materials in this study, from the fabrication of the CNW itself to the formation of the COL-CNW nanocomposite hydrogel, reveal no immediate sign of toxicity.

In summary, the size distribution and specific morphology of nanoparticles as well as the porosity, elasticity, and biocompatibility of microstructure are critical parameters to design a feasible nanostructured scaffold. A nano-fibrous assembly has a beneficial impact on guiding cell adhesion by increasing the particle surface area, porosity, and available binding sites of a substrate [8, 9, 31, 32]. The availability of a porous microstructure directly affects the mass transport for cell nutrition, channels for cell migration, and surface features for cell attachment [33]. Likewise, a substrate with larger elasticity can effectively sustain greater contractile force while going under deformation and can subsequently offer a better mechanical support system for cell growth [27]. This was also evidenced in previous studies showing that the stiffer collagenous gels increased the rate of cell survival by allowing the spreading of their lamellipodium

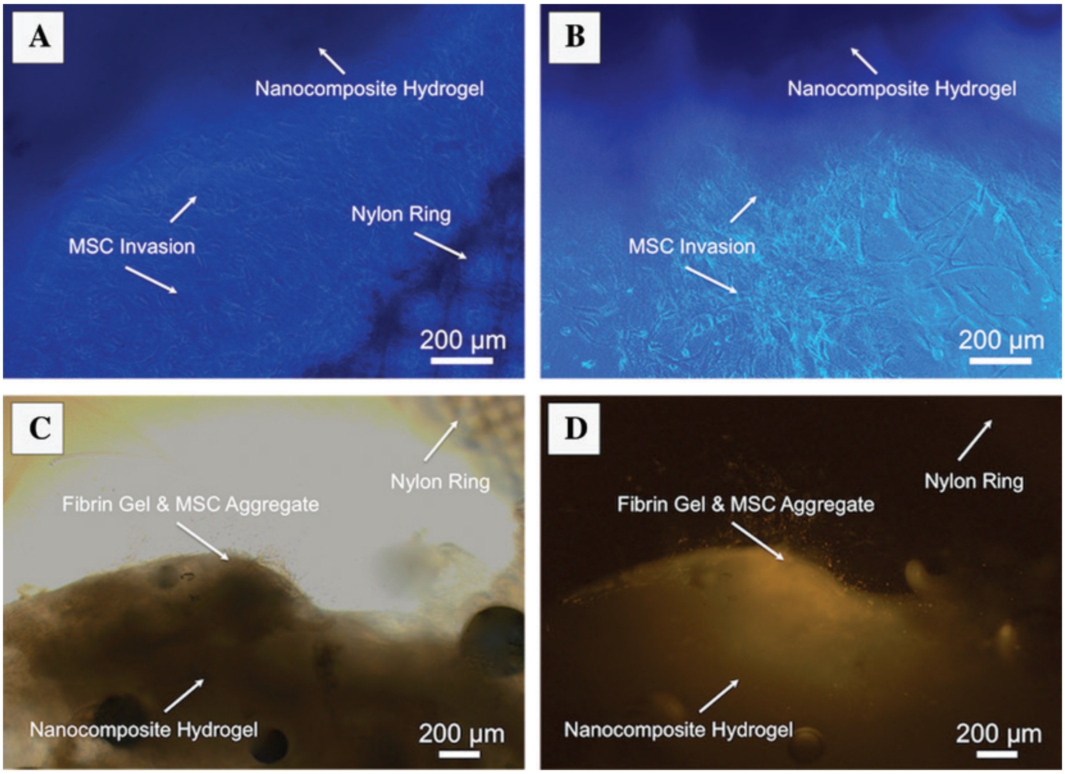

Figure 8: (A) and (B) The outgrowth of the MSCs with fibroblastic morphology and their invasion surrounding the COL-CNW nanocomposite hydrogel on the frames of blue fluorescent staining at day 5 (A) and day 8 (B) of culture with 10× magnification. (C) and (D) The migration and invasion of the PKH-labeled MSC aggregates from fibrin hydrogel into the surrounding COL-CNW nanocomposite hydrogel under bright field (C) and fluorescent (D) imaging both at day 8 of culture with 4× magnification.

while reducing their apoptosis [27, 34, 35]. Ultimately, a biocompatible platform promotes the availability of a suitable interface between cells and a substrate in order to regulate the essential functions and normal activities of the cells while reconstructing a three-dimensional tissue [36].

Nonetheless, these factors are often in conflict with one another, and to design a feasible ECM bioscaffold material, a balance must be obtained. As a result of such considerations, the fully bio-based COL-CNW nanocomposite hydrogel in this study with the tailored viscoelastic functionalities described earlier could present a potential green nanomaterial candidate for scaffolding in tissue engineering.

4 Conclusions

The objective of this work was to biomimetically design a fully bio-based nanostructured scaffold material reinforced with renewable polysaccharide nanoparticles, in the form of CNWs, without impairing the inherent material's biocompatibility. A bionanocomposite film of CAP and a bionanocomposite hydrogel of type I COL were fabricated to investigate their mechanical property improvements as subjected to the increase of the CNW concentrations. The most significant aspect of this study was reflected on the fabrication and characterization of a series of homogenized bionanomaterials with well-dispersed CNWs under controlled processing conditions, exhibiting excellent property improvements with CNW content as low as 3.0 wt.%. This was believed to be due mainly to the formation of a rigid CNW percolating network as well as the strong van der Waals interactions between the surfaces of the CNWs and the biopolymer matrices. While both bionanomaterials offered considerable mechanical enhancements, the bionanocomposite hydrogel provided a more ductile yet flexible substrate for potential scaffolding applications, in which the MSC engraftment within the bionanocomposite hydrogel exhibited good biocompatibility and reasonable viability. We believe that our biomimetic platform presented in this work could open new perspective designs in the self-assembly of bionanomaterial that could be useful for scaffolding in tissue engineering, and it could make the design of the next generation of fully green functional bionanomaterial a reality.

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