Chitin Nanofibers, Preparations and Applications

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1. Introduction

Chitin nanofibers (CNFs) are mainly extracted from crab and prawn shells [1, 2] and recently found in small amount in edible species of mushrooms [3]. CNFs are composed of chitin compound. Chitin in powder form is obtained from fish industry wastes which is otherwise thrown as industrial waste. Since CNFs are biodegradable having typical width 10-20 nm and large surface-to-mass ratio thus they are being prepared, studied, and applied more recently worldwide along with rapidly growing field nanotechnology dealing with the better properties of materials when their sizes are smaller in the range 1-100 nm. Fibrilated chitin in the form of highly viscous gel suspension in water has found scope in pharmaceuticals [4], chiral separation [5], fillers in silsesquioxane [6]. When blended with inorganic metals to prepare advanced hybrid organic-inorganic composites they can have applications in electronics, electrical, optical devices and much needed solar energy production.

To introduce NFs, cellulose NFs are most important as cellulose is most abundant and readily available from plant cell walls and also produced by bacteria. Thus cellulose NFs must be most existing and easily available in nature. Attempt was successful to apply the cellulose NFs by using bacterial cellulose of the width 50 nm [7]. Though the diameter of NFs was 50 nm, larger compared to latter extracted by researchers [8], fibers worked as nanofillers in the cavities of polymerized acrylic resin. A visible light (400-800 nm) transparent flexible sheet of cellulose NFs reinforced acrylic resin polymer was obtained that showed a transmittance value of 85% at 600 nm wavelength when NFs content was 60 wt%. Prepared sheet was highly transparent due to the nanosized effect of NFs as the size of fibers was one-tenth of the wavelength of light that made material free from scattering therefore sheet was transparent. Thus authors claimed that the NFs of 50 nm width can have scope in optical devices such as displays. In year 2007, Abe et al. [8] extracted cellulose NFs of 15 nm width from Radiata pine tree wood powder.
using a series of chemical treatments followed by mechanical grinding. The width of the fibers was measured by field emission scanning electron microscopy (FE-SEM). The authors were first successfully reduced the size of extracted NFs from 50 to 15 nm from any natural resources.

Chitin is second most plentiful biomaterial [9] next to cellulose exists on earth with yearly production of $10^{11}$ tons. Chitin raw dried powder is manufactured from exoskeleton of sea food shellfish, crabs, shrimps, and insects and edible mushrooms of fungus species and sea weed algae. Chitin content in fish industrial waste is 8-33% which is thrown if not used. Thus our group is actively engaged in developing chitin research to make a number of products from atomized or fibrillated chitin in the form of chitin nanofibers (CNFs) and its derivatives [10-13]. Chitin obtained from its natural resources is highly crystalline and most of it is α-chitin conformation though the contents of α- and β-chitin depends on the source.

We have published a number of review articles [11, 14, 15] covering back ground of CNFs in detail, method of preparation, sources, composition, physical and chemical properties, characterization, their composites and derivatives preparations, surface modification. Commercialization of dry chitin powder and CNFs has also been described. For atomization or fibrillation of 1 wt.% wet chitin to CNFs three types of methods were used and compared. A very recently developed [10] Star Burst atomization system which employed high pressure water jet system where slurry of chitin in high acetic acid medium is introduced in chamber of Star Burst system machine where it is fibrilized into NFs of width (18.0-19.0 nm). Atomization occurred in this newly developed machine chamber by collision to ceramic ball that throws out fine fibrillated NFs at extremely high pressure of 245 MPa through an outlet nozzle. The two other commonly used apparatus used for fibrillation are a blender and grinder. The advantages of Star Burst system over blender or grinder for fibrillation have been described in article [10] published recently. CNFs obtained by Star Burst system were studied thoroughly recording FE-SEM images of fibers obtained after a number of passes up to ten. The width of NFs decreased from 19.0 nm to 16.5 nm when number of passes increased from one to ten, respectively. Effect of number of passes on the CNFs properties was investigated by FT-IR, XRD profiles of chitin. In review article [11] molecular structure of chitin, hierarchical organization on the surface of crab shell exoskeleton and isolation from crab and prawn shell has been described. Method of isolation of CNFs from crab or prawn shell using a number of chemical treatments followed by grinder treatment has been explained. The width of NFs was determined by FE-SEM recordings, NFs of 10-20 nm diameter with high aspect ratio were obtained after one pass. FE-SEM images were recorded of stepwise isolation of NFs, just after removing the matrix components and after one pass treatment in acetic acid and without acetic acid condition. Without grinding treatment the fibers were like accumulated ribbons, after one pass treatment without acid the fibers were not separated but in acidic condition after one pass the fibers separated due to repulsion among the positive charges generated on the surface of fibers in acidic conditions. Chitin NFs were modified to produce novel green materials into nano-whiskers of width 6.2 nm and length 250 nm when fibers were deacetylated by treating with 33% NaOH. This contribution discusses most recent advances in preparation, derivatization, characterization and applications of CNFs. Most of the work has been conducted in our laboratory and we have also discussed the results from other groups as well.
2. Preparation of CNFs from crab and prawn shells, and mushrooms

2.1. CNFs from crab shells

Commercial grade dried crab shell flakes of species Paralithodes camtschaticus (red king crab) were used as a raw starting material to isolate NFs. Flakes from red king crab shell are so cheap and abundant that they are used in fertilizer industry. Crab shells were crushed to powder and purified according to the well established method. 1 wt.% slurry of crab chitin was prepared by a series of chemical treatment described in a previous chapter [14]. In brief minerals were removed by HCl treatment, suspension was filtered and washed thoroughly with distilled water, removal of proteins was done by refluxing the suspension with NaOH, pigments and lipids were removed by ethanol. After completion of above the treatments, suspension was filtered washed with distilled water and kept wet for mechanical grinding for fibrillation, this wet slurry was made to a concentration of 1 wt.% and called chitin slurry. Chemical treatment loosened the tightly bonded fibrils bundles to larger extent apart from removal of minerals, proteins, pigments, and lipids as shown in Fig. 1a and b.

Figure 1. FE-SEM images of crab shell surface after removal of matrix from shell surface by chemical treatment without mechanical grinding at different magnification scales; a) 1000 nm; b) 100 nm. Reprinted with permission from ref. 1. Copyright 2009, American Chemical Society.

Bundles of NFs of width 30 nm are visible in micrographs without mechanical grinding. For fibrillation, 1 wt.% slurry was passed through a grinder of the model (MKCA6-3; Masuko Sangyo Co., Ltd.). After passing through the grinder, chitin slurry changed to highly viscous stable wet gel of CNFs. To record FE-SEM picture of sample, sheet of chitin material was prepared. Sheet was coated with 2 nm layer of platinum by an ion sputter coating before recording SEM micrographs. Chitin slurry was passed for one cycle through grinder at pH 7 and 3. As shown in Fig. 2a at neutral pH, fibers had width in wider range 10-100 nm. The bundles of embedded chitin-protein fibers were fibrillated successfully by grinding of wet chitin. It was easy to remove protein from water soaked chitin to isolate chitin fibrils. Authors [16] reported preparation method of CNFs from wet squid pen β-chitin at pH 3–4. In acidic
condition at low pH cationization of C2 amino groups in β-chitin occurred resulted in more dispersed and stable phase because of electrostatic repulsion. Similar electrostatic phenomena occurred at low pH of 3 when amino groups cationized in preparation of CNFs from α-chitin obtained from crab shell in one grinding pass in acidic condition by our research group [1]. Fine fibrils in the narrow range of 10-20 nm were obtained as shown in Fig. 2b and c. Chitin slurry of 1 wt.% became a highly viscous gel phase after one cycle of grinding treatment is due to large surface area of NFs. Viscous gel phase formation is the indication that fibrillation was successful in one cycle of grinding and it was more facilitated in acidic medium as unbroken high aspect ratio NFs were visible in FE-SEM images.

![Figure 2. FE-SEM pictures CNFs prepared from crab shell after one cycle of grinding at pH values; a) pH 7; b and c) pH 3; the scale is a and b) 400 nm, c) 200 nm. Reprinted with permission from ref. 1. Copyright 2009, American Chemical Society.](image)

### 2.2. CNFs from prawn shell

In section 2.1, CNFs were isolated successfully from crab shells flakes in acidic medium with uniform width (10-20 nm) and high aspect ratio after series of chemical treatments followed by one pass of mechanical grinding. Acidic pool and excess acetic acid, however, in CNFs is a matter of worry for applications of NFs especially in pharmaceutical, cosmetic, biomedical industries. Acidic contamination of NFs for above applications is an important issue to address from toxicity viewpoint. Removal of acid from NFs is difficult, complicated, and make products expensive. Therefore preparation of CNFs in normal condition of neutral pH is utmost and immediate necessity to apply them for above products. CNFs from prawn shells have been extracted under neutral conditions without addition of any acid. Fresh shells of prawn species *Penaeus monodon* (black tiger prawn) was used to prepare CNFs. Prawns are cultivated on large scale world wide and its shells are thrown as industrial waste. Chemical treatments to remove minerals, pigments and proteins and lipids are same for prawn [2] as described for isolation of chitin from crab shells. In brief, addition of NaOH and HCl removed proteins and minerals, respectively leaving the chitin and pigments in shell. Pigments were removed with ethanol extraction. Yield of dry chitin from prawn shells was 16.7%. Degree of deacetylation (DDA) of prepared samples determined by the content of C and N by elemental analysis was 7%. SEM
micrograph of the black tiger prawn shell surface after removal of the matrix components is shown in Fig. 3. SEM picture shows exocuticle which is the main part of the prawn shells. It is important that uniform CNFs with an elaborate interwoven structure is clearly visible in the image. 1 wt.% chemically treated chitin suspension was crushed by a domestic blender and passed through a grinder for fibrillation without addition of acid. Chitin slurry obtained was high viscous gel after a single grinding treatment similar to that observed in CNFs from crab shell. Fig 4a and b are the SEM images of one pass NFs at different magnifications. The width of NFs was 10–20 nm. In crab shell NFs at neutral pH, width of the fibers was widely distributed in range 10-100 nm after single grinding passes. Thus preparation of NFs from prawn shells is much advantageous than crab shell. Using prawn shells, thin, homogeneous, uniformly distributed, well separated, and large aspect ratio CNFs were successfully prepared in neutral medium with much superiority over acidic crab shell preparations to apply for a number of industrial applications. The explanation we have given of fibrillation of prawn shell achieved at neutral pH unlike to crab shell that occurred at pH 3 is following. The outer most skeleton (exoskeleton) of prawn or crab shell is made up of two parts exocuticle and endocuticle. Exocuticle has a very fine interwoven plywood type structure, endocuticle is rather more coarse and has thick fibers as shown in Fig.1. 90% of crab shell is made up of these thicker endocuticular fibers [17]. Thus a low pH of 3 medium is used to obtain nanofibris in crab shell. On the other hand the exoskeleton of prawn including black tiger prawn made up of mostly semitransparent soft shell of fine exocuticle as shown in Fig. 3, thus their fibrillation occurs easier than crab shell at neutral pH. The preparation for CNFs from prawn shells in neutral pH is also valid to other species of prawn as described in article [2].

Figure 3. FE-SEM image of black tiger prawn after chemical treatment. Reproduced with permission from ref. 2. Copyright 2011, Elsevier.
2.3. Nanofibrillation of dry chitin powder by Star Burst system

Authors [10] used new fibrillation machine Star Burst system (SBS) developed by Sugino Machine Co., Ltd. to prepare NFs from commercially available dry α-chitin powder from crab shell with and without acetic acid medium. The working principle of SBS instrument has been described in introduction part of the review. Instrument uses high pressure water jet to atomized the chitin slurry into NFs. FE-SEM showed that NFs became thinner as the number of SB passes increased. Fibers were thinner in acidic medium than neutral conditions. NFs prepared in SBS were thinner than reported earlier [1] using grinder in acidic medium. XRD recording showed that SBS did not damage NFs and did not reduce crystallinity.

Fig. 5 shows FE-SEM micrographs of CNFs after SB treatments under a neutral aqueous condition. After one pass, the chitin was not fibrillated (Fig. 5(a)). Thick aggregates of CNFs were observed. There was a significant change in the morphology of CFs after the treatment with five and ten passes (Fig. 5b, c, d, and e). In five and ten passes the NFs are fibrillated as shown in Fig. 5c and e on magnification of 300 nm. The width of fibers in five passes is 18.2 and it reduced to 17.3 nm in ten passes. Thus SBS is powerful tools to give fibers of very thin diameter even without acetic acid solution pool. If we consider the atomization of CNFs in SBS with acetic acid then even in one pass fibrillation occurred as shown in Fig. 6a, b.). Fibrillation completed in five passes as can be seen from SEM pictures Fig. 6d, c.), while processing the fibers in ten passes the thickness of fibers decreases while aspect ratio reduced as fibers breaks.
due to over processing as can be seen from SEM pictures Fig. 6e and f.). Fiber thickness in one, five, and ten passes are 19.0, 18.0, and 16.5 nm, respectively. It is very noteworthy that advantage of very recently developed advanced technology high pressure jet SBS can atomized chitin slurry with or without acetic acid in just five passes to give NFs of small diameter (18.0-18.2 nm) and with high aspect ratio. Increasing number of passes to ten that is considered over processing decreases the width of NFs to very smaller extent. So five passes are optimum with or without acetic acid medium.

Figure 5. FE-SEM of CNFs; a) one pass, b and c) 5 passes, d and e) 10 passes prepared by SBS instrument without acetic acid. The scales are shown in the pictures. Reprinted from ref. 10.

2.4. Characterization of CNFs by FT-IR and XRD recording

Fig. 7 shows the FT-IR spectra of chitin fibers treated by the Star Burst system after 1, 5, and 10 passes under both neutral and acidic conditions. All spectra of obtained of CNFs showed that spectral features are in excellent agreement with the spectrum of commercial chitin. In particular the OH stretching band at 3424 cm$^{-1}$, NH stretching band at 3259 cm$^{-1}$, amide band I at 1652 and 1621 cm$^{-1}$, and amide II band at 1554 cm$^{-1}$ of the CNFs are observed. These absorption peaks are especially characteristic of chitin. This suggests that original chemical structures of chitin were maintained even after 10 passes of Star Burst mechanical treatments with or without acidic pool. Fig. 8 are the XRD pattern of commercial chitin and processed CNFs. X-ray diffraction profiles of CNFs processed by the Star Burst system after several passes under both neutral and acidic conditions. All diffraction patterns coincide closely with
original chitin powder. The four diffraction peaks of the CNFs observed at two θ= 9.2, 19.1, 20.9, and 23.1° corresponded to 020, 110, 120, and 130 planes, respectively [18]. They were typical antiparallel crystal patterns of α-chitin. Thus, the original crystalline structure was maintained after the purification process followed by the Star Burst treatments. Following are the relative crystalline indices of CNFs determined from X-ray diffraction profiles. Original chitin powder has a comparatively high crystallinity of 83.7%. After the Star Burst process under both acidic and neutral conditions, there was no significant difference in the relative degree of crystallinity after the various numbers of passes. This result indicates that at least 10 mechanical treatments with the SBS did not damage the CFs, even though the system used a super high pressure water jet operated at 245 MPa.

2.5. Preparation of CNFs from edible mushrooms

CNFs were isolated and characterized [3] from cell wall of edible mushrooms by a number of chemical treatments to remove glucans, minerals, and proteins associated with mushrooms followed by grinding treatment in acidic medium. NFs width was in the range 20-28 nm depending on the type of mushroom used. The goal of extraction of CNFs from edible mushrooms of nano-sized scale fibers is to develop novel functional food materials. The detailed extraction method and final SEM images of NFs extracted and methods employed to characterize them have been described below. The mushrooms species Pleuotus eryngii (king trumpet mushroom), Agaricus bisporus (common mushroom), Lentinula edodes (shiitake), Grifola frondosa (maitake), and Hypsizygus marmoreus (buna-shimeji) commonly used for human

Figure 6. FE-SEM of CNFs; a and b) one pass, c and d) 5 passes, e and f) 10 passes prepared by SBS instrument with acetic acid. The scales are shown in the pictures. Reprinted from ref. 10.
**Figure 7.** FT-IR spectra of chitin fibers after 1, 5, and 10 passes through Star Burst with and without acetic acid. Reprinted from ref. 10.

**Figure 8.** X-ray diffraction profiles of chitin fibers after 1, 5, and 10 passes through Star Burst with and without acetic acid. Reprinted from ref. 10.
consumption were used in this study. The purification was done by a series of chemical treatments to remove associated compounds: proteins, pigments, glucans, and minerals according to the following procedure. Sodium hydroxide was used to dissolve, hydrolyze, and remove proteins and alkali soluble glucans. Hydrochloric acid was used to remove minerals. At this stage partial neutral saccharides and acid soluble protein compounds were also removed. The extraction step with sodium chlorite and acetic acid removed pigments from the sample. At final stage, the sample was treated with sodium hydroxide again to eliminate and remove the residual glucans including trace amount of proteins. After chemical treatment if the extracted mass allowed to dry, it causes strong hydrogen bonding between CNFs when all matrix substances are washed away which makes it difficult to fibrillate chitin to NFs. Thus the sample was kept wet after removal of the matrix for preparation of CNFs. The purified sample with 1 wt.% content of chitin was passed through a grinder for nano-fibrillation in acetic acid medium at pH 3. After grinder treatment, the chitin homogeneous stable dispersed slurry of chitin with high viscosity was obtained resulted due to high surface-to-volume ratio of NFs thus finally the sample was successfully fibrillated. Fig. 9 shows SEM images of CNFs from five mushrooms after removing matrix components and one pass though the grinder. The isolated chitins are well fibrillated and uniform. The width of the fibers was in the range 20-28 nm depending on the species of mushroom. The yield of CNFs contents in mushrooms was not so high as in crab or prawn shells, it was merely in the range 1.3-3.5 wt.% depending on the species of mushrooms.

Figure 9. FE-SEM images of CNFs prepared from a) Pleurotus eryngii, b) Agaricus bisporus, c) Lentinula edodes, d) Grifola frondosa, e) Hypsizygus marmoreus. The scale bars are 200 nm. Reprinted from ref. 3.
FT-IR and XRD spectrometry were employed to characterize the CNFs from mushrooms. FT-IR spectra (Fig. 10) of commercially available chitin derived from crab shell and CNFs from 5 types of mushroom were compared for analysis. The major bands of the spectra of CNFs are in agreement with commercial chitin. Similarly XRD of commercially available chitin and the CNFs prepared from five types of mushrooms were compared (Fig. 11). The four diffraction bands of CNFs are typical crystal patterns of α-chitin. Thus, CNFs extracted from several types of mushroom maintained α-chitin crystalline structures after the removal of matrix substances followed by grinder treatment. However, in the case of Hypsizygus marmoreus, X-ray diffractogram (Fig. 11f) contains crystal patterns of cellulose (Fig. 11g). The diffraction peaks observed from 15° to 17°, and 22.5°, corresponding to the 110, 1–10, and 200 planes, respectively are typical for the cellulose I crystal.

Figure 10. FT-IR spectra of a) commercial chitin, and CNFs from mushroom source of species b) Lentinula edodes, c) Pleuotus eryngii, d) Hypsizygus marmoreus, e) Grifola frondos, and f) Agaricus bisporus. Reprinted from ref. 3.

Figure 11. XRD pattern of a) commercial chitin, and CNFs from mushroom source of species b) Pleuotus eryngii, c) Agaricus bisporus, d) Lentinula edodes, e) Grifola frondos, f) Hypsizygus marmoreus, and g) commercially available cellulose. Reprinted from ref. 3.
3. CNFs nanocomposites

Very recently [6] new nanocomposite films of CNFs reinforced silsesquioxane-urethaneacrylate (SSQ-UA) copolymer were prepared. CNFs-SSQ-UA nanocomposite films were highly transparent due to filling of nanometer sized (10-20 nm) CNFs inside the hybrid inorganic-organic SSQ-UA copolymer. CNFs due their crystalline structure drastically increased the Young’s moduli and the tensile strengths of the composite and decreased the coefficient of thermal expansion (CTE). High thermal stability of polysilsesquioxane improved heat resistance of CNFs. The composite in the ratio of SSQ/UA = 5/0, 4/1, 3/2, 2/3, and 1/4, was prepared and blended with CNFs and copolymerized using a photo initiator 2-Hydroxy-2-methylpropiophenone then cured for free radical polymerization by UV irradiation for 8 min at 40 mW cm\(^{-2}\) (SPOT CURE SP-7, Ushio Inc).

3.1. Optical properties of CNFs composites

Fig. 12 shows % transmittance vs wavelength (nm) of composite film. Neat CNF sheet was not transparent as % transmittance is nil in visible region and interpreted at 600 nm for all composites. While neat poly-SSQ film had approximately 90% transmittance. After SSQ-UA matrix impregnation and subsequent polymerization, the obtained CNFs nanocomposites in different ratio of SSQ/UA became highly transparent for visible light. CNFs sheets blended with SSQ-UA had good transparency (85% at 600 nm) in case of SSQ/UA ratio 5/0. Blending with 1/4 ratio of SSQ/UA, CNFs sheet transparency decreased slightly to 80% compared with 85% for 5/0 blending ratio of SSQ/UA. The composite films became transparent due to nano-sized composition of CNF sheet. Since the width (10-20 nm) of CNFs was much shorter than the wavelength of visible light (approximately 400-800 nm), the nanocomposites cause less light scattering than a microfiber reinforced composite at the interface between nanofiber and SSQ-UA matrix. At 600 nm since transmittance of nanocomposites were 85-80%, the optical loss caused by nanofiber reinforcement were only in the range 5-10% despite the high fiber content of 50 wt.%. The transmittance of nanocomposites increased as ratio of SSQ increased. The chitin nanofiber sheet obtained in this study can be available like a paper, though the novelty of the paper is composed of nano-meter thick fibers. Several patterns can be printed on the nanofiber paper that we have prepared using a domestic inkjet printer (Fig. 13a). The printed NF paper became transparent (Fig. 13b) after matrix impregnation. This newly established technique of transparent printing on such a thin (70 μm) composite sheet can have application in printing of wiring used in electronic devices or electronic papers.

3.2. Thermal properties of composites

Fig. 14 shows the CTE of neat CNFs and its composites. Although neat poly-SSQ (SSQ/UA = 5/0) was too fragile to measure the thermal expansion, the CNF reinforced nanocomposite was tough for CTE measurement. CTE of CNF sheet without SSQ matrix was only \(8.0 \times 10^{-6}\) K\(^{-1}\). While CTE of SSQ-UA copolymer films without CNFs were high in the range 96.2-164.0 \(\times 10^{-6}\) K\(^{-1}\) depending on the ratio of SSQ/UA as shown by bars in Fig. 14. CTEs of all nanocomposites decreased significantly to a constant value of 30 \(\times 10^{-6}\) K\(^{-1}\). These values corresponded to 66 to
81% decreased compared to the corresponding neat SSQ-UA matrices. Thus, CNFs with low CTE worked effectively to decrease the thermal expansion of SSQ-UA copolymer film as a result of reinforcement.

3.3. Mechanical characterization of composites

Fig. 15 shows Young’s moduli and tensile strengths of SSQ-UA copolymer films and their CNFs composites. The Young’s moduli of SSQ-UA with the ratio of 3/2, 2/3, and 1/4 without CNFs decreased from 1,571 to 128 MPa with increasing the ratio of reactive diluent UA oligomer. This is due to decrease in crosslinking density with decreasing the amount of strengthening hybrid component SSQ. The SSQ-UA films with the ratio of 5/0 and 4/1 were too fragile to measure the mechanical properties so their bars are not shown in the Young’s
Moduli plot. Nanocomposites were tough enough for the testing due to CNF support. The Young’s moduli of these nanocomposites significantly increased and reached in the range 3.36 to 4.29 GPa. The tensile strengths also significantly increased in the range 31 to 59 MPa. It is important to notice that each Young’s moduli and tensile strength of the chitin nanofiber composites were higher than that of CNF sheet or SSQ-UA copolymer. The higher Young’s moduli and tensile strength of composite is due to SSQ-UA matrix embedded in every space of CNF sheet and strongly interacts with NF at the interface thus resulted in the increase of the reinforcement effect. The enhancements of mechanical properties of composite strongly support that a CNF sheet with a high Young’s modulus (1.80 GPa) and a high tensile strength (30 MPa) worked effectively as a reinforcement filler for SSQ-UA copolymer.

![Figure 15. Coefficient of thermal expansion (CTE) of SSQ-UA copolymer films and SSQ-UA-CNFs composites. Reproduced with permission from ref. 6. Copyright 2012, Elsevier.](image1)

![Figure 14. Coefficient of thermal expansion (CTE) of SSQ-UA copolymer films and SSQ-UA-CNFs composites. Reproduced with permission from ref. 6. Copyright 2012, Elsevier.](image2)
4. Preventive effect of CNF on dextran sulfate sodium (DSS)-induced ulcerative colitis (UC)

In this section we describe the medical aspect of CNFs taking a model of DSS-induced colitis in mouse as investigated by Azuma et al [4]. The effect of CNFs on disease activity index such as weight loss, loose stools, and bleeding symptoms in colitis were studied. CNFs administered mouse exhibited a significant reduced in disease activity index. Colon length increased that was shortened due to DSS induction by administration of CNFs compared to control. Damage in intestinal mucosa was microscopically monitored as shown in Fig. 16. In CNFs group on 6th day erosion, crypt destruction, and edema were markedly suppressed compared to control. The number of myeloperoxidase (MPO)-positive cells lowered significantly compared to control group. Thus CNFs improved clinical symptoms in DSS-induced acute UC mouse model.

![Figure 16](image)

**Figure 16.** Effect of CNFs administration on histopathologiccedal changes in DSS-induced acute UC mice; a) control, b) CNFs, and c) chitin powder. Reproduced with permission from ref. 4. Copyright 2012, Elsevier.

5. Conclusion

Preparation of CNFs from crab, prawn shells, and a number of species of mushrooms have been discussed. Both chemical treatments and mechanical processing have been described in detail. CNFs prepared from crab shell, the presence of acidic medium was important to reduce the size of NFs. While in case of prawn shells the fibrillation was achieved in neutral conditions. Width of CNFs was 10-20 nm with high aspect ratio. After completion of fibrillation the CNFs were in physical state of wet gel of very high viscosity. Size of NFs was determined by recording FE-SEM of flakes or thin film of NFs. Apart from using grinder a newly developed high pressure jet atomization machine (Star Burst System; SBS) was also employed to fibrillate the NFs. Fibrillation was more effective when SBS was used that gave more thinner (19.0-16.5 nm) and homogeneous NFs compared to girder. NFs were characterized for chitin content by XRD and FT-IR measurements. CNFs were also prepared from five different species of edible Chitin Nanofibers, Preparations and Applications

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mushrooms, NFs of width 20-28 nm were obtained. But the chitin yield in mushroom was lower (1.3-3.5 wt.%) compared to crab (12.2 wt.%) or prawn (16.7 wt.%). SSQ-UA copolymer-CNFs transparent composite sheets or thin film ware prepared and their optical, thermal, and mechanical properties were investigated. The properties improved on blending CNFs with copolymer to larger extent which has increased the scope of CNFs. CNFs were found effective to DSS-induced UC disease in mouse colon, the UC symptoms removed and lowered the MPO-positive cell count decreased significantly.

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