

# Nano-chitin in 3D Printing Technology: Its Structure, Preparation and Application

Zhaowen Lin<sup>1,\*</sup>

<sup>1</sup>School of Materials and Energy, South China Agricultural University, Guangzhou Guangdong 510000, P. R. China

\*Corresponding author's e-mail: audi6236@icloud.com

**Abstract:** Chitin nano-materials derived from natural organisms are receiving much attention due to reduced fossil resources. In order to reduce carbon emissions and waste of valuable resources, nano-chitin materials manufacturing technology has been developed. Studies of the preparation and characterization of 3D printing functional nano-chitin materials can provide insights into desirable properties such as biocompatibility, high surface area. In this review, we compare and highlight the different methods for nano-chitin extraction from renewable resources and the conversion of the obtained nano-chitin into compound material with high 3D printing capabilities. Finally, the application of nano-chitin in the field of 3D printing is described with future prospects.

**Keywords:** Nano-structure, Chitin, Nano-whisker, 3D printing.

## 1. Introduction

Among the many renewable resources, shrimp shells and other Marine biological shells are abundant reusable biological wastes. But this renewable waste has not been effectively reused. In many countries, abandoned shrimp shells are often discarded directly in the ocean. Therefore, it is of great interest to transform these huge potential shrimp shells into social resources. The path of transformation is diversified, which can directly convert the shrimp shell into a carbon material, or then synthesize chitosan by extracting chitin from the shrimp shell. Chitin is abundant in the crustacean of marine arthropods such as shrimp and crab, its internal structures are interconnected mesh nano-structures. Chitosan is the only polymer basic polysaccharide in the natural polysaccharide, which has various biological properties such as anti-tumor, antioxidant and trauma healing. With the widespread application of chitosan in the biomedical field, people began to apply chitosan in other fields. Recently, the application of chitosan in 3D printing has also attracted the attention of scholars. In this review, we focus on recent methods for the preparation of nano-chitin and its advances in 3D printing applications.

## 2. Literature Review

Liang et al. Introduced the steps of extracting nano-cellulose in detail, such as pretreatment, separation of nano-cellulose, and then discussed the method of turning nano-cellulose into composite materials with good 3D printing performance. In addition, they also introduced the application of 3D printing and nano-cellulose in 3D printing, such as in biomedical field and environmental engineering field.[1] Their work provides good ideas for this paper.

Yang et al and sun et al both introduced the preparation methods of nano-chitin, such as hydrochloric acid solution[2], electro-spinning method [3, 4], tempo hydro-carbonylation [5, 6], etc., and reviewed the application progress of nano-chitin in medical treatment, water treatment, industrial fillers, etc. in recent years.[2, 7] They set up a framework for the preparation method of nano-chitin in this paper. Besides, their

application is very extensive. On their basis, we need to supplement and refine its application in 3D printing.

## 3. The Structure of Chitin

Chitin is a natural and hierarchical biomaterial(Figure 2). Chitin has three crystal structure, including:  $\alpha$ -chitin,  $\beta$ -chitin, and  $\gamma$ -chitin [8,9]. All of them are highly ordered. Researches show that shrimp and crab shells in crustacean, spider in insects, aspergillus niger in fungi are  $\alpha$ -chitin.  $\beta$ -chitin can be extracted from squid rings and cuttlefish bone [10, 11, 12].  $\gamma$ -Chitin is mainly found in the *Orgyia dubia* and *Ptinus*[9, 13].  $\gamma$ -Chitin has three glycoside chains, two in the same direction and one in the opposite direction, so it has a unstable structure and ready to change to other crystalline forms.

Although chitin has widespread existence and high biocompatibility characteristics, the low solubility and especial structure of chitin limit its applications in many fields. Therefore, scientists began to explore the possibility of its use as nano-materials. Nano-chitin not only retains its original characteristics, but also has high surface area and good functionality, which contribute to being widely used in biomedicine. Because the natural chitin has a special structure (linear(1,4)- $\beta$ -N-acetyl glycosaminoglycan structure), so it is highly crystalline and has a strong hydrogen bond [7].

## 4. Preparation Methods of Nano-chitin

The two ways to prepare nano-chitin are generally "from top to bottom" and "from bottom to top".

### 4.1. From top to bottom

In this "from top to bottom" approach, nano-chitin is heavily extracted from natural chitin materials, such as the shell or exoskeleton of crustaceans. Extraction generally has two steps: Firstly, to purify chitin. It mainly contains alkali treatment or enzymatic digestion to remove proteins, acidic treatment to remove inorganic minerals, and have a bleaching treatment ( $\text{CH}_3\text{CH}_2\text{OH}$ ) to reduce pigments and lipids [8,14]. In the next step, chitin is degraded to nano-chitin using acid hydrolysis, mechanical treatment, TEMPO oxidation, and partial deacetyl.

#### 4.1.1. Hydrochloric acid solution

The hydrochloric acid solution can be used to prepare nano-whiskers of chitin. Chitin has a semi-crystalline structure, crystal zone and amorphous region. According to the chitin crystalline part and amorphous part in acid has a different kinetic effect, amorphous part is dissolved by acid first, the remaining high crystal region under the action of 3 mol/L HCl amine glucose unit C2 amine cation electrostatic repulsion or ultrasonic or other shear force to obtain chitin nano-crystal [2].

#### 4.1.2. Mechanical treatment method

The mechanical treatment preparation method is mainly used to prepare chitin nano-fibers. In acidic or neutral conditions, the wet or dry chitin was nano-fibrotic by crushing, grinding, high-pressure homogenization, or combining several ways. Under acidic conditions, a small amount of amine group in the wet chitin structural unit is protonated into cations, generating electrostatic repulsion to destroy the hydrogen bonds between chitin molecules and nano-convert chitin by mechanical treatment. Ifuku et al dispersed the purified dried crab shell powder in water to form a 1% mass fraction solution, adding acetic acid to make pH to 3, rough crushing using a mixer, and then grinding to nano-render chitin [15]. Chitin nano-fibers prepared under acidic conditions are uniformly and stably scattered in water, but due to the high viscosity, it is very hard for the chitin nano-fiber suspension to be got rid off acid completely, resulting in its limited application in acid-sensitive materials, such as biomaterials. Under neutral conditions, salaberria used high-speed dispersion, ultrasonic, multiple dynamic high pressure homogenization treatments to produce chitin nano-fibers [16].

#### 4.1.3. TEMPO hydrocarbonylation

This method can prepare chitin nano-crycetes and chitin nano-fibers. TEMPO system refers to the 2,2,6,6-tetramethylpiperidine-1-oxygen radical (TEMPO) / NaBr / NaClO system, NaClO is the main oxidant. It first reacts with NaBr to form NaBrO, NaBrO oxidizes TEMPO to produce nitroso ions, and then uses the amorphous part of chitin to form polyturonic acid. The crystalline part of the surface hydroxyl group is oxidized to carboxylic acid or carboxylate, resulting in a surface containing anion chitin, which is insoluble with water. A stable nanochitin suspension liquid is formed by electrostatic repulsion or osmosis under mechanical force [5,6].

#### 4.1.4. De-N-acetyl method

People using this method can only make chitin nano-whisker. The N-acetyl group on the chitin structural unit is hydrolyzed under alkaline conditions to form the amine-based glucose unit, which can be protonated into cations at a pH of 3 to 4, and disintegrate the fiber through mechanical shear to form the chitin nano-crystal [17]. The morphology, size, crystallinity and other properties of the prepared nano-chitin are related to the purification method, the sources and preparation methods of chitin.[2]

### 4.2. From bottom to top

In this way, crustacean materials are firstly dissolved to form chitin solutions, and then nano-chitin is prepared using electrospinning, self-assembly, dissolution-regeneration, and other methods.

#### 4.2.1. Electrospinning method

People use electrospinning to produce chitin nano-fibers. The equal-width chitin nano-fibers can be prepared from the

chitin solution under a strong electric field. Because of its poor solubility and large molecular weight, chitin should be degraded before electrospinning to increase its solubility. The degradation methods include Co60 ray, microwave radiation, etc. [3,4] The commonly used solvents are hexafluoroisopropanol (HFIP), mesonic acid, etc. It has also a report that direct spinning without degrading chitin molecular weight. Barber et al used microwave-assisted ionic liquid 1-ethyl-3-methimidazole acetate to increase molecular weight of the obtained chacen, and Chitin nano-fibers were prepared by electrospinning of ionic liquid chitin solution [18]. This method not only saves the chemical reagents and energy consumption needed for conventional chitin extraction, but also saves the chemical reagents, time and energy for preparing electrospinning solutions.

#### 4.2.2. Self-assembly method

Scientists make Chitin nano-fibers using this method. Using water-based solvents and toxic solvents is easy to cause hydrogen bond fracture. In recent years, scientists have studied sodium hydroxide urea solution as a solvent to dissolve chitin. Duan et al used heat-induced self-assembly way to get chitin nano-fiber microspheres in this sodium hydroxide urea solution [19]. When high temperatures, the urea-NAOH sheaths are ruined and chitin chains crash together ,self-aggregate and forming nano-fibers.

#### 4.2.3. Solution regeneration method

This solution regeneration method can produce both chitin nano-whiskers and chitin nano-fibers. Chitin is dissolved into special solvents, such as 1-butyl-3-methimidazole bisulfite, 1-methimidazole bisulfate and other plasma liquids (IL), and then regenerated by self-assembly or water or methanol.

## 5. The Application in 3D Printing

Nano-chitin has lots of advantages, for example, good physical properties, nontoxic, biodegradable, large surface area, so these properties of nano-chitin can be fully utilized in 3D printing.

### 5.1. Used as a viscosity-modifying agent

The alginate saline gel has received attention in the field of bioprinting due to its easy crosslinking and excellent biocompatibility. However, their gel precursor solutions have low viscosity properties at low shear rates, compromising its application in the printing field. Bernardo found that this problem could be solved using adding nano-cellulose or nano-chitin as viscosity modifications, which could increase the viscosity of the precursor solution. In their experiments, the gel curves confirmed that CTNW alginate bioink made of chitin nano-crytes obtained from acid hydrolysis can increase more viscosity compared to the CNC alginate bioink. The gel curve also confirms the crosslinking effect of CTNW alginate bioink, and can be printed with high concentration CTNW bioink [20].

### 5.2. Used as an enhanced modification to the PLA

Li found that enhanced modified CHNFs / PLA composite can be prepared by wet mixing and the PEG dispersant method. When the amount of nano-chitin is above 30%, the mechanical properties of the composite produced by wet mixing method are better than that of PEG dispersant [21]. Therefore, nano-fiber reinforced thermoplastic polymer composite can be prepared by the wet mixing fiber.

### 5.3. Used as the rheological modifier

The pre-poly(1, 8-octanediol-co-Pluronic F127 citrate) (POFC) emulsion couldn't be printed, because citrate-based thermoset bioelastomer is insoluble and unmeltable. However, through experiments, Gu found that the flow behavior of pre-POFC emulsion can be adjusted by chitin nano-crystal (ChiNC) as a rheological modifier, and then realize direct ink writing printing of POFC frame; In addition, ChiNC can be the support agent in thermal hardening processes to prevent the collapse of filaments. Their work shows the pre-POFC/ChiNC inks are suitable for direct ink writing printing [22]. The product has advantages as good mechanical performances, and low swelling.

### 5.4. Used as a mechanical thermal enhancer for stereo lithography and 3D printing

Reyemark D. Maalihan prepared chitin nano-whiskers(CNWs) using chitin from crab as raw material [23]. After comparing methacrylate with different molecular weight of CNWs, they found that the addition of 1.0wt % CNWs to NEAT MA enhances the mechanical property and maximum thermal degradation temperatures of the nano-composites. As a result, CNWs improve mechanical and thermal performance, and also maintain the good properties of 3D-printed MA/CNWs nano-composites.

### 5.5. Used as a 3D printing raw material with PBAT

Balaji Sadhasivam's research shows that Poly(butylene adipate-co-terephthalate) (PBAT), filled with different percentages of raw nano-chitin, can be used to print 3D biodegradable fillers [24]. Through thermal and mechanical properties tests, it was found that the rigidity of nano-composites increases as the nano-chitin mass fraction increases. Meanwhile, the thermal stability of PBAT can be improved by adding nano-chitin, but the effect was not obvious. Their product --PBAT-NC nano-composite can be processed into useful items such as plastic supports under conditions similar to LDPE processing.

## 6. Conclusion and Outlook

This paper reviews the structure, preparation methods and application of nano-chitin in 3D printing. Nano-chitin research has made great progress, nano-chitin has high mechanical strength, surface area, non-toxic, antibacterial, biodegradable, biocompatible properties, but its application in 3D printing is still auxiliary, research number is less, hope that this paper for nano-chitin in 3D printing application, and to accelerate the efforts to the sustainable development of the world.

## References

- [1] Liang, Y.E. & Sam, F.Y.L. (2021). Recent advances in 3D printing of nanocellulose: structure, preparation, and application prospects. *Nanoscale Advances*, 1155-1504.
- [2] Sun, Xubing., Du, J.C. & You, Y.H. (2016). Research Progress in Preparations, Modifications and Applications of Nano Chitin. *Chinese Polymer Bulletin*. 8: 71-80.
- [3] Min, B.M., Lee, S.W., Lim, J.N., et al. (2004). Chitin and chitosan nanofibers: electrospinning of chitin and deacetylation of chitin nanofibers. *Polymer*, 45: 7137-7142.
- [4] Noh, H.K., Le, S.W., Kim, J.M., et al. (2006). Electrospinning of chitin nanofibers: Degradation behavior and cellular response to normal human keratinocytes and fibroblasts. *Biomaterials*, 27: 3934-3944.
- [5] Fan, Y.M., Saito, T. & Isogai, A. (2008). Chitin Nanocrystals Prepared by Tempo-Mediated Oxidation of  $\alpha$ -chitin. *Biomacromolecules*, 9: 192-198.
- [6] Fan, Y.M., Saito, T. & Isogai, A. (2009). Tempo-mediated oxidation of  $\beta$ -chitin to prepare individual nanofibrils. *Carbohydrate Polymers*, 77: 832-838.
- [7] Yang, X.F., Liu, J., Pei, Y., et al. (2020). Recent progress in preparation and application of nano-chitin materials. *Energy&Environmental Materials*, 3:492-515.
- [8] Tharanathan, R.N. & Kittur, F.S. (2003). Chitin: The Undisputed Biomolecule of Great Potential. *Food Science and Nutrition*, 43: 61-87.
- [9] Jang, M.K., Kong, B.G., Jeong, Y.I., et al. (2004). Physicochemical characterization of  $\alpha$ -chitin,  $\beta$ -chitin, and  $\gamma$ -chitin separated from natural resources. *Journal of Polymer Science*. 42: 3423.
- [10] Teng, W.L., Khor, E., Tan, T.K., et al. (2001) Concurrent production of chitin from shrimp shells and fungi. *Carbohydrate Research*, 332: 305.
- [11] Fan, Y., Saito, T. & Isogai, A. (2008) Preparation of Chitin Nanofibers from Squid Pen  $\beta$ -chitin by Simple Mechanical Treatment under Acid Conditions. *Biomacromolecules*, 9: 1919.
- [12] Shushizadeh, M.R., Pour, E.M., Zare, A., et al. (2015) Persian gulf  $\beta$ -chitin extraction from sepia pharaonis sp. cuttlebone and preparation of its derivatives. *Bioactive Carbohydrate and Dietary Fibre*, 6: 133.
- [13] Kaya, M., Mujtaba, M., Ehrlich, H., et al. (2017). On chemistry of  $\gamma$ -chitin. *Carbohydrate Polymers*, 176-177.
- [14] Salaberria, A.M., Labidi, J. & Fernandes, S.C.M. (2015). Different routes to burn chitin into stunning nano-objects. *European Polymer Journal*, 68: 503.
- [15] Ifuku, S., Nogi, M., Yoshika, M., et al. (2010). Fibrillation of dried chitin into 10-20 nm nanofibers by a simple grinding method under acidic conditions. *Carbohydrate Polymers*, 81: 134-139.
- [16] Salaberria, A.M., Fernandes, S.C.M., Diaz, R.H., et al. (2015) Processing of  $\alpha$ -chitin nanofibers by dynamic high pressure homogenization: Characterization and antifungal activity against *A. niger*. *J. Carbohydrate Polymers*, 116: 286-291.
- [17] Fan, Y.M., Saito, T. & Isogai, A. (2010). Individual chitin nano-whiskers prepared from partially deacetylated  $\alpha$ -chitin by fibril surface cationization. *Carbohydrate Polymers*, 79: 1046-1051.
- [18] Barber, P.S., Griggs, C.S. & Bonner, J.R. (2013). Electrospinning of chitin nanofibers directly from an ionic liquid extract of shrimp shells. *Green Chemistry*, 15: 601-607.
- [19] Duan, B., Zheng, X., Xia, Z., et al. (2015). Highly Biocompatible Nanofibrous Microspheres Self-Assembled from Chitin in NaOH/Urea Aqueous Solution as Cell Carriers. *Angewandte Chemie*, 127: 5241-5245.
- [20] Bernardo Noiva Raposo Fernandes de Melo. (2019). Chitin nanocrystals-alginate bioinks for bioprinting of 3D structure[D]. Universidade Nova de Lisboa.
- [21] Li, J.J., Zhao, J.F., Sun, J.G., et al. (2017). Preparation and Properties of Chitin Nanofibers/Poly(lactic Acid) Composite. *Polymer Materials Science and Engineering*, 33:161-167.

- [22] Gu, S.H., Tian, Y.L., Liang, K., et al. (2020) Chitin nanocrystals assisted 3D printing of polycitrate thermoset bioelastomers. Elsevier, 256:117549.
- [23] Maalihan, R.D., Pajarito, B.B. & Advincula, R.C. (2020) 3D-printing methacrylate/chitin nanowhiskers composites via stereolithography: Mechanical and thermal properties. Materials Today: Proceedings, 33:1819-1824.
- [24] Sadhasivam, B., Ramamoorthy, D., & Dhamodharan, R. (2018). Scale-up of non-toxic poly(butylene adipate-co-terephthalate): Chitin based nanocomposite articles by injection moulding and 3D printing. International Journal of Biological Macromolecules, 165:3145-3155.