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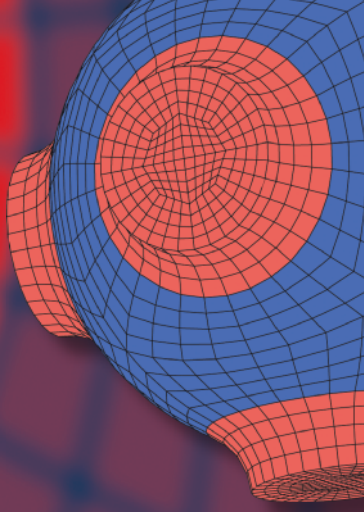

Visakh P. M. *Editor*

Biodegradable and Environmental Applications of Bionanocomposites

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Volume 177

Common engineering materials are reaching their limits in many applications, and new developments are required to meet the increasing demands on engineering materials. The performance of materials can be improved by combining different materials to achieve better properties than with a single constituent, or by shaping the material or constituents into a specific structure. The interaction between material and structure can occur at different length scales, such as the micro, meso, or macro scale, and offers potential applications in very different fields.

This book series addresses the fundamental relationships between materials and their structure on overall properties (e.g., mechanical, thermal, chemical, electrical, or magnetic properties, etc.). Experimental data and procedures are presented, as well as methods for modeling structures and materials using numerical and analytical approaches. In addition, the series shows how these materials engineering and design processes are implemented and how new technologies can be used to optimize materials and processes.

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Visakh P. M.
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Biodegradable and Environmental Applications of Bionanocomposites

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Preface

Biodegradable polymers and their bionanocomposites based on layered silicates: Environmental applications are given in Chap. 1. Chapter 2 offers a review of chitosan/poly (ethylene glycol)/ZnO bionanocomposite for wound healing application. This chapter covers the introduction to chitosan, its properties, and some novel potential applications of chitosan bionanocomposite for wound healing application. Preparation and applications of chitosan–gold bionanocomposites are described in Chap. 3. Preparation strategies for chitosan–gold nanocomposite and applications of chitosan–gold nanocomposites are described in this chapter. Different applications of chitosan–gold bionanocomposites such as textile industry, improvement in textile functionalities by chitosan–gold nanocomposite, wound healing by chitosan graft scaffoldings composite, and wound healing by peptide conjugate-chitosan/derivatives are also described.

Chapter 4 deals with the environmental properties and applications of cellulose and chitin-based bionanocomposites. In this chapter, the sources and properties as well as the extraction and preparation methods of the corresponding cellulose and chitin bionanocomposites are introduced. The environmental characterizations of cellulose and chitin-based bionanocomposites and their applications in various fields are reported.

Chapter 5 discusses the polylactic acid/halloysite nanotube bionanocomposite films for food packaging. In addition, the polylactic acid/halloysite nanotube bionanocomposite preparation and characterizations are also described. Food packaging application of polylactic acid/halloysite nanotube bionanocomposite is also discussed. A detailed review of preparation of ZnO/chitosan nanocomposite is covered in Chap. 6.

"Polymer Composites Scaffolds for Bone Implants: A Review" is discussed in Chap. 7. In this chapter, strategies and techniques to engineer new kind of polymer surface to promote osteoconduction with host tissues will be discussed. Also, benefits and applications of polymeric composite scaffolds for orthopedic surgery will be discussed. Biodegradable polyvinyl alcohol/starch/halloysite nanotube bionanocomposite are discussing in final chapter, Preparation and Characterization of Biodegradable nanocomposites are also reported.

In this book, chapter authors have reviewed different aspects of biodegradable polymers and their bionanocomposites. This book is a valuable reference source for faculties, professionals, research fellows, senior graduate students, and researchers working in the field of biodegradable polymers and their bionanocomposites. The use of biodegradable bionanocomposites is considered as a promising area of research; a lot of research activities are going on, and some knowledge of bionanocomposites is helpful to bring a change in the current techniques and applications. Finally, we would like to express our sincere gratitude to all the contributors of this book, who made excellent support to the successful completion of this venture. We also thank the publisher Springer for recognizing the demand and importance of biodegradable bionanocomposites.

Tomsk, Russia Visakh P. M.

Contents

1 Biodegradable Polymers and Their Bionanocomposites Based on Layered Silicates: Environmental Applications 1
Julia Martín, María del Mar Orta, Santiago Medina-Carrasco, Juan Luis Santos, Irene Aparicio, and Esteban Alonso
1.1 Introduction 2
1.2 Biodegradable Polymers 3
1.3 Biodegradable Polymer-Based Bionanocomposites 4
1.3.1 Chitosan/Clay Nanocomposites 9
1.3.2 Starch/Clay Nanocomposites 10
1.3.3 Alginate–Clay Nanocomposites 11
1.3.4 Cellulose/Clay Nanocomposites 12
1.3.5 Protein–Clay Nanocomposites 13
1.3.6 Polylactic Acid/Clay Nanocomposites 14
1.4 Preparation of Biopolymer–Clay Nanocomposites 14
1.4.1 Solution-Blending Method 15
1.4.2 Melt-Blending Method 15
1.4.3 In Situ Polymerization Method 16
1.5 Characterization Techniques 17
1.6 Environmental Applications 20
1.6.1 Bionanocomposites with Layered Silicates in Soil and Water Treatment 20
1.6.2 Bionanocomposites with Layered Silicates for Food Packaging 22
1.6.3 Bionanocomposites with Layered Silicates for Agricultural Applications 23
1.7 Conclusion 23
References 24

2 Chitosan/Poly (Ethylene Glycol)/ZnO Bionanocomposite for Wound Healing Application 31
Zahra Emam-Djomeh and Mehdi Hajikhani

2.1 Introduction 31
2.2 Chitosan 32
2.3 Poly (Ethylene Glycol) 41
2.4 Chitosan/Poly (Ethylene Glycol)/ZnO Bionanocomposite 44
2.5 Wound Healing Application 47
2.6 Conclusion 53
References 53

3 Preparation and Applications of Chitosan–Gold Bionanocomposites 67
Rishabh Anand Omar and Monika Jain
3.1 Introduction 67
3.2 Chitosan–Gold Nanocomposite 69
3.3 Preparation Strategies for Chitosan–Gold Nanocomposite 70
3.3.1 General Synthesis 71
3.3.2 Physical Methods 71
3.3.3 Radiolysis, Sonochemistry and Photochemical (UV, Near-IR) Strategies 71
3.3.4 Chemical Synthesis 72
3.3.5 Reduction by Borohydride 72
3.3.6 Citrate Reduction 73
3.3.7 Synthesis by Seeding Growth Technique 73
3.3.8 Biosynthesis Technique 73
3.4 Applications of Chitosan–Gold Nanocomposites 74
3.4.1 Textile Industry 74
3.4.2 Improvement in Textile Functionalities by Chitosan–Gold Nanocomposite 77
3.4.3 Effluent Treatment Application 78
3.4.4 Bioremediation 79
3.4.5 Application in Biomedical Field 80
3.5 Future Applications 86
References 87

4 Environmental Properties and Applications of Cellulose and Chitin-Based Bionanocomposites 99
Renyan Zhang and Hui Xu
4.1 Introduction 99
4.1.1 Cellulose 99
4.1.2 Chitin 104
4.2 Environmental Properties 106
4.2.1 Mechanical Properties 107
4.2.2 Thermal Properties 107
4.2.3 Barrier Properties 108
4.2.4 Biodegradability 108
4.2.5 Antibacterial and Antifungal Properties 109
4.3 Applications of Cellulose-Based Bionanocomposites 109

4.3.1 Electronics Device Industry 110
4.3.2 Biomedicine Industry 111
4.3.3 Food Industry 116
4.3.4 Environmental Protection 117
4.4 Applications of Chitin-Based Bionanocomposites 120
4.4.1 Biomedicine Industry 120
4.4.2 Environmental Protection 122
4.4.3 Food Industry 124
4.4.4 Agriculture 126
4.4.5 Cosmetics 126
4.5 Conclusion 127
References 128

5 Polylactic Acid/Halloysite Nanotube Bionanocomposite Films for Food Packaging 141
Zahra Emam-Djomeh and Hajikhani Mehdi
5.1 Introduction 141
5.2 Polylactic Acid 143
5.3 Polylactic Acid/Halloysite Nanotube Bionanocomposite 148
5.4 Food Packaging Application of Polylactic Acid/Halloysite Nanotube Bionanocomposite 152
5.5 Conclusion 158
References 158

6 Preparation of ZnO/Chitosan Nanocomposite and Its Applications to Durable Antibacterial, UV-Blocking, and Textile Properties 169
Tanmoy Dutta, Abdul Ashik Khan, Nabajyoti Baildya, Palas Mondal, and Narendra Nath Ghosh
6.1 Introduction 169
6.2 Preparation of ZnO/Chitosan Nanocomposite 173
6.2.1 General Mechanism of the Formation of ZnO/Chitosan Nanocomposite 176
6.3 Antibacterial Activity of ZnO/Chitosan Nanocomposite 176
6.3.1 Probable Mechanism of the Antibacterial Activity of ZnO/Chitosan Nanocomposite 180
6.4 Applications of ZnO/Chitosan Nanocomposite in Textiles 181
6.5 Applications of ZnO/Chitosan Nanocomposite as a UV-Blocker 182
6.6 Conclusion 183
References 183

7 Polymeric Nano-Composite Scaffolds for Bone Tissue Engineering: Review 189
Lokesh Kumar and Dheeraj Ahuja
7.1 Introduction 190

7.1.1 Scaffold for Bone Tissue Engineering ... 190
7.2 Properties of Scaffold ... 192
7.2.1 Biocompatibility ... 193
7.2.2 Biodegradability ... 194
7.2.3 Porosity ... 195
7.2.4 Targetability ... 195
7.2.5 Binding Affinity ... 195
7.2.6 Stability ... 196
7.2.7 Loading Capability and Deliverance ... 196
7.2.8 Mechanical Properties ... 196
7.2.9 Scaffold Architecture ... 197
7.3 2Dimesional (2D) Versus 3Dimensional (3D) Culture Scaffold ... 197
7.4 Polymer Scaffold and Processing Techniques ... 198
7.4.1 Conventional Techniques ... 198
7.4.2 Rapid Prototyping Technique ... 202
7.4.3 Combination of Techniques ... 203
7.5 Biomaterials for Tissue Engineering Polymeric Scaffold Manufacturing ... 204
7.5.1 Ceramics ... 204
7.5.2 Natural Polymers ... 205
7.5.3 Synthetic Polymers ... 206
7.5.4 Composites ... 206
7.6 Applications ... 207
7.7 Conclusion and Future Prospective ... 208
References ... 209

8 Biodegradable Polyvinyl Alcohol/Starch/Halloysite Nanotube Bionanocomposite: Preparation and Characterization ... 221
P. Manju and P. Santhana Gopala Krishnan
8.1 Introduction ... 221
8.2 Poly(Vinyl Alcohol) ... 222
8.3 Starch ... 223
8.4 Halloysite ... 224
8.5 PVOH/ST/HNT Bionanocomposite: Preparation ... 226
8.5.1 Solution Casting ... 226
8.5.2 Electrospinning ... 226
8.5.3 Melt Processing ... 228
8.6 PVOH/ST/HNT Bionanocomposite: Characterization ... 228
8.6.1 Chemical Interaction Analysis ... 228
8.6.2 XRD Analysis ... 229
8.6.3 Morphological Analysis ... 229
8.6.4 Mechanical Properties ... 230
8.6.5 Thermal Analysis ... 232
8.6.6 Water Absorption Capacity ... 233
8.7 Conclusions ... 233

References 234

9 Environmentally Friendly Bionanocomposites in Food Industry 237
Subajiny Sivakanthan and Podduwala Hewage Sathiska Kaumadi
9.1 Introduction 237
9.2 Environmentally Friendly Bionanocomposites 238
9.2.1 Properties and Applications of Bionanocomposites 240
9.3 Bionanocomposites in the Food Industry 241
9.4 Properties of a Bionanocomposite that Make It Suitable as a Food Packaging Material 243
9.4.1 Mechanical Properties 243
9.4.2 Barrier Properties 245
9.4.3 Thermal Properties 246
9.5 Application of Bionanocomposites in the Packaging of Food 249
9.5.1 Dairy Products 249
9.5.2 Fruit and Vegetable 250
9.5.3 Meat and Poultry 251
9.5.4 Application of Bionanocomposites in Novel Food Packaging Systems 252
9.6 Safety Concerns 258
9.7 Conclusion 258
References 259

Chapter 1
Biodegradable Polymers and Their Bionanocomposites Based on Layered Silicates: Environmental Applications

Julia Martín, María del Mar Orta, Santiago Medina-Carrasco, Juan Luis Santos, Irene Aparicio, and Esteban Alonso

Abstract Bionanocomposites are hybrid materials comprising inorganic nanoparticles or nanofillers disposed in a biopolymer matrix. Different functional materials have been prepared using a wide type of biopolymers (naturally or synthetic) and inorganic particles (silica, metal, carbon nanotubes, cellulose nanowhiskers or layered silicate clays) with different compositions and topologies. In this chapter, special attention is paid in layered silicates because of their availability, low cost and their easy intercalation chemistry. The natural polysaccharides (chitosan, starch and alginate), proteins and the synthetic polylactic acid incorporating to layered silicates of the smectite group constitute the bionanocomposites most studied for environmental applications. In this work, the physicochemical and structural properties of developed bionanocomposites including the different methods of preparation and characterization techniques have been discussed. Finally, environmental applications of bionanocomposites based on layered silicates in the field of food, agriculture, soil and water treatments, both in cleaning and desalination, are contemplated.

Keywords Bionanocomposites · Biopolymers · Layered silicates · Adsorption · Water remediation

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1.1 Introduction

An important part of the increase in well-being in developed countries produced by technological development is due to the incorporation of fossil fuels, the main source of production of plastic products, which are in part responsible of the exponential environmental deterioration. Plastics from fossils are synthetic polymeric products that are chemically inert and therefore resistant to degradation. Reversing this damage is only possible through the use of knowledge. The advancement of polymer technology has allowed the development of renewable resources, giving rise to sustainable green products made from natural and synthetic materials. These compounds have the ability to be metabolically degraded in the environment.

Biodegradable polymers are those in which chain cleavage occurs leading to mineralization. For this, specific conditions are required in terms of pH, humidity, oxygenation and the presence of some metals to ensure its biodegradation. Properties such as greater mechanical strength and rigidity, light weight, adjustable shapes, easy handling, durability and low susceptibility to environmental degradation make biopolymers the right choice to be used as matrices for a wide range of applications [1].

Much of the properties of biodegradable polymers are excellent when compared to petroleum-based plastics. Being easily biodegradable, they can be a real alternative to basic plastics. Therefore, biodegradable polymers have great commercial potential for bioplastic, but some of the properties, such as brittleness, low distortion due to increased temperature, high gas permeability, low melt viscosity for further processing, etc., could restrict its use in a wide range of applications. Nanoreinforcement of pristine polymers to prepare nanocomposites has already proven to be an effective way to further enhances these properties [2]. Therefore, the modification of biodegradable polymers by innovative technology is a great challenge for materials scientists.

The utility of inorganic nanoparticles as additives to improve polymer performance has been demonstrated in last years [2–9].

Organically modified layered silicate and polymers are of particular interest due to their demonstrated significant enhancement of a large number of nanocomposites physical properties, including barrier, flammability resistance, thermal and environmental stability, solvent absorption, and biodegradability rate of biodegradable polymers relative to an unmodified polymeric resin [2].

The main reason for the improvement of these properties of the polymer/silicate in nanocomposites layers is the strong interfacial interactions between the polymer matrix and the layered silicates [10]. Among the layered silicates commonly used for the preparation of bionanocomposites belong to the same general family of 2:1 layers or phyllosilicates [11, 12]. Its crystalline structure consists of layers formed by two tetrahedrally coordinated silicon atoms and central octahedral sheets with a shared edge of either aluminum or magnesium hydroxide. The thickness of the layer is around 1 nm, and the lateral dimensions of these layers can vary from 30 nm to several microns or more, depending on the particular layered silicate. The stacking

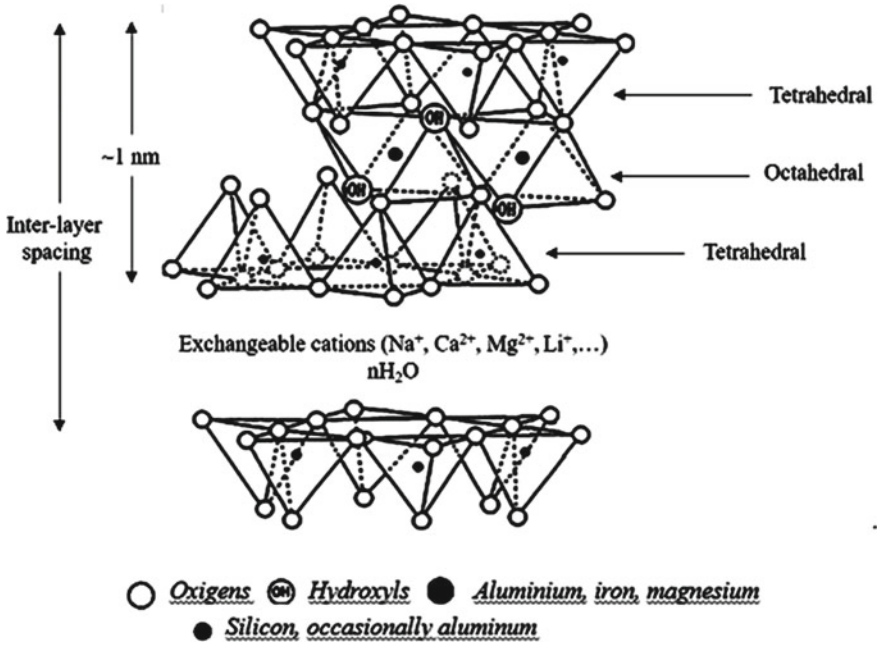

Fig. 1.1 Structure of 2:1 phyllosilicates (from [13] with permission)

of the sheets leads to a van der Waals space between the layers called the middle layer or gallery. Isomorphic substitutions within the layers are possible; thus, the tetrahedral silicon can be partly substituted by Al^{3+} or Fe^{3+} and Al^{3+} of octahedral sheet can be replaced by Mg^{2+} or $Fe^{2+}$and more rarely by others cations as Li^+, etc. This generates negative charges that are counteracted by alkali and alkaline earth cations located inside the galleries. The local surface charge that is generated is not constant in all the layers of the silicate; it is considered an average value that is known as cation exchange capacity (CEC) and is generally expressed as mequiv/100 g.

Montmorillonite (MMT), hectorite and saponite are the most widely used phyllosilicates 2:1. In the case of substituted tetrahedral silicates in the layer, the negative charge is on the surface of the silicate layers and therefore the polymer can more easily interact with the matrices than in the case of the material with substitutions in the octahedral layer. A scheme of the structure of these layered silicates is shown in Fig. 1.1.

1.2 Biodegradable Polymers

According to the preparation of nanocomposites, biopolymers can be classified as follows [14, 15].

Naturally occurring biopolymers are those produced from biosources including polysaccharides (cellulose (CELL), chitosan (CTS), starch (ST), alginate (ALG), gums, carrageenan etc.), proteins originated from plants or animals (zein, gelatin, collagen, egg proteins, milk proteins, etc.) and lipids (triacylglycerides and waxes, etc.).

Biopolymers derived from microorganism products such as polyhydroxybutyrate and polyhydroxyvalerate, poly (hydroxyalkanoates) or poly (3 hydroxybutyrate-co-3-hydroxyvalerate), among others.

Synthetic biodegradable polymers are those manufactured by industrial technologies such as polylactic acid (PLA), polyglycolic acid, polyvinyl alcohol, polybutylene succinate or polycaprolactone. Studies have shown its potential to improve physical (strength, flexibility, durability, etc.), optical (photosensitivity, color, gloss, etc.) and thermal (glass transition, conductivity, melting) properties.

1.3 Biodegradable Polymer-Based Bionanocomposites

Because biopolymers have poor mechanical and barrier properties, its reinforcement with nanosized materials have attracted the interest of material researchers achieving notable enhancements of their native properties. Bionanocomposites consist of a hybrid materials derived from a biopolymer matrix reinforced with inorganic nanofillers [16]. Three types of nanocomposites can be differentiated based on the shape and dimensions of the dispersed particles [17]: (i) particulate nanocomposites (isodimensional silica, metal or metal oxide nanoparticles); (ii) elongated-particle-nanocomposites (two dimensions in the nanometer scale such as carbon nanotubes or cellulose nanowhiskers); and (iii) layered-particle-nanocomposites (one dimension in nanometer range such as layered silicate clays). The introduction of nanoparticles in the polymer matrix has improved the properties of virgin polymers in terms of mechanical (strength, elastic modulus, stability), heat (resistance) and permeability (gases and water) properties [15, 18–20].

For environmental applications, layered silicate clays have been commonly studied owing to their properties of availability, affordability, high surface area and ionic exchange capacity. Recent studies have proven that the intercalation of polymers and organic molecules in the interlayer space enhance their mechanical, thermal and adsorption properties. The extent of nanofiller dispersion and intercalation depends on the affinity between the silicate and the polymer matrix, as well as the preparation technique [17, 21–23]. Four different morphologies can be obtained one mixed both components (Fig. 2.2).

Phase-separated microcomposites (conventional microcomposite): The polymer is unable to penetrate and intercalate between the silicate layers, acting as microparticles dispersed in the polymeric matrix. Their properties are particularly poor due to the lack of uniform dispersion.

Intercalated nanocomposites: The polymer is penetrated and inserted between the silicate layers giving rise to a significantly expansion in the interlayer distance of the layers.

Flocculated nanocomposites: Slightly similar to the intercalated structure and appear when there is hydroxyl interactions between the silicate sheets.

Exfoliated nanocomposites: The individual sheets are separated completely and uniformly dispersed in a continuous polymer matrix. To get a complete exfoliation is desirable since provide materials with enhanced mechanical, thermal, and barrier properties (Fig. 1.2).

Table 1.1 shows the biopolymers and clays most commonly used for the preparation of bionanocomposites as adsorbent materials in environmental applications for water remediation.

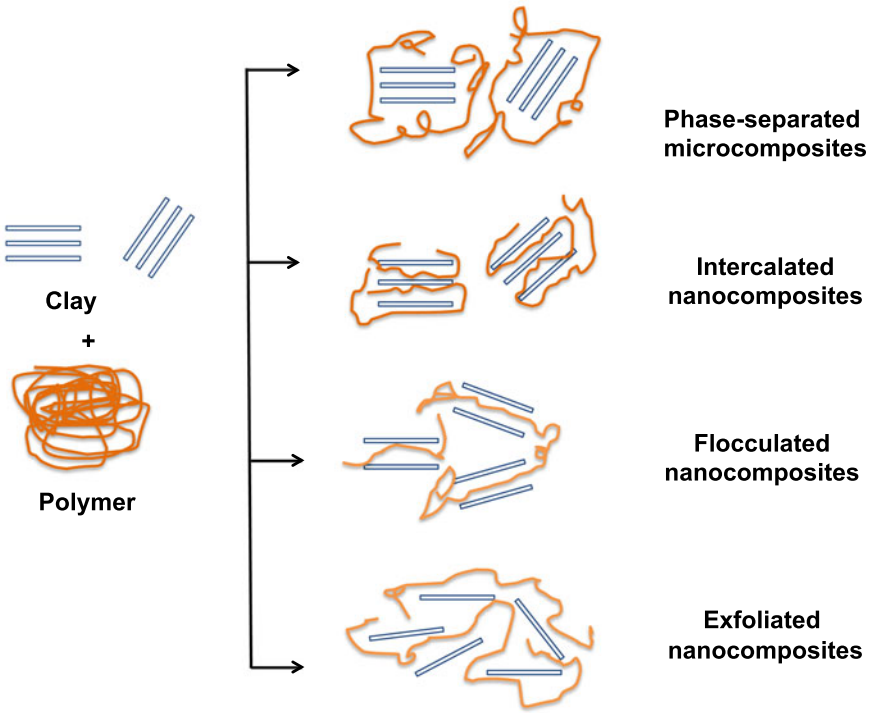

Fig. 1.2 Illustration of four possible polymer–clay morphologies (adapted from [13] with permission)

Table 1.1 Biopolymers and clays used in the synthesis of bionanocomposites (modified from [13] with permission)

Biopolymer	Clay/organoclay	Preparation methods	References
Chitosan	Montmorillonite	Solution blending method	[24–30]
	Bentonite	Solution blending method and microwave heating	[31–34]
	Vermiculite	Solution blending method and solution blending involving ultrasound irradiation	[35–37]
	Hexadecyl trimethyl ammonium-Vermiculite	Solution blending method involving ultrasound irradiation	[36]
	Na-mica-n	Solution blending method	[38]
	LDH	Solution blending method	[39]
	Attapulgite	Solution blending method	[40]
Carboxymethyl chitosan	Montmorillonite	Solution blending method	[41]
Modified chitosan (ethylenediaminetetraacetic acid, glutaraldehyde, sodium dodecyl sulfate and cetyltrimethyl ammonium bromide)	Gluconite	Solution blending and crosslinking method	[42]
Starch	Montmorillonite	Solution blending method; Melt intercalation method	[26, 43, 44]
	Organomontmorillonite	Melt intercalation method	[43]
	Kaolinite and Kaolinite/ dimethyl sulfoxide	Solution blending method	[45] [46]
	Bentonite	Solution blending method	[47]
	Palygorskite	Solution blending method	[37]
	LDH	Solution blending method; Hydrothermal treatment	[48, 49]

(continued)

Table 1.1 (continued)

Biopolymer	Clay/organoclay	Preparation methods	References
Starch acetate	Organomontmorillonite	Melt intercalation method	[50]
Cationic starch	Montmorillonite	Solution blending method	[51, 47]
	Bentonite	Solution blending method	[47]
Carboxymethyl cellulose	LDH	Solution blending method	[52]
Carboxymethyl cellulose-Starch	LDH	Solution blending method	[53]
Carboxymethyl cellulose-CTS	Exfoliated montmorillonite	Hydrogen-bond, amidation and chains interleaving interaction	[54]
Alginate	Montmorillonite	Solution blending method	[26, 55–57]
	Mica	Solution blending method	[58]
	Palygorskite	Solution blending method	[37]
	LDH	Solution blending method	[59]
Alginate/Xylan	Bentonite	Solution blending method	[59]
	Halloysite	Solution blending method	[59]
Hydroxyethylcellulose	Montmorillonite	Solution blending method	[60]
C16-hydroxyethylcellulose	Montmorillonite	Solution blending method	[60]
Cellulose-graft-Polychloromethylstyrene-graft-Polyacrylonitrile	Organomontmorillonite	Radical polymerization and solvent blending methods	[61]
Cellullose	Mica	Solution blending method	[62]
Cellullose	Montmorillonite	Solution blending method	[63, 64]
Guar gum	Bentonite	Solution blending method	[65]
Xanthan gum/n-acetyl cysteine	Mica	Solution blending method	[66]

(continued)

Table 1.1 (continued)

Biopolymer	Clay/organoclay	Preparation methods	References
Sacram	Sepiolite	Solution blending method	[37]
Zein	Palygorskite	Solution blending method	[67]
	Sepiolite	Solution blending method	[67]
	Montmorillonite	Solution blending method	[68]
Gelatin	Montmorillonite	Solution blending method	[26]
Polylactic acid	Montmorillonite	Melt-blending method	[69, 70]
	Organomontmorillonite	Solution blending method and melt-blending method	[71, 72]
	LDH	Solution blending method	[73]
Polylactic acid-chitosan	Organomontmorillonite	Solution blending method	[74]
Polylactic-co-glycolic acid	Montmorillonite	Solution blending method	[75]

1.3.1 Chitosan/Clay Nanocomposites

CTS (poly-alfa(1,4)-2-amino-2-deoxy-D-glucose) is the second most abundant polysaccharide in nature derived from *N*-deacetylation of chitin. CTS contains chemically amine and hydroxyl groups in their structure which are potentials that has been harnessed in the field of adsorption of substances when it is intercalated onto clay minerals [27, 29, 30, 76]. CTS requires an acidic medium to be dissolved and, at this pH CTS the protonated amine groups (−NH3+) promoting strong electrostatic bonds with the clay by replacing a exchangeable cations of the pristine clay located in the interlayer space [77].

The most intensive researches on CTS-based nanocomposites are focused on 2:1-type layered silicates, especially on MMT. Darder et al. [29, 30] discovered that CTS chains can form mono- or bilayer structures within the MMT interlayer depending on the relative amount of CTS with respect to the CEC of the clay. Authors observed an increase in d spacing from 1.20 nm to 1.45 nm and 2.04 nm, mono- or bilayer structures, respectively. The CTS interaction mechanism resulted different; the first CTS layer is adsorbed through a cationic exchange mechanism, while the second one is adsorbed in the acetate salt form [29]. Authors also observed that when there is an exchange higher than the CEC of the MMT, the adsorption mechanism shifts to an anionic exchange capacity (AEC) since the protonated amino groups of CTS, not involved in the interaction with the MMT, act as anionic exchangers improving the adsorption of anions [27, 77, 78].

The structure of the CTS-based nanoncomposites is also affected by the concentration of clay in the CTS matrix. Wang et al. [78] prepared CTS/MMT nanocomposites using clay quantities from 2.5 to 10 wt%. The X-ray diffraction (XRD) pattern and transmission electron microscopy (TEM) images corroborated the formation of intercalated and exfoliated morphologies when the content of MMT was low and an intercalated and flocculated structure at higher concentrations. Later, same authors [41] studied the structures and properties using three different CTS derivatives (CTS, carboxymethyl CTS, and *N*, *N*, *N*-trimethyl CTS). The carboxymethyl groups in the CTS matrix increased the interaction with the hydroxylated edge groups of MMT through hydrogen-bonding reaction. Intercalation and slight flocculation structure was observed for the CTS/MMT nanocomposites. The use of carboxymethyl CTS enhanced the extent of flocculation, and the quaternization of the amino group of CTS gives a nearly exfoliated structure.

Other 2:1 layered silicates have also been prepared using CTS. For example, Chen et al. [35] prepared a CTS/vermiculite bionanocomposite using epichlorohydrin (ECH) as crosslinking agent. According to the characterization results, CTS cannot intercalate into the interlayer space of the vermiculite but crosslink with the external surface of clay. ECH produces an improvement of the mechanical resistance, chemical stability, pore size and adsorption/desorption properties of the bionanocomposite establishing covalent bonds with the carbon atoms of the OH groups of CTS [79]. More recently, Alba et al. [38] synthesized several CTS/mica (2–4)-based bionanocomposites by ion-exchange reaction. The bionanocomposite prepared using

Na-Mica-4 contains more CTS than those from Na-Mica-2. Both bionanocomposites exhibited higher thermal stability than the pure CTS. The characterization results indicated that the adsorption of CTS in the interlayer space was successful, although a CTS portion remains in the outer surface being hydrogen-bonded to the mica. The zeta-potential values change from negative to positive for both bionanocomposites. More recently, da Silva et al. [31] prepared CTS/Bentonite nanocomposites (at different CTS proportions, from 50 to 300%) using microwave heating. Various types of nanocomposites (in the interlayer space) were obtained varying the CTS proportions: monolayer for 50% of CTS; bilayer for 100–200% of CTS; mono- and bilayer for 300% of CTS. Increasing the heating reaction time up to 30 min improved the CTS intercalation efficiency without altering the morphology.

LDHs are classified as negative clays and have been previously assessed as effective adsorbents of anions [80]. Li et al. [39] prepared CTS/LDH bionanocomposites by two methods: direct mixing of LDH nanopowder with CTS gel and in situ method by adding LDH into CTS gel. A better dispersion of the nanoparticles was observed with the second method, which resulted in better adsorption properties.

1.3.2 Starch/Clay Nanocomposites

ST combined two types of polymers: amylase (10–30%) and amylopectin (70–90%) [46, 81]. It is a neutral molecule containing hydroxyl groups (susceptible to substitution reactions) and acetal groups (susceptible to chain breakage). The reinforcement of ST with clays produces a better dispersion of the structure and enhanced its mechanical, thermal stability, and barrier properties without altering the biodegradability [44, 46, 50]. Chivrak et al. [43] compared the properties of MMT and organomodified montmorillonite (OMMT) incorporated into the ST matrix by a melt blending process. Characterization results showed that MMT leads to intercalated bionanocomposites, while OMMT allowed the elaboration of well-exfoliated bionanocomposites displaying better mechanical properties. Ruamcharoen et al. [45] prepared two nanocomposites incorporating various amount of kaolinite and kaolinite modified with dimethyl sulfoxide into sago starch via solution blending method. The XRD and TEM images revealed intercalate and exfoliate structures attesting well-dispersed kaolinite layers in the ST matrix. Authors observed a decreased of the water vapor transmission of the native ST and an improvement in the tensile strength and modulus of ST-based bionanocomposites.

The modification of ST matrix with cationic groups has been proposed to get functional bionanocomposites for the removal of contaminants of anionic character from aqueous media [51, 82]. Koriche et al. [47, 83] revealed that the incorporation of cationic quaternary ammonium groups in the modified ST facilitated its intercalation in MMT through a cationic exchange mechanism. The excess of positively charge from ST produced an enhancement in the adsorption capacity of nanocomposite toward anionic pollutants. Similar results were reported recently by

Lawchoochaisakul et al. [51] whose use cationic ST/MMT as potential adsorbents for basic dyes.

ALG-nanocomposites based on synthetic LDH clays have been studied although to a lesser extent than natural clays. Chung et al. [49] synthesized LDH crystallites into the ST (or acid-modified ST) matrices by hydrothermal treatment obtaining well-dispersed structures. Wu et al. [53] synthetized first LDH with carboxymethyl cellulose (CMCELL) as the stabilizer and then used it to prepare LDH/CMCELL/ST nanocomposites. The incorporation of CMCELL in LDH enhanced the stability in water giving its hydrophilic character which were also helpful to get a uniform dispersion of the LDH/CMCELL/ST.

1.3.3 Alginate–Clay Nanocomposites

ALG is a biopolymer formed by linear copolymers units of 1,4-β-D-mannuronic acid and 1,4-β-L-guluronic acid. It is negatively charged polymer containing carboxylate groups in its structure. Reported studies indicate that the incorporation of clays into ALG beads matrix improves the mechanical and thermal stabilities of the ALG beads [55, 56, 84–86].

Alcântara et al. [37] carried out a study to compare the preparation and interaction mechanisms of bionanocomposites using sepiolite and palygorskite fibrous clays and polysaccharides of neutral (ST), cationic (CTS) and anionic (ALG) character. Overall, in the three cases the interaction of the OH groups present in the biopolymers backbone and the SiOH groups on the silicate surface is observed. Moreover, in the case of ALG and CTS biopolymers, the presence of negatively carboxylate and positively amino groups, respectively, may be also implicated leading to strong interactions between both components. The resulted bionanocomposites displayed good mechanical properties, improved water resistance and reduction of water absorption, which make them very attractive as adsorbents for water remediation. Recently, Naidu and Jhon [59] prepared xylan/ALG/bentonite or halloysite nanocomposites by solution blending method. When the load of lays was 5 wt% a significant decrease (49%) of the water vapor, permeability was observed explained by the impermeable nature of the silicate layers. Overall, nanocomposites prepared with bentonite clays exhibited superior mechanical and optical properties than halloysite-based nanocomposite and native ALG. Thermal stability and solubility were not significantly influenced by the intercalation of the clays.

Reese et al. [87] prepared five LDH/ALG hybrid composites, four possessing different guluronic/mannuronic acid ratios ALG and one acetylated ALG by coprecipitation method. Aluminum nitrate and zinc nitrate were used as a LDH precursor to form hybrid composites with ALG solution. An increase in the d spacing was observed by XRD when increasing guluronic acid content (1.28–1.85 nm) and acetylated ALG (to 1.72 nm). The nuclear magnetic resonance (NMR) spectroscopy revealed the interaction of negatively charged carboxylic groups from the biopolymer with the positively charged inorganic main layer while, scanning electron microscopy

(SEM) confirmed the highly flexible nanofoil morphology of the hybrid composites which could function as reinforcement to the concrete applications.

1.3.4 Cellulose/Clay Nanocomposites

CELL is the most abundant biopolymer in nature consisting of linear beta-1,4-linked D-glucopyranose monomers. CELL aerogels have been applied as adsorbents in various contaminant treatments [54]. Despite its biodegradability, affordability, biocompatibility and its absence of toxicity, CELL is insoluble in water or in common solvents doing complex its processing. Moreover, CELL has chains very packed through inter- and intramolecular hydrogen bonds and suffers poor dimensional stability, mechanical strength and functionality [13, 54, 61]. The chemical modification of its structure is necessary to improve its properties [88]. To that end, graft copolymerization is a method commonly used. Park et al. [89] synthesized maleic anhydride grafted CELL acetate butyrate (CAB-*g*-MA) as the compatibilizer in order to obtain a better interaction between CELL acetate and the OMMT. A mixture of exfoliated and intercalated structures is obtained when the compatibilizer is used at 5 wt%, while without it an intercalated structure was observed. Because CELL tends to decompose at $T^a < 260–270$ °C (below its melting point), the free radical polymerization and solution-casting techniques instead of melt compounding are currently used to prepare grafted CELL/clay bionanocomposites. Abbasian et al. [61] prepared a CELL-graft-polychloromethylstyrene-graft-polyacrylonitrile/organoclay through metal catalyzed radical polymerization and solvent blending methods.

Other simply and most advantageous, for economic reasons, modification of CELL is the production of CMCELL. CMC is an anionic polymer, which contains a hydrophobic polysaccharide backbone and numerous hydrophilic carboxyl groups, hence showing an amphiphilic characteristic. Cukrowicz et al. [90] prepared two nanocomposites using CMCELL of different viscosity to modify the structure of MMT. The FTIR and XRD analysis confirmed the constituent materials react by the hydrogen bonds formation, which results in the polymer adsorption on the surface of mineral particles and results also showed that MMT forms a more stable system with the lower viscosity CMCELL. However, both types of CMCELL caused a partial or full delamination of the MMT structure, which may be related to the similarity of mineral surface and polymer charges or to the rigidity of the polymer chain backbone making it difficult to change its conformation. Yadollahi et al. [52] intercalated CMC into Mg–Al LDH and Ni–Al LDH by coprecipitation methods. XRD pattern revealed an increase in d spacing from 0.862 to 1.73 nm for Mg–Al LDH and from 0.816 to 2.23 nm for Ni–Al LDH. TEM images showed the presence of intercalated and non-intercalated layers in both Mg–Al LDH/CMC and Ni–Al LDH/CMC nanocomposites, while the thermal stability was higher for Mg–Al LDH/CMC than Ni–Al LDH/CMC nanocomposites. The introduction of long alkyl chains has also been proposed to modified CELL making hydrophobic derivatives. Simon et al. [60] introduced a low amount of C16 alkyl groups in hydroxyethyl CELL that presented

a higher affinity for the MMT surface than its precursor hydroxyethyl CELL. More recently, Wang et al. [54] prepared a CMCELL/CTS/ exfoliated MMT nanosheets (MMTNS) composite hydrogel very effective as adsorbent for dye effluent remediation. The hybrid material was synthesized via hydrogen bond, amidation and chains interleaving interaction. MMT is first mechanically exfoliated into nanosheets with single or few layers, which improve its adsorption performance. MMTNS present some properties (positive charge on edge and negative charge on surface, small size or strong dispersion among others), which make possible that MMTNS could then be fully dispersed in the CELL matrix.

1.3.5 Protein–Clay Nanocomposites

Proteins (zein, collagen, gelatin, wheat gluten, soy, etc.) are hetero-biopolymers containing different kinds of amino acids. Protein molecules are amphoteric molecules and present good gas barrier properties but lower water vapor permeabilities. The incorporation of proteins into clays has been used to improve the water vapor barrier properties or with the end of reduce the hydrophilic character of original clays. Alcântara et al. [67] prepared Zein/sepiolite and palygorskite. Fibrous clays do not present intercalation properties but exhibit a large specific surface area, microporosity, the ability to adsorb a large variety of molecules. Zein is a hydrophobic molecule that was assembled on the external surface of clays through the interaction of its amide groups with the OH groups at their external surface. The quantity of zein in the clay affects the structure. Zein/Sepiolite showed a larger amount of assembled protein than those based on Zein/Palygorskite due to its higher external surface area. Authors also tested that improved properties of water resistance are obtained when ALG is incorporated as bioadditive. The resulting ALG/Zein/Zlay nanocomposite improved some properties like flexibility and good water vapor barrier properties and reduced the water uptake. Same authors [68] prepared a Zein/MMT nanocomposite with excellent compatibility, homogeneity and mechanical properties and without the need to add compatibilizers or plasticizers. Azhar et al. [26] prepared a gelatin/MMT nanocomposites, and the coexistence of both intercalated and flocculated structures was observed. TEM images showed that a large amount of MMT was stacked together in gelatin–MMT nanocomposite, while a few intercalated clay platelets were observed.

During the protein intercalation process by clays, it is very important to adjust and control the pH below the isoelectric point of the biopolymer. For example, the incorporation of smectite clays in the gelatin matrix occurs at low pH values when the protonated amine groups of the protein can replace totally or partially the cations situated in the interlayer space of the smectites [77]. Overall, proteins have the ability to form a good film that makes them an ideal material for biodegradable packaging and make them best suited for packaging and biomedical applications rather than environmental remediation.

1.3.6 Polylactic Acid/Clay Nanocomposites

PLA is a synthetic biopolymer derived from cornstarch by fermentation. Its basic unit is lactic acid. PLA presents promising qualities of strength, thermal plasticity, biodegradability and biocompatibility. However, its brittleness, low miscibility, gas permeability and slow crystallization rate may limit its use. Clays have been proposed to improve some of these last properties without affect its natural one [15, 91].

To enhance the miscibility with PLA by the solution blending method, some researches have proposed the modifications of the clay surface [72, 74, 92]. Wu and Wu [74] get exfoliated structures enhancing the interaction between PLA and OMMT. First, authors carried out the organofunctionalization of the MMT with n-hexadecyl trimethylammonium bromide cations and then modifying it with CTS. McLauchlin and Thomas [72] used of a novel surfactant to modify the MMT, cocamidopropyl betaine (CAB) containing both quaternary ammonium and carboxyl moieties, which resulted in a proper dispersion in the PLA matrix. CAB presents a negative charge in basic conditions and a positive charge at lower pH values. The XRD pattern and TEM images showed an ordered intercalated structure. To improve the chemical compatibility between PLA and LDH, Chiang and Wu [73] modified the surface of LDH using PLA with carboxyl end group through an ion-exchange process. XRD pattern and TEM images of synthesized nanocomposites denote that modified LDHs are randomly dispersed and exfoliated into the PLA matrix.

Recently, Gomez-Gamez et al. [69] studied the technical properties of PLA/MMT nanocomposites (using different clay loadings). Authors observed an increase in thermal stability and Young's modulus at increasing clay loading. Similar results were obtained by Lopes Alves et al. [71] when incorporated different OMMTs in PLA matrix. Neppalli et al. [70] compared the structure and morphological changes when different types of clays (cationic (MMT) and anionic (perkalite)) are incorporated in PLA matrix. The results showed that perkalite-based nanocomposites presented a faster crystallization rate and a higher crystallinity, besides a faster degradation, while the MMT-based nanocomposites resulted in disordered lamellar stacks.

1.4 Preparation of Biopolymer–Clay Nanocomposites

In order to improve the intercalation/exfoliation process in a polymeric matrix, it is very common to carry out a change in the chemical composition of clays on their surface to make its polarity coincide with that of the polymer [17]. In order to achieve this purpose, the most used technique is cationic exchange and has been employed mechanisms among which we can highlight adsorption of block copolymers [93], grafting of organosilane [94], ionomers applications [95], as reported in previous works [22, 23].

A review work [23] showed a detailed analysis of the ways of working established to develop bionanocomposites based on polysaccharides with MMT, explaining their state of dispersion and properties.

There are three main techniques commonly used to carry out biopolymer/layered silicate bionanocomposites [15, 17, 23, 81], which are involved in the incorporation of clay in a polymer previously swollen with solvent. These methods are: (i) solution blending [2], (ii) polymer melt (or melt blending) [96] or (iii) adding modified clay to a polymerization reaction (in situ polymerization method) [97]. The analysis of the data presented in Table 1.1 shows that the most used and generally the most appropriate method is the first one on the list.

1.4.1 Solution-Blending Method

For this way of preparation, a solution containing the polymer is initially prepared by using a solvent that allows it, being water the most used in case of biopolymers. At the same time, the layered silicate is dissolved in the solvent used or in another if necessary to achieve a miscible solution. Once this step is completed, the polymer solution is mixed with the phyllosilicate dispersion, allowing an intercalation of both structures and the resulting solution is allowed to homogenize, to finally evaporate the solvent [22]. In the case of non-water-soluble polymers, the method is carried out with the use of organic solvents in large quantities, therefore with a high cost both economically and for the environment [81]. An added drawback is that a very low amount of solvent can be kept in the final product at the polymer–clay interface, which as has been shown in previous work will result in less interactions between the surfaces of the clay and the polymer [98]. It has been previously commented that the solution blending method is the most widely used (as shown in Table 1.1), and this is a consequence of the fact that biopolymeric materials that are used in the production process of bionanocomposites (such as be CELL, pectin or CTS) do not allow the possibility of being processed by fusion due to high thermal degradation or by the thermomechanical process. Preparing the polymers in situ also has limitations in the way they are obtained, since in practice most biopolymers are available from natural formation processes showing generally in polymeric form compared to the much less common monomeric form [23]. Figure 1.3a represents a schematic view of the preparation procedure by solution-blending.

1.4.2 Melt-Blending Method

This preparation technique makes a better mix possible of the clay materials and biopolymer in comparison with the solution-blending. The procedure involves a process where high temperatures are reached in which the clay material is placed at the same time into the heated polymeric material matrix in an equipment where the

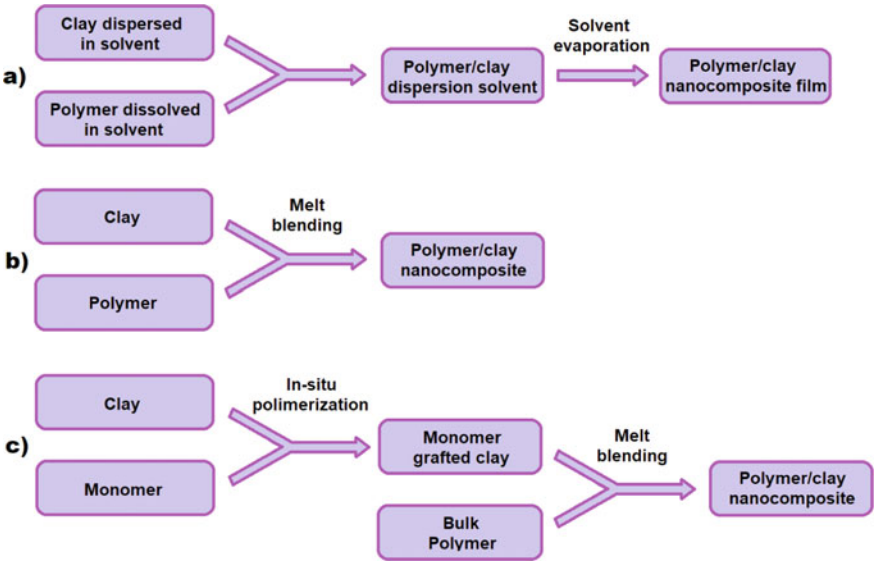

Fig. 1.3 Bionanocomposite preparation methods: **a** solution blending, **b** melt blending and **c** in situ polymerization (adapted from [13] with permission)

mixture is produced by melting and the resulting compound is prepared by kneading to obtain a final product of high homogeneity [22]. By controlling two conditions, the residence time and the shearing, the dispersion of the clay can be optimized [99]. The residence time is necessary to enable the chains of polymeric material to diffuse into the interlaminar spacings of the clay to obtain a highly exfoliated and disorderly morphology. The shearing collaborates in the delamination of clays. This technique has advantages for the conservation of the environment because no solvent is used, but the thermal or thermomechanical treatments cause partial degradation of the compounds and, as long residence times are necessary for the exfoliation of the clay, a degradation in the matrix is caused. These disadvantages make this mode of operation unsuitable for preparing biopolymeric materials, with the exception of additive modified ST thermoplastic or PLA. [70, 100, 101]. Figure 1.3b schematizes a representation of the melt-blending preparation method.

1.4.3 In Situ Polymerization Method

In a first stage of this method, the clays swell in a monomer solution where polymerization begins and from that situation it propagates [23]. Heat or a suitable chemical can be used to start the polymerization process [99]. With polymerization, there is an increase in the molecular weight of the macromolecules, producing an increase in the basal spacing d001 and, on occasions, to a morphology exfoliated practically in

its entirety [2]. This technique has been used as another way of preparation instead of melt blending method as a mechanism for an in situ polymerization preparation of intercalated lactide monomers for the production of compounds in exfoliated form [102]. As previously explained, the polysaccharide chains are obtained from nature itself in the growth process of plants and are obtained from these already in their polymeric conformation. Because of that, this way of preparation is not suitable to obtain bionanocompounds that are prepared from a polysaccharide base [23]. In Fig. 1.3c, a representation of the in situ polymerization preparation method can be seen.

There are other ways to obtain this type of materials using a mode that does not involve the traditional way, such as microwave induction, intermatrix synthesis, self-assembly, template targeting, electrospinning or preparation under supercritical conditions (e.g., supercritical CO_2) to obtain the final compound [81].

1.5 Characterization Techniques

One of the main aspects in the research of polymer–clay nanocomposites is the characterization and understanding of the physicochemical properties enveloped with adsorption capacity, such as expansion properties, morphology, layer loading and charge distribution, structure and pore size. The most commonly applied techniques for the characterization of clays are XRD, TEM, SEM, Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and thermal gravimetric analysis (TGA) [17].

The study of lamellar spacing by XRD has been used to a great extent to study the resulting biopolymer–clay structure after the possible preparation methods indicated in the previous section, allowing the technique also to obtain crystallographic structural and microstructural information [21]. By evaluating the position and possible displacements of the basal planes can be determined the type of nanocomposite in terms of its structure, which can be separated by phases, with the biopolymer and the clay intercalated or with exfoliated sheets in general. XRD could be used as a way to see and understand the kinetic properties of the molten polymer mixture. The study of the intensity and shape of the peaks corresponding to the basal planes is a way of interpreting the degree of dispersion of the clay inserted in the matrix containing the polymer or polymers in more complex cases. One of the characteristics that can be understood for exfoliated or disordered structures is that the disappearance of the reflections leads as the most likely interpretation the complete distribution of the phyllosilicate sheets in the matrix formed by the biopolymeric part with the breakdown of the structure of the sheets or layers of the phyllosilicate or clay. For this technique in general and applying it to intercalated structures, spacing expansion is observed through the displacement of peaks corresponding basal spaces toward lower 2Theta diffraction angles, and it is so observed after intercalation of the polymer. On the contrary, for conventional microcomposite structures, the d values and consequently those of 2Theta will not change. This is interpreted to mean that the clay

compound retain its laminar conformation since the chains of polymeric material are not adsorbed in the interlaminar space [21].

Nevertheless, sometimes the use of XRD analysis alone does not allow determine with certainty the structure of the nanocomposite because in general it only provides information on the orientation and spacing of the clay sheets, which makes it difficult to interpret the diffractogram. Another additional problem is that when there are mixtures of different clays, the value obtained from the spacing can be inaccurate due to the overlapping of the peaks of the different contributions. Furthermore, the possible absence of peaks corresponding to reflections of intercalated clays that are randomly distributed could lead to the erroneous interpretation that the structures are exfoliated. Finally, dilution of the clay can also be causing broadening or no peaks even without a delamination phenomenon [21].

The XRD technique is usually complemented by TEM to a large extent because this microscopic technique has the possibility of providing qualitative information on the spatial arrangement and morphological characteristics of the clay, in addition to allowing any internal defects to be observed visually. Combining the visualization of the samples with the contribution of diffraction data, the main advantage of TEM is its magnification (between 50,000 and 10,000,000x). The micrographs obtained by TEM allow the direct observation of the spatial distribution of the phyllosilicate sheets, which is not possible by other microscopic techniques. In the images obtained, the clay sheets show a strong contrast because they are basically composed of Si, Al and O with higher atomic numbers than those of the polymer containing C, H and N. In this way, the interlaminar spaces of clay layers are usually represented as dark lines, while the presence of heavier atoms as lighter areas. In this sense, TEM images of separated phase compounds are represented as darker due to the presence of clay aggregates, while for exfoliated composites brighter images are obtained. In the same way as the TEM technique, SEM also allows to obtain micrographs of the surface areas of solid samples, in addition to providing information on their dispersion, structural conformation and elemental composition [21]. Notwithstanding, the magnification of the SEM technique is of the order of 1 nm, making it difficult to obtain detailed and precise information on the distribution of nanoparticles in the polymeric matrix, although it is useful for obtaining images of surfaces that are fractured. There is also the possibility of a combined use of SEM with the technique of energy dispersion X-ray spectroscopy to study the dispersion range of nanosized particles on bionanocomposites surfaces that are fractured [15].

The FTIR technique, like those previously described, is widely used and is a well-accepted method to study the structure of the nanocomposite polymer, which makes it possible to differentiate whether the chemical bonds belong to the nanocomposite or are simply part of the polymer. As a drawback, the differentiation of the chemical bonds that appear can present complexity in the interpretation and understanding even though the intercalation has already been carried out. As previously explained, many studies have been conducted using FTIR to characterize the biopolymer introduced into the clays structure [29, 38, 78]. It is a characterization technique with great capacity and utility to determine electrostatic relationships and interactions between clays and biopolymers. Darder et al. [19] used this spectroscopic technique to evaluate

the interactions between sepiolite, starch and alginate and were able to observe an appreciable change in the vibration band of the silanol-type groups placed on the surface of the clay, results that seem to indicate that the Interaction relationships occur between the OH groups on the external surface of the clay and the groups belonging to the polysaccharide that have hydroxyl character. The FTIR results showed that the corresponding band appeared displaced showing a decrease in the frequency values, which implies a lower value of absorbance with respect to that assigned to the Mg–OH stretching vibration in which no changes were observed. Other work done by Darder et al. [80] and Leroux et al. [103] were able to find high-intensity bands by FTIR corresponding to the interactions that occurred between carboxylate groups that were negatively charged from pectin biopolymers and ALG and LDH sites presenting positive charge, while a weak interaction was observed between LDH and i-carrageenan.

The NMR technique is also used to study the morphology, the surface at the chemical level and the dispersion of the clay using the signals of spin–lattice relaxation time for ^{1}H, ^{13}C, ^{23}Na, ^{27}Al or ^{29}Si [2]. NMR (^{1}H and ^{13}C) was used to obtain values that allowed quantifying the degree of exfoliation of nylon 6-MMT nanocomposites and established a possible correlation between obtained results and the quality of the clay dispersion. For cases where the phyllosilicate is stacked and not sufficiently dispersed in the polymer matrix, the polymer–clay average distances are higher and the average paramagnetic contribution shows a lower value [2]. NMR (^{29}Si and ^{23}Na) spectroscopy was used to demonstrate the assimilation of chitosan into mica, while chemical bonds of chitosan are formed to Al–O and Si–O of the basal sheets [38]. Combined use of FTIR and ^{13}C NMR measurements allow to verify the ability of the chitosan–clay nanocomposite to act as an anion exchanger when the biopolymeric material is distributed in the form of a bilayer in the interbasal zone (using in this case smectite) [30].

As the last of the techniques presented, TGA gives information on thermal behavior of bionanocomposites, where many works have allowed understand the greater stability of biopolymeric materials that are part of bionanocomposites. The introduction of biopolymeric materials between sheets of phyllosilicates causes an increase in the decomposition temperature in the order of 40–50 °C. This is because the layered silicate works as a thermal shield when the bionanocomposite is structurally formed, causing a greater thermal stability of the entire system and helping a greater carbon formation once the thermal decomposition is completed [2]. The TGA results obtained demonstrate decomposition or degradation, in the form of weight loss, depending on time or temperature, such as isothermal or dynamic heating. Between room temperature and around 170 °C, a weight loss is observed, which is interpreted as the loss of water that can come from the interlayers or from the surface of the phyllosilicate, from the water retained in the biopolymeric materials or the interstitial water. The origin of the water described above will depend on the thermal decomposition in the layered silicates or biopolymeric materials used. As a normal mechanism, the decomposition of the system starts at about 250 °C with the degradation of components of organic origin and ends at about 600–630 °C with the decomposition of the crystalline structure and the phyllosilicate [2, 19, 26, 39, 48]. To

finish this section, different works have studied the thermal stability of starch-based nanobiocomposites to understand the interactions that take place from the evaluation of the thermal stability and the behavior of the system once the clay dispersion occurs. For example, thermal analysis was used to highlight the increase in temperature at which the degradation of potato starch-MMT system occurs in comparison to the laminar silicate material. In the same sense, the system with organically modified MMT was analyzed and compared with the previous one. It should also be taken into account that the stability as a function of temperature of the potato starch-MMT system was greater than in the case with organically modified MMT, which could be explained as a relationship between the dispersion of MMT and the stability of the system as a function temperature [44].

1.6 Environmental Applications

The development of bionanobiocomposite materials systematically and tailor fashion based on layered silicates have offered improved functionality and properties, such as enhanced barrier properties, elasticity, strength, optical clarity and antimicrobial properties, and which make them optimal for a large number of environmental applications in the field of food, agriculture, soil and water treatment, besides in the biomedical field, sensor technology, drug delivery and applied sciences [13, 16, 77, 82]. The main environmental applications of bionanocomposites based on layered silicates are shown below.

1.6.1 Bionanocomposites with Layered Silicates in Soil and Water Treatment

The better adsorption capacity, selectivity and stability of bionanocomposites with respect to nanoparticles give them numerous applications in many areas, such as soil and water treatment among others.

Bionanocomposites prepared from 2:1-type layered silicates and natural polymers by cation-exchange reaction are especially interesting in the removal of anionic contaminants [27]. The interaction between the polymer in the cationic state and the surface of the mineral clay changes the nature of the clay surface from hydrophilic to hydrophobic. Acidic pesticides, at the pH of soil and natural waters, are in an ionic state. This modification with organic cations is a strategy to favor the affinity between the bionanocomposite and the anionic pesticides [104–110].

The concern of heavy metal contamination due to its magnitude, toxicity and non-biodegradability is high. Industrial wastewater derived from paints, pigments, fertilizers, fungicides, the manufacture of metals and batteries are the potential origin of the presence of heavy metal ions in soils and waters. There are many investigations

that show the advantage of bionanocomposites with layered silicates in cleaning polluted waters. So, as examples, CTS/vermiculite biocomposite has the potential to be utilized as an ecofriendly adsorbent for removing Cd(II) and Pb(II) [35]. The ecofriendly novel ALG-Au-mica bionanocomposite was also found to exhibit good adsorption capacity for the adsorption of Pb(II), Cu(II) in single and binary system from aqueous solution [58]. Xanthan gum-glutathione/ zeolite bionanocomposite has been proved to be a promising adsorbent of Ni (II) (85%), Pb (II) (93%) and Congo red (80%) from aqueous solution, Guar gum/bentonite for Pb (II) and crystal violet dye and Xanthan gum/ n-acetyl cysteine-modified mica bionanocomposite for Pb(II), Ni(II) and Cu(II) [58, 65].

Water is considered the most essential natural resource; however, freshwater systems are directly threatened by various human and industrial activities that cause pollution by different agents such as sewage and domestic waste, heavy metals, detergents and soaps, fertilizers, industrial effluents, pesticides, etc.… The use of advanced technology for water purification that is cost effective has been and continues to be the subject of research by the scientific community. The membranes show attractive characteristics due to the minimum energy consumption, easy operation, control and maintenance, great selectivity and permeability, good adaptability, satisfactory performance and stability in various conditions. Important advances have been made in water management with the improvement of the characteristics and performance of membranes prepared with clays and various types of polymers, generally presenting a synergistic effect in the elimination of contaminants. Membranes prepared from different polymers and layered silicates are useful in the removal of heavy metals, dyes, and organic and inorganic contaminants from water, as well as for desalination and as superabsorbents [111]. Mavrova et al. [112] proposed new hybrid process of electro-coagulation/membrane filtration with very good results of Se As, Cu, Pb removal and others metals in the treatment of industrial wastewater. Ma et al. [113] prepared for the first time zeolite-polyamide thin film nanocomposite membranes on a porous polysulfide substrate for use in forward osmosis for water decontamination, reported that incorporation of zeolite-polyamide in the range of 0.02–0.1 wt/v% caused an increase in the permeability of the membrane possibly due to the porous nature of the zeolite. These membranes also exhibit antimicrobial, antifungal and antifouling properties. However, there is a need to improve these membranes such as reduce membrane fouling, improve selectivity, shelf life and permeability. Thermal, mechanical and chemical resistance can be improved and help reduce energy consumption. Research should be focused on assessing profitability of nanomaterials, perfecting techniques for incorporating nanoclay in the polymer matrix and monitor the long-term stability of these membranes. The development of a single effective membrane should be advanced for the largest possible number of contaminants from a specific source [114].

1.6.2 Bionanocomposites with Layered Silicates for Food Packaging

The use of bionanocomposites for food packaging can, in addition to protect food and increase shelf life, due to improved mechanical, thermal and gas barrier properties, be seen as a green solution to the growing problem of food packaging waste disposal on a global scale [115–117].

By varying the quantity of the components of the formulations in the bionanocomposites based on layered silicates, it is possible to improve the stability and biodegradability of the resulting materials. This is especially important for modulating the mechanical properties, but also another important property of food packaging materials, such as their ability as a mass transfer barrier to and from food products, permeability of gases, such as oxygen and carbon dioxide, as well as water vapor, are essential in many food packaging. Applications, such as modified atmosphere packaging and its consideration for the packaging of carbonated beverages, may also be key. Its use can be extended to foods with high moisture content. It can also spread to foods with high moisture content. Barrier functions can be enhanced by incorporating lipids and/or polysaccharides. The incorporation of nanoparticles for the improvement of physicochemical and barrier properties has been investigated [118–120].

The barrier properties of gases that do not interact in nanocomposites depend mainly on two factors: (1) the aspect ratio of the layered silicate particles and (2) the extent of dispersion of the silicate within the polymer matrix [121]. When the nanoclays are used at moderate levels (4–7% by weight) and processed correctly, the degree of dispersion of the layered organoclay is maximized (and consequently an exfoliated morphology is achieved); the barrier properties depend only on the aspect ratio of the particles. In most cases, the reduction of oxygen permeability through the incorporation of organoclay falls in the range of 50–60%, and up to 65% in the case of synthetic fluorinated mica at 4% by weight [122].

Equally interesting results have been obtained for the water vapor permeability (WVP), increasing the permeability linearly with the increase in the proportion of layered silicate in the formulation [123, 124].

The mechanical and functional characteristics of bionanocomposites based on lamellar silicates can present the following advantages for food packaging:

- Improved organoleptic characteristics of foods such as appearance, odor and flavor.
- Reduction of volume, weight and packaging waste.
- Longer shelf life and improved quality of items generally not packaged.
- Control over intercomponent.
- Individual food containers with small particles, such as nuts and raisins.
- They function as carriers of antimicrobial agents and antioxidants.
- Controlled release of active ingredients.
- Materials from renewable resources.
- Biodegradability.

These unique characteristics of nanobiocomposites allow the development of new products in the food industry [125, 126].

1.6.3 Bionanocomposites with Layered Silicates for Agricultural Applications

The high worldwide consumption of plastics for improving agricultural production and crop protection causes a large amount of plastics waste into the environment, which are buried in the ground or burned by farms that release harmful substances. Bionanocomposites prepared with nanomaterial additives such as TiO_2 and MMT have been a good alternative to non-biodegradable plastics since they can be directly disposed of in the soil or in a composting system at the end of their useful life [127, 128].

Some advantages of these nanobiocomposites films are [129]:

- They are biodegradable in the soil by microorganisms such as bacteria, fungi and algae.
- They are degraded by the action of sunlight and water.
- They control the release of active substances such as pesticides and insecticides.
- Controlled degradation allows optimal crop development.
- They have a greater durability against UV, visible and infrared light radiation.
- Materials from renewable resources.

1.7 Conclusion

The possibility of assembly biopolymers with clay minerals affords the preparation of new materials with favorable properties for environmental applications. Clay minerals are well known for their adsorption properties, while biopolymers can be great allies regarding their biocompatibility and biodegradability. The reinforcement of biopolymers with layered silicates produce a better dispersion of the structure and enhanced its mechanical, thermal and adsorption properties without altering the biodegradability. The natural polysaccharides (CTS, ST, ALG or CELL) together with the synthetic PLA incorporating into layered silicates of the smectite group and the synthetic LDH clays have the materials more relevant for environmental applications. Published results highlight the high influence of both types of materials as well as their quantities in their structure and morphologies as well as the need to explore other adsorption parameters and operating conditions. Therefore, a holistic approach must be taken to optimize the use of certain biopolymer/clay nanocomposites for a specific contaminant.

The techniques or methods that have been used for obtaining layered silicates that are functionalized are of vital importance to obtain a correct dispersion of clay materials in the polymeric matrices. Clay nanocomposites have properties and characteristics that are due in the first place to the methods and therefore to the mechanisms used for the modification of layered silicates. As shown in this work, the solution-blending method provides, in general, the best dispersion of clays in the polymeric matrix compared to melt-blending method and is the most widely used in the literature. This can be associated mainly with high agitation power and the low viscosity associated with solution-blending method. From another point of view, melt-blending proves to be the most ecofriendly and viable technique from an industrial perspective while providing good economic potential.

The potential applications of materials based on layered silicate nanobiocomposites in the food packaging industry offer a real alternative to the problem of packaging and waste disposal. In agriculture, the use of these biodegradable and renewable plastic materials is also a way to improve crop production and protection.

Applications of bionanocomposites in soil and water treatment, both in cleaning and desalination, are being studied extensively by materials science researchers due to its promising future. Reported studies have shown that its efficiency and effectiveness in the recovery of organic and inorganic pollutants through adsorption processes are very high. There is a clear trend in the study of the design of effective membranes in comprehensive water treatment. However, most of these applications have been assessed at the laboratory scale, but their use must be extended to industrial applications and large-scale production.

The potential applications of materials based on layered silicate nanobiocomposites in the food packaging industry offer a real alternative to the problem of packaging and waste disposal.

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Chapter 2
Chitosan/Poly (Ethylene Glycol)/ZnO Bionanocomposite for Wound Healing Application

Zahra Emam-Djomeh and Mehdi Hajikhani

2.1 Introduction

The rapid growth of science and technology has led us to see new achievements in the field of biomedical every day. Recent studies have identified polymers as an important member of the biomedical sciences used in various fields and various forms [1, 2]. In general, polymers can be classified into two groups: biopolymers and synthetic polymers [3]. Synthetic polymers have advantages such as easy production, processability, and lower cost than biopolymers [4]. Common synthetic polymers include polyethylene, polypropylene, polytetrafluoroethylene, and polymethyl methacrylate [5]. The next group of polymers is biopolymers that originate from living organisms [6, 7]. In simpler terms, these polymer compounds are biological molecules. These biomolecules can be of plant origins such as corn or beans, animal origin, or even bacterial origin [8]. Biopolymers can be classified as follows. Based on monomeric units, they can be divided into the following three categories: The first group is polynucleotides, which are long polymers and consist of 13 nucleotide monomers or more. The first group includes RNA and DNA. The second group is polypeptides, which are short polymers of amino acids. The second group includes collagen, actin, and fibrin. The last group is polysaccharides, which are polymeric structures of carbohydrates, which generally have a linear structure. The third group includes starch and cellulose [9].

Biopolymers can also be classified into five categories based on their origin, including [9, 10]: The first category includes polyesters such as polylactic acid and

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polyhydroxyalkanoates. The second category includes proteins, which are divided into two categories, plant, and animal. In the case of plant proteins, gluten and zein can be mentioned, and in the case of animal proteins, we can mention collagen, gelatin, elastin, and serum albumin. The third category includes polysaccharides, which are divided into four subcategories. The first subcategories contain bacterial polysaccharides that include xanthan, dextran, dellan, levan, curdlan, polygalactosamine, and cellulose. Fungal polysaccharides such as pullulan and elsinan glucans are divided into the second subcategories. The third subcategories belong to polysaccharides of plant or algal origin, including starch, cellulose, agar, alginate, carrageenan, pectin, konjac, and various gums. Finally, animal polysaccharides such as chitin and hyaluronic acid are divided into the fourth subcategories. The fourth category includes lipids/surfactants, among which are acetoglycerides, waxes, and emulsions. The last category includes polyphenols, which include lignin, tannin, and humic acid [11]. Over the last decade, numerous studies by researchers on wounds and wound healing have shown the importance of this issue. Biocompatibility, biodegradability, non-toxicity, and non-allergenicity are considerations for polymers used in wound healing research [12–14].

Zinc oxide is an inorganic compound that has various applications in industry. This substance is found naturally, but most of it is produced synthetically [15]. It is also used in many skin diseases [16]. Various studies have shown that topical application of zinc oxide regenerates epithelium, reduces infection, and heals wounds [17]. Zinc oxide also accelerates wound healing [18]. In this chapter, we have tried to introduce different properties of two polymers, chitosan, and polyethylene glycol, which are among the common polymers used to prepare different types of wound dressings. Biocompatibility, availability, reasonable price, and processability have led to these polymers being considered in wound dressing. Also, the production of bionanocomposites from chitosan/PEG containing zinc oxide nanoparticles for wound healing has been studied in this chapter.

2.2 Chitosan

The main component in the exoskeleton of insects and crustaceans such as shrimp is composed of chitin [19]. Chitin is an abundant mucopolysaccharide, which ranks second after cellulose in terms of biosynthesis per year [20]. However, less attention has been paid to chitin than cellulose [21]. As shown in Fig. 6.1, chitin is a large, structural polysaccharide made from glucose-modified *N*-acetyl-glucosamine chains [22]. Chitin is converted to a copolymer called chitosan by alkaline deacetylation [23]. Chitosan is a linear polysaccharide consists of 2 acetamido-2-deoxy-D-glucopyranose (acetylated unit) and 2-amino-2-deoxy D-glucopyranose (deacetylated unit), which they randomly distributed by β-(1 → 4) linked [24]. Chitosan has many structural similarities to cellulose, but unlike cellulose, it is considered a new source. Chitosan is insoluble in water and organic solvent. However, it is soluble in dilute aqueous acetic, lactic, malic, formic, and succinic acids (acidic solution

pH < 6.5) [25]. Unlike most commercial polysaccharides (such as starch, cellulose, dextran, pectin, agar, and agarose) that are neutral or negatively charged (acidic), chitosan has a polycationic structure and is known as a weak base. Chitosan at neutral or base pH contains free amino groups that prevent the solubility of this polysaccharide in water, whereas these amino groups in glucosamine units are protonation at an acidic pH and cause the solubility of chitosan in water [26]. In general, the solubility of chitosan in water depends on the distribution of free amine and N-acetyl groups in the molecule. Differences in chitosan (polycationic structure) electrostatic properties at pH < 6.5 make this polysaccharide interact with anionic polysaccharides such as carrageenan and alginate. This tendency to interact with other negatively charged molecules, such as proteins, fatty acids, bile acids, and phospholipids, also applies [27]. Recent studies show that chitosan tends to chelate metal ions such as iron, copper, cadmium, and magnesium [28]. In biomedical research, natural materials are always preferred to synthetic materials due to their high compatibility. Properties such as biocompatibility, non-toxicity, biodegradability, and antibacterial effect have made chitosan an interesting polymer in biomedical applications [29]. Numerous studies have been published on the effect of chitosan on accelerating wound healing. Studies have shown that chitosan regenerates wound tissue by stimulating homeostasis [30]. In recent years, chitosan has been proposed as an ideal polymer in the fabrication of tissue engineering scaffolds because *N*-acetylglucosamine, the monomers that make up chitosan, is common in hyaluronic acid, which is an extracellular macromolecule that plays an essential role in tissue repair [31]. On the other hand, chitosan polysaccharide can be degraded by some human enzymes such as lysozyme, so its biodegradability properties apply [32]. Various methods have been developed for the preparation of chitosan nanocomposites, some of which are mentioned below (Fig. 2.1).

The physical method in the preparation of polymer composites is the simplest method that has been widely used in the last two decades [34]. In this simple method, two or more polymers are mixed to modify properties such as mechanical properties, permeability, biodegradability, antimicrobial properties, and other properties, and the resulting composite has properties between the constituent polymers [34]. Using this method, the properties of polymers can be directed to the required direction based on the need. This feature is a significant feature of the industry. The most common methods of preparing polymer composites include cast polymer molding, annealing method, and extrusion method [35, 36]. Nanotechnology has been used to improve the properties and create more properties of composites and has led to the manufacture of nanocomposites with unique properties. Numerous studies have been conducted in recent years to prepare chitosan nanocomposites and use them in various fields such as food packaging [37], medical applications [38], drug delivery [19], wound dressing [39], preparation of membrane filters [40], and other applications [41].

Numerous methods such as photochemical methods, radiolysis, ultrasound, and application of electrospray technology can be used to synthesize nanoparticles required to prepare nanocomposites and homogenize them in the chitosan matrix [42]. Silver nanoparticles have been used for antioxidant and antibacterial properties in nanocomposites prepared from chitosan or chitosan combined with other

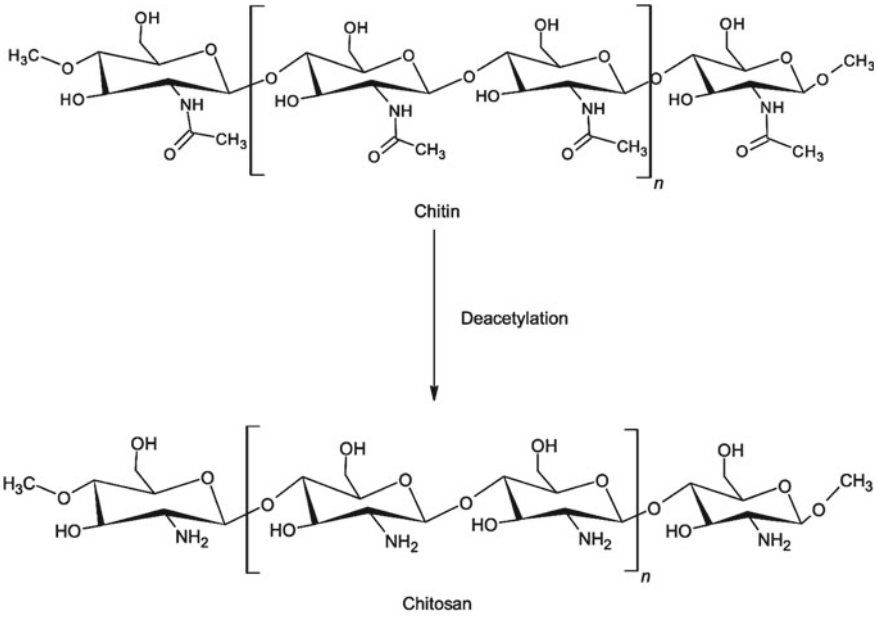

Fig. 2.1 Chemical structure of chitin polymer which is converted to chitosan polymer by deacetylation [33]

polymers [43]. Zinc oxide is also known as another common nanoparticle used in nanocomposites prepared for wound healing [44]. The critical point in preparing nanocomposites is how to prepare nanoparticles and how to add them to the polymer matrix. The nanoparticles used must have a normal distribution in their particle diameter. Uniformity in size leads to uniform release patterns and better performance of nanocomposites [45]. They also play an important role in the dispersion of nanoparticles in the polymer matrix. In order to disperse nanoparticles properly in the polymer matrix, the concentration of the polymer solution should not exceed a certain limit, and the nanoparticles should first be properly homogenized in a secondary solution and then added to the polymer solution to homogenize properly. The use of physical methods such as ultra-turrax homogenizers and ultrasound is another way to homogenize nanoparticles. If nanoparticles have non-uniform sizes, it is more difficult to homogenize them in nanocomposites [46].

In an innovative method published by Qiu et al. [47], chitosan and zinc oxide flexible nanocomposites were prepared (Fig. 3.2). In this study, zinc ions were dispersed in a chitosan polymer solution and then prepared by the chitosan/zinc film casting method. The film's treatment with sodium hydroxide at a high temperature (80 °C) causes zinc oxidation and conversion to ZnO. Microscopic examination and AFM showed good dispersion in zinc oxide nanoparticles. Studies have shown that ZnO nanoparticles were successfully synthesized in situ in chitosan films. The main purpose of this work is to provide an easy and green way to make chitosan/ZnO

nanocomposite films with good compatibility and antibacterial properties, which shows applications as antibacterial packaging and dressing [47].

The most important property of chitosan that is highly regarded is the antimicrobial properties of this polycationic polymer. The molecular weight of chitosan, the degree of acetylation, the degree of substitution, and the properties of the microorganism wall are among the influential factors in the antimicrobial power of chitosan [48]. Nanocomposites made of chitosan polymer have a positive surface charge that bonds better to the mucosa [49]. A positive surface charge causes chitosan to attach to the cell wall of microorganisms negatively charged by phosphate groups in phospholipids [50]. The amino groups in chitosan bind to the microorganism wall, causing a change in cell permeability, resulting in osmotic disruption by the influx of ions and proteins between the cytoplasm and extracellular space, and ultimately inhibiting microbial activity [51]. The main factor in the antimicrobial activity of chitosan is the positive charge density of this polymer and its electrostatic interaction with the negative charge of the cell wall surface of microorganisms [52]. Other properties such as the degree of deacetylation and degree of substitution affect the positive charge density of the polymer [53]. Since many free amine groups are available in the polymer backbone, polymer modification and preparation of chitosan nanocomposites have shown acceptable results [54]. Recent studies have shown that a decrease in pH and molecular weight can exacerbate the antimicrobial properties of chitosan by considering the degree of protonation [55]. This fact means that the higher the degree of protonation, the lower the pH, and the lower molecular weight of chitosan should be used to achieve antimicrobial properties [56]. Other studies on chitosan have shown that low molecular weight in chitosan has a more significant antimicrobial effect on gram-negative bacteria than gram-positive bacteria [57] (Fig. 2.2).

Numerous studies have been performed on the positive effect of chitosan on wound healing. For example, hydrogels made from chitosan and polyacrylic acid on rats showed that wounds healed within 14 days, and regular cell formation was seen [58, 59]. Further results showed that chitosan increases adhesion between cells and therefore accelerates healing [60, 61]. The surface properties of the chitosan films can be modified with the help of different treatments that create different applications for this biopolymer [54]. For example, the chemical modification of chitosan with stearoyl increases protein adsorption due to increased hydrophobicity.

On the other hand, the chemical modification of chitosan with phthalic/succinic anhydride increases the absorption of lysozyme due to increasing the hydrophilicity of this biopolymer [59]. Studies also show that the use of heparin stimulates growth factor-related healing at the wound site and accelerates wound healing [62]. Chitosan-sericin nanofibers were prepared using electrospinning technology to investigate their effect on wound healing [63]. A polymer solution is charged under very high voltage in the electrospinning technique and flies to the opposite pole. The polymer solution undergoes many twists during the movement between the two poles, which causes it to stretch and narrow the small flow of the polymer [64].

For this reason, nanofibers are formed with a very small diameter, and the solvent evaporates rapidly due to the ratio of surface to the large volume of nanofibers (Fig. 2.3). The resulting scaffolds are usually much more effective than films due

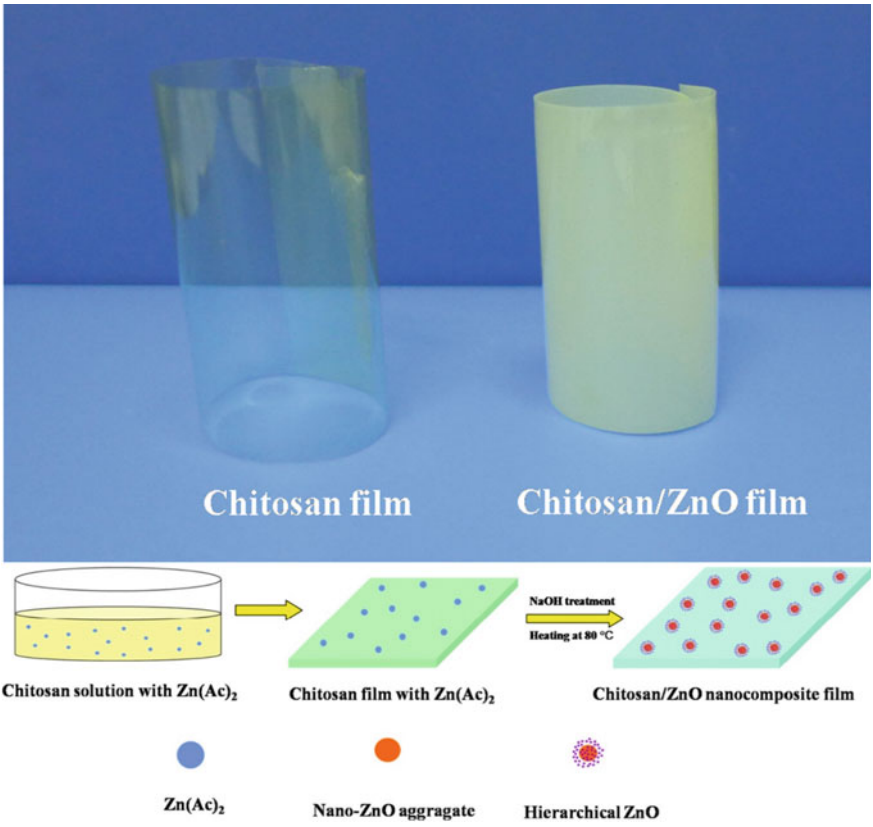

Fig. 2.2 Structure of the chitosan film containing zinc oxide nanoparticles is shown in the vicinity of the control chitosan film. Also, the steps of chitosan nanocomposite treatment with sodium hydroxide to oxidation Zn nanoparticles within the chitosan matrix can be seen [47]

to the high surface-to-volume ratio [65]. For example, chitosan–sericin nanofibers have been shown to have high antimicrobial properties against gram-positive and gram-negative bacteria [63]. Alginate and chitosan nanocomposites along with silver sulfadiazine nanoparticles show acceptable performance in wound healing [59]. It was observed that if the alginate content to chitosan is 50%, the release of nanoparticles will be longer and more common, and therefore, the effectiveness of the wound dressing will be longer [59, 66]. The alginate and chitosan nanocomposite had sufficient swelling ability and showed good compatibility with cells [59]. The effect of this nanocomposite on fibroblast cells in mice and humans did not show any signs of adverse effects and caused the formation of fresh epithelium, which leads to wound healing. Chitosan alginate scaffolding showed antimicrobial effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

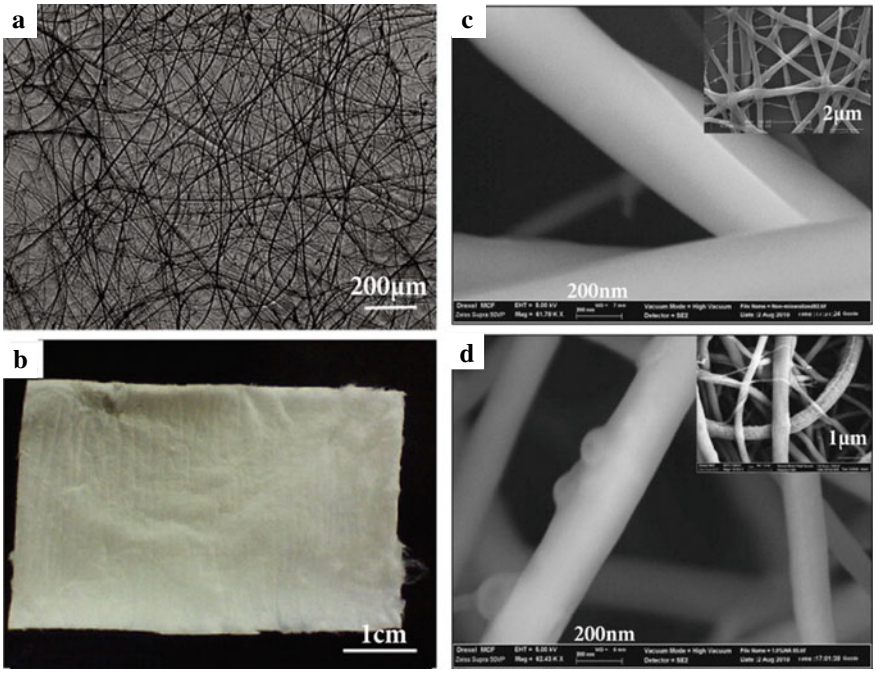

Fig. 2.3 **a** Macroscopic image, **b** fibrous mat, and **c** and **d** structure and dispersion of chitosan scaffold nanofibers for use in tissue engineering [67]

Further studies also indicate the high compatibility and non-toxicity of this nanocomposite [59]. In another study, a chitosan hydrogel patch containing encapsulated silver nanoparticles was investigated. Chitosan hydrogel is highly swollen, effectively increases blood clotting efficiency, and non-toxic [68]. This hydrogel showed a good effect against *S. aureus* and *E. coli* microorganisms [69]. Having suitable mechanical properties such as elasticity is one of the properties of this hydrogel [59]. Other nanocomposites studied include gelatin–chitosan scaffolds and polyvinyl alcohol–chitosan nanocomposites with similar results in antimicrobial, biocompatibility, and non-allergenic properties [70].

Zinc oxide nanoparticles and castor oil in chitosan film were used to evaluate the effect of inhibiting gram-positive and gram-negative bacteria. Results showed that the wound dressing and antibacterial properties have properties such as water absorption, biodegradation, biocompatibility, and wound healing [71]. The results showed that increasing the concentration of zinc oxide nanoparticles increases the inhibitory power of nanocomposites against the growth of microorganisms. Plant extracts such as *Juglans regia* and *Salix alba* encapsulated in chitosan hydrogel were studied to evaluate the effect on wound healing. Studies have shown that the compounds obtained from the above plants positively affect wound healing, but due to the lack of inhibition of microorganisms, there is a possibility of infection in the wound [59].

Chitosan/*Aloe vera* nanocomposite was proposed as a suitable option due to its anti-inflammatory and moisturizing properties [72]. The resulting nanocomposite showed higher efficiency and had more adhesion to wound surface fibroblasts. In uncomfortable wounds, chitosan/*A. vera* nanocomposite can be used as a good dressing [73]. In other studies, adding bioactive substances such as lupeol, cinnamonic acid, salicylic acid, phenols, and sulfur on the growth inhibitor of microorganisms was investigated [59]. Insolubility or low solubility in water at biological pHs is considered to be the biggest problem of chitosan. Therefore, many efforts have been made to modify the properties of chitosan through the chemical modification of this biopolymer. Carboxymethyl chitosan, trimethyl chitosan, and carboxymethyltrimethyl chitosan are among these modified polymers widely used in targeted drug delivery systems to improve solubility and water absorption properties [74, 75].

Further studies have identified modified chitosan as a suitable dressing due to its strong cationic properties derived from trimethylation derivatives [76]. This chitosan-derived biopolymer also showed excellent mucosal adhesion and a good drug loading effect [77]. The binding of the carboxymethyl moiety to the chitosan backbone increases the solubility and biocompatibility of the nanocomposite. The addition of the carboxymethyl group causes the polymer to have good solubility in a wide range of pH and creates an excellent gel-forming ability [78].

Chitosan/polyethylene oxide scaffold was fabricated using the electrospinning technique to investigate the effect on wound healing. Scanning electron microscopy evaluation showed that with an increasing amount of chitosan, the diameter of nanofibers increased, and also the elongation strength of the scaffold decreased, but increasing the amount of chitosan increased the tensile strength. On the other hand, the amount of polyethylene oxide determined the solubility of the scaffold. Bioburden studies showed that the 2:1 ratio of polyethylene oxide and chitosan has a sufficient inhibitory effect against the growth of *S. aureus*. This polymer ratio also showed good compatibility in in vivo tests and caused fibroblast proliferation in mice [79, 80].

In 2015, Shahzad et al. examined the complex of polyvinyl alcohol, hydroxyapatite, and chitosan in the preparation of nanofibers, hydrogels, mats, and films [81]. It was observed that the morphology of the polymer complex changes under heat treatment. SEM studies have shown that the polymer matrix has a porous texture. It was also found that hydrogel has higher water absorption in phosphate buffer salt solution at pH 7.4 than other forms. Studies of encapsulation drug release showed that lyophilized hydrogels did not have a suitable drug release pattern. Microbial studies showed that polymer composites have suitable antimicrobial activities against *E. coli* and *S. aureus*. At the end of the study, it was concluded that the composite of polyvinyl alcohol, hydroxyapatite, and chitosan with high biocompatibility, proper drug release, and high microbial inhibition capacity has a high potential for use in the manufacture of various wound dressings [81].

The high viscosity of the chitosan solution makes it difficult to use this polymer in the production of scaffolds with electrospinning technology because the polymer has low spinning capacity due to its high viscosity and does not tend to form jets during the process [82]. Various solutions have been introduced to overcome this

problem in chitosan, including reducing the polymer solution concentration, using modified chitosan, and using a copolymer with chitosan [83]. The most suitable way is to use polymers with high spinning properties in the mixture with chitosan, which causes the physical properties of the chitosan polymer solution to be modified and spinning [84]. Different copolymers such as polyethylene oxide and polyvinyl alcohol have been used to produce scaffolds with chitosan in various studies [85]. The results showed that high compatibility between polymers is observed in nanofibers, and nanofibers' morphology is appropriate. The use of copolymers increases the mobility of ions and increases the electrical conductivity by reducing the viscosity of chitosan. Electrical conductivity is considered one of the most important physical properties of the polymer solution in the electrospinning process and has a high impact on the morphology and diameter of nanofibers. By reducing the diameter of the nanofibers, the surface-to-volume ratio in the scaffold increases and improves the efficiency of the wound dressing in attaching to the tissue [82]. Encapsulation of wound healing compounds in nanofibers causes the release to be done from a larger surface and in the form of a linear and controllable pattern [86].

In 2013, Archana et al. developed a chitosan/polyvinyl pyrrolidone nanocomposite with titanium oxide nanoparticles [87]. Thermodynamic properties, chemical structure, were investigated by differential scanning calorimetry and Fourier transform infrared spectroscopy. Observation of the microstructure with the help of SEM reported the proper dispersion of titanium oxide nanoparticles in the nanocomposite. Nanocomposite mechanical strength studies showed that titanium oxide nanoparticles have a direct effect on increasing tensile strength. The study of the effect of nanocomposites to inhibit the growth of pathogens had a very positive result. The use of nanocomposites in the wounds of albino mice to study the effectiveness of wound healing showed that the chitosan/polyvinylpyrrolidone nanocomposite with titanium oxide nanoparticles has excellent biological compatibility against fibroblasts and accelerates wound healing. High water vapor transfer rate, good compatibility, excellent antimicrobial properties make this nanocomposite ideal as a wound dressing [59, 87].

Gels consist of two phases, liquid and solid, which in the case of hydrogels, the liquid phase consists of water [88]. Hydrogels have many applications for use in living tissues due to their high water content [89]. The hydrogel's high flexibility and soft structure make it possible to tolerate the hydrogel on the damaged tissue for a long time, while the adjacent tissues suffer the least damage [90]. Chitosan does not require any additives to form a gel and can form a gel network by inhibiting repulsion between amino groups in its chain and creating hydrogen bonds and hydrophobic interactions [91]. Hydrogel scaffolds have received a great deal of attention in the field of tissue engineering in the last decade due to their biodegradability, high biocompatibility, high drug delivery ability, and controlled release. Wang et al. [92] produced honey/gelatin/chitosan hydrogels and evaluated the properties of this product. The antimicrobial activity against *S. aureus* and *E. coli* was completely successful, and the hydrogel was safe against the growth of microorganisms. The effect of hydrogel on the healing of burn wounds during 12 days was investigated, and MEBOVR ointment was used as a control sample [92]. Honey/gelatin/chitosan hydrogels were

found to accelerate the healing of burn wounds, but these results were not repeated in the control sample. Histological studies showed the non-toxicity of this hydrogel to wound tissue [59]. Therefore, it could be concluded that this hydrogel has a high potential for use as a wound dressing due to its appropriate effect on wound healing, non-toxicity, biocompatibility, and biodegradability [92, 93]. Lih et al. tested poly (ethylene glycol)/tyramine/chitosan hydrogels along with hydrogen peroxide and horseradish peroxidase for use as tissue adhesives [94]. It was observed that polyethylene glycol can increase the solubility of this biopolymer through cross-linking with chitosan [94]. The study on the mechanical properties of hydrogels showed that by creating these transverse pins, the adhesion property was strengthened between 3 and 20 times. The study of the effect of hydrogel on the wound for two weeks showed that polyethylene glycol increases the hemostatic properties of chitosan and accelerates wound healing [59, 94].

The casting method is known as a simple method in designing various nanocomposites. This method uses a high proportion of solvents, which are generally organic [95]. For example, sodium carboxymethylcellulose (CMC) and propyl-3 trimethylammonium chitosan chloride (HTCC) nanocomposite films were prepared in combination with PVA *N*-(2-hydroxyl) [96, 97]. This nanocomposite's physicochemical and mechanical properties were evaluated, and it was concluded that hydrogen bonds bonded together with the polymer matrix. It was observed that moisture permeability, mechanical properties, swelling properties, and water absorption of composite films change significantly with changes in CMC content [97]. Thus, increasing CMC permeability to water, the percentage of swelling and water absorption capacity increased, and mechanical properties such as flexibility and tensile strength were strengthened [96]. Antimicrobial studies on nanocomposite films showed good antimicrobial activity. In a similar study, the properties of chitosan/bentonite nanocomposites were investigated. The films were evaluated for physicochemical properties, including WVTR, water absorption capacity, flexibility, and thickness. The interaction of positively charged chitosan and negatively charged bentonite was also investigated by FTIR and film surface morphology using SEM. It was observed that the hydrophilic nature of bentonite increases the water absorption capacity and mechanical strength of the films [98]. Antibacterial studies examined the positive effect of films on the growth of gram-positive and gram-negative bacteria and found that the films potentially inhibited the growth of microbes, making them ideal for use in wound dressings [98]. Another study conducted in 2015 by Archana et al. [99] examined the physicochemical, mechanical, thermal, and microbial properties of chitosan/polyvinyl pyrrolidone films along with silver oxide nanoparticles. Due to silver nanoparticles and chitosan polymer, it had high antimicrobial activity for nanocomposites, and the films also had a high swelling capability. In vivo studies of the wound showed that the film containing silver oxide nanoparticles had a higher wound healing ability than the pure chitosan film, which the author identified a synergistic ability between silver nanoparticles and the chitosan polymer [99].

The use of organic solvents to prepare films by the casting method makes it possible to prepare the desired polymer matrix easily and even use polymers with different polarity properties in a polymer solution. The high ability to dissolve

different polymers makes it possible to produce countless different composites. However, the toxicity of these solvents is one of the limiting problems of this method. Solvent residue in the final dressing is an important point that is rarely mentioned in studies, but due to the rapid absorption and high toxicity of these compounds must be considered. For example, in the preparation of dressings from composites containing collagen, the hexafluoro-2-propanol solvent is used, which is highly toxic and carcinogenic [100]. Various other forms such as membranes, sponges, and emulsions with chitosan polymer have been studied for use in dressings with the aim of accelerating the wound healing process. In addition, loading of bioactive compounds into chitosan composites yielded acceptable results such as high encapsulation efficiency and a suitable release pattern, which introduces chitosan as a suitable polymer for use in dressings.

2.3 Poly (Ethylene Glycol)

Polyethylene glycol is a polyether compound derived from petroleum with many applications in various industries, including the medical industry [101]. This polymer is named depending on its molecular weight. When the molecular weight of this polymer is less than 20,000 g/mol, it is called polyethylene glycol or PEG, but when the molecular weight is higher than 20,000 g/mol, it is called polyethylene oxide or PEO. Polyoxyethylene or POE is another name for PEO [101, 102]. As shown in Fig. 3.4, PEG structure is usually expressed as $H(OCH_2CH_2)_nOH$, which is the average repetition of oxyethylene groups in the polymer chain, and n can vary from 4 to 180 [103]. The properties of this polymer vary depending on its molecular structure, but they are all jointly soluble in water. Other properties of polyethylene glycol include non-toxic, odorless, neutral, lubricating, non-volatile, and non-irritating properties that can be used in many pharmaceutical products [104]. PEG, for example, can reduce protein uptake and prevent the formation of the protein corona, which is critical in drug delivery systems [105]. PEGylation or pegylation is another application of this polymer in which PEG is covalently or non-covalently bonded to a macromolecule such as a protein [106]. This phenomenon keeps the drug functional groups hidden from the immune system and prevents immune and allergic responses. It also increases the hydrodynamic size of the drug in solution, which reduces drug clearance due to increased circulatory time [107]. Also, with the help of this technique, hydrophobic drugs can be converted into the water-soluble form [107] (Fig. 2.4).

Various studies have shown the solubility of polyethylene glycol in water, ethanol, dichloromethane, acetonitrile, and benzene. Also, this polymer is insoluble in hexane and diethyl ether [109]. The polyethylene oxide molecule has a semi-crystalline structure and is easily soluble in water due to its high affinity for hydrogen bonding with the water molecule [110]. High solubility and adhesion properties have led to the use of polyethylene oxide as an essential material in the design of bioadhesive and mucoadhesive systems, especially in drug delivery systems and wound healing

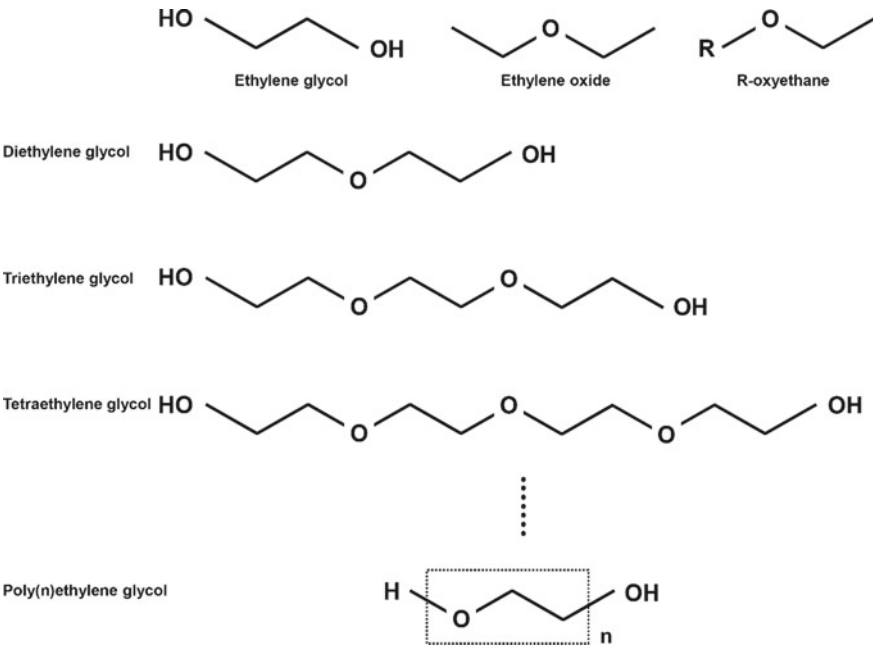

Fig. 2.4 Different forms of ethylene glycol polymerization [108]

[61]. The big problem in using PEO is the lack of sufficient mechanical strength and hydrophobicity properties [111]. Numerous studies have been published on polymer composite with PEO in which attempts have been made to modify this polymer's physicochemical and mechanical properties [112]. In this regard, conducted studies on the properties of chitosan/polyethylene oxide composites [112]. It was observed that a 50/50 ratio of two polymers reduces the tendency to spherulitic crystallization. As a result, the transparency of the films is reduced, and the films are seen as opaque. The study on the mechanical properties of chitosan/polyethylene oxide film showed that the addition of chitosan is significantly effective in improving mechanical properties such as tensile strength (TS), elongation (E), and puncture strength (PuSt). Also, by doubling the ratio of chitosan to polyethylene oxide, PuSt and TS increased by 80% and 56%, respectively. The barrier properties of the film were also tested. Compared to PEO control films, the results are a tenfold increase in water vapor permeability (WVP) in chitosan/polyethylene oxide films [113].

The reason for this is the compactness of the film structure and the better placement of the polymer chains in the PEO in a special region. However, the addition of secondary polymers disrupts the order in PEO polymer chains and increases the intermolecular distance, so water vapor permeability should be increased. Finally, the author described the presence of chitosan as essential for the mechanical properties of PEO [113, 114]. Polyethylene glycol is known in biomedicine as an antifouling polymer [115]. According to research, PEO, with its surface hydration ability and

steric hindrance effect, prevents sedimentation. PEG is attached to the surface of the material from one side, and due to its high solubility in water, it increases the solubility of materials in water [116]. Polyethylene glycol is widely used to prepare dressings due to its biocompatibility, low toxicity, hydrophilicity, flexibility, and non-immunogenic properties [117]. PEG macromers can bind to growth factors such as epidermal growth factor, which delivers this polymer to the wound site. PEG-based dressings have been widely used to heal diabetic wounds by strengthening and inducing skin cell growth and collagen deposition [5]. Acceleration of wound healing by collagen deposition also reduces scar formation. The biggest problem in making a dressing from this polymer, as mentioned above, is its poor mechanical properties, which can be eliminated with the help of a copolymer [118]. Combining PEG with other polymers significantly enhances the mechanical properties of PEG-based dressings and even alters the thermodynamic properties of this polymer. Improving thermodynamic properties can be helpful in drug delivery systems by encapsulating bioactive compounds that are sensitive to environmental conditions [119].

In another study by Rengifo et al. [120], chitosan/polyethylene oxide nanofibers were used to treat skin cancer. Carboxymethyl-hexanoyl chitosan and dodecyl sulfate nanoparticles were used in the preparation of nanofibers (Fig. 2.5). Pyrazoline was loaded into the nanoparticles as an anticancer agent and then added to the polymer solution. Morphological studies showed a homogeneous structure of nanoparticles within chitosan/polyethylene glycol scaffolds. The release pattern of nanoparticles and pyrazoline was investigated for 120 h. It was observed that when carboxymethyl-hexanoyl chitosan and dodecyl sulfate nanoparticles are used as carriers, the rate of pyrazoline transport through the epidermis should also be increased. Therefore, chitosan/polyethylene oxide scaffolds can be used for controlled drug release in topical chemotherapy for skin cancer [120].

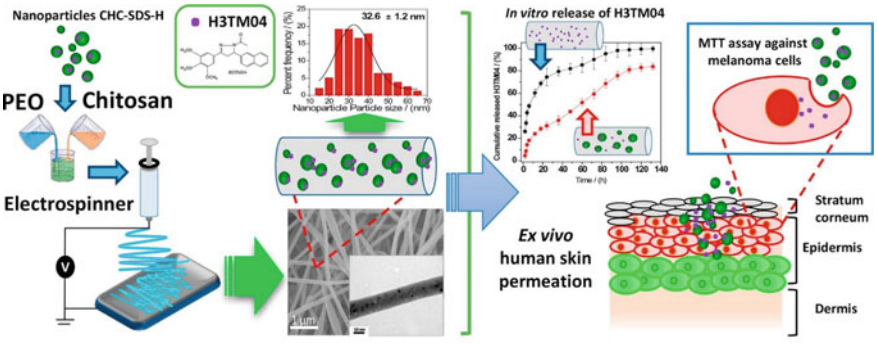

Fig. 2.5 Steps for preparing a scaffold from a polymer mixture of chitosan and polyethylene oxide. These nanofibers contain carboxymethyl-hexanoyl chitosan/sodium dodecyl sulfate nanoparticles loaded with pyrazoline to treat skin cancer [120]

2.4 Chitosan/Poly (Ethylene Glycol)/ZnO Bionanocomposite

Zinc oxide is an inorganic compound, insoluble in water and the form of a white powder. The chemical formula of this substance is ZnO, and it is used as an additive in countless products such as food, medicine, and even building materials [121]. Zinc oxide is a natural form of zinc in nature, but most of it is produced in synthetic form worldwide [122]. ZnO is considered a non-toxic and non-allergenic substance that is used in skin and eye care products. The use of ZnO in skin wounds has been shown to inhibit IgE secretion and suppress allergic reactions [123]. The effects of ultraviolet light absorption and refraction have been demonstrated by zinc oxide nanoparticles, which is why zinc oxide nanoparticles are used in sunscreen products. Recent studies have reported the toxic effects of high doses of zinc oxide nanoparticles on human skin [124]. The study showed that if ZnO nanoparticles are too small, it disrupts intramolecular biomolecules, causing protein unfolding, fibrillation, thiol cross-linking, and ultimately loss of enzymatic activity [125]. Zinc oxide can inhibit the growth of microorganisms through the process of photoinduced oxidation. Numerous studies have shown the positive effect of ZnO against the inactivation of gram-positive and gram-negative bacteria [126]. The use of ZnO and other metals also improves the efficiency of solar energy and enhances the antibacterial effect of other metals [127]. For example, the effect of Fe/ZnO nanowires in comparison with ZnO and TiO_2 against the growth of *E. coli* was studied [128]. Their results showed that ZnO/Fe nanowires have higher photocatalytic activity than pure ZnO. The addition of cobalt to Fe/ZnO nanoparticles increased the particle size and showed antibacterial activity against four strains of bacteria. Attractive properties of zinc oxide cause it to be used in the form of nanoparticles in polymer composites to improve the film's functional properties such as mechanical, barrier, and antibacterial properties [129].

The attractive properties of zinc oxide due to its application in the form of nanoparticles in polymer composites improve the film's functional properties such as mechanical properties, barrier, and antibacterial [130]. Liu and Kim [131] studied the properties of genipin-cross-linked chitosan (GC) and polyethylene glycol (PEG) composites along with zinc oxide and silver nanoparticles on wound healing. It was observed that at high doses of nanoparticles, there is a good dispersion in the composite. The swelling properties of the nanocomposite were completely pH-dependent, which is due to chitosan. On the other hand, the nanoparticles significantly improved the mechanical strength of the nanocomposite. It was observed that with increasing ZnO content, the antimicrobial activity of the films increased [131]. The antibacterial activity of GC/PEG nanocomposite samples was tested in the form of containing and without nanoparticles against the bacterial species *E. coli*, *P. aeruginosa*, *S. aureus*, and *Bacillus subtilis*. It was observed that GC/PEG composite films containing zinc oxide and silver nanoparticles had higher antibacterial activity than GC/PEG nanocomposite films without nanoparticles and GC/PEG containing zinc

oxide [132]. This antimicrobial effect is intensified by silver, which is due to the doping of zinc oxide with silver [133].

In 2020, Sithique and Sakthiguru [134] investigated the physicochemical properties of carboxymethyl chitosan nanocomposites and zinc oxide and lawsone intending to be used as wound dressings. The chemical properties and bonding between nanoparticles and carboxymethyl chitosan were investigated by Fourier transfer infrared spectroscopy (FT-IR). The amorphous and crystalline properties of the nanocomposite were investigated using X-ray diffraction (XRD). As shown in Fig. 2.7, the microstructure of the nanocomposite and the distribution of nanoparticles in the film were investigated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Finally, thermogravimetric analysis (TGA) was used to analyze the temperature resistance of the film. Encapsulation and release patterns in phosphate buffer saline were investigated, and it was observed that the nanocomposite has efficient properties in encapsulation and release. In vivo studies showed no signs of allergenic properties, and the nanocomposite had high biocompatibility. Microbiological studies showed high antimicrobial properties for nanocomposites. These biological properties indicate that the carboxymethyl chitosan/zinc oxide/lawsones nanocomposite has a high potential for acting as an excellent biological material in wound healing applications [134]. In a recent study by Masud et al. [12], the bionanocomposite properties of cross-linked polyethylene glycol with sodium tri polyphosphate containing chitosan-ZnO nanoparticles were investigated to act on wound healing. The antibiotic gentamicin was used as a widely used substance in preventing wound infection and assisting wound healing in bionanocomposite, and the release of this antibiotic was investigated. Cytotoxicity analysis was performed on Vero cells and BHK 21 cells, and both confirmed the biocompatibility results of the bionanocomposite. Studies on antimicrobial properties have reported the ability of bionanocomposites against the growth of *E. coli* and *Salmonella enterica.* In vivo evaluation showed that drug-containing bionanocomposite has better therapeutic properties than natural dressing and drug-free bionanocomposite due to the synergistic effect of drug and ZnO nanoparticles (Fig. 2.6). Bionanocomposite showed an optimal loading efficiency of 76% with a drug concentration of 300 ppm, which is a significant efficiency among similar studies. High compatibility, biodegradability, high resistance to microorganism growth, good release pattern, and high encapsulation capability make this bionanocomposite suitable for use in wound dressings [12].

In another study by Preethi et al. [135], *Solanum lycopersicum* leaf extract was loaded into a chitosan/zinc oxide nanocomposite. Characteristics of the obtained nanocomposite were examined by various tests such as visible, ultraviolet spectroscopy, X-ray diffraction (XRD), field irradiation scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), and scattered energy X-ray spectroscopy (EDS). Chitosan/zinc oxide nanocomposite showed significant antibacterial activity against *S. aureus*-induced skin infection, making this nanocomposite an ideal wound dressing. In addition, chitosan/zinc oxide nanocomposite was studied for antibacterial activity against *S. aureus*, *B. subtilis*, and *E. coli*, which showed acceptable results in inhibiting the growth of microorganisms [135].

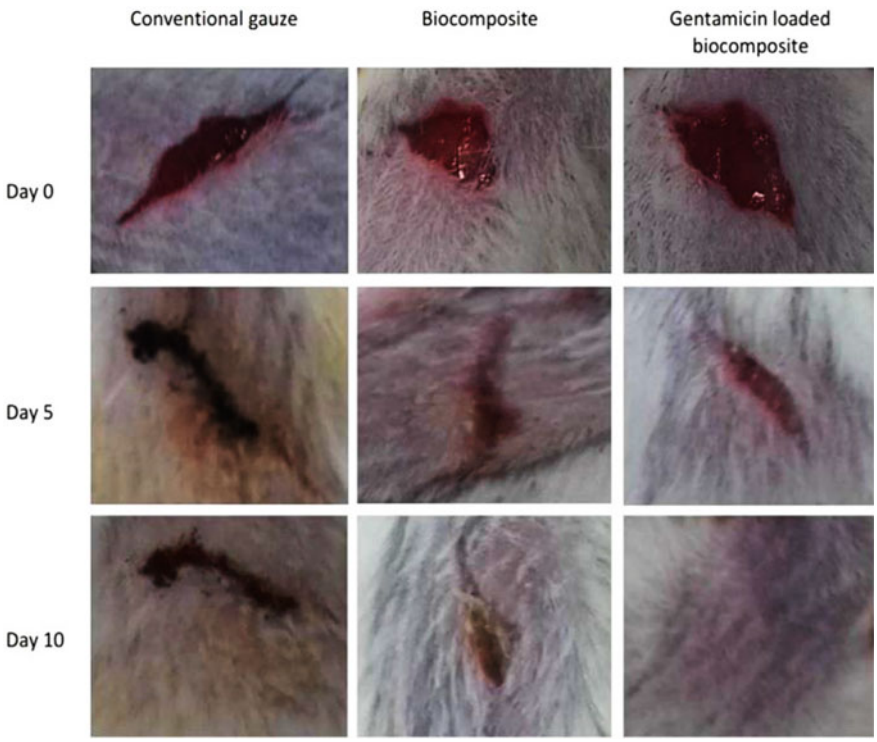

Fig. 2.6 Demonstration of effect of chitosan/polyethylene glycan nanocomposite containing zinc oxide nanoparticles on wound tissue repair in an animal model [12]

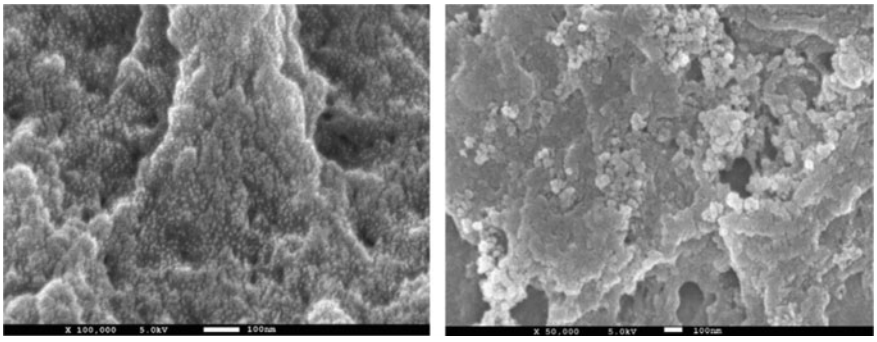

Fig. 2.7 Dispersion of zinc oxide nanoparticles in the chitosan/polyethylene glycol bionanocomposite by FESEM [12]

Teicoplanin-containing PEO/chitosan scaffold was developed by Amiri et al. [136] using electrospinning technology. The purpose of this scaffold was to create antibiotic-loaded drug delivery systems to overcome several antibiotic-related limitations. The antibiotic-loaded scaffold can be applied topically for skin and wound healing and post-operation implants to prevent abdominal adhesion and prophylaxis and treat infections in orthopedic surgery. The author recommends doses of 2 and 4 wt% teicoplanin for bead-free nanofibers. The antibiotic release study showed that the scaffold was able to release teicoplanin within 12 days. This long period of time increases the lifespan of the wound dressing and makes it cost-effective to use due to the small number of dressings required in a treatment period. Antibacterial testing against *S. aureus* showed that loading teicoplanin into the chitosan-PEO nanofibers maintained the antibiotic's antibacterial activity and increased it by 1.5–2 times. Cellular studies did not show any toxicity against human fibroblasts. In addition, an in vivo study of a wound model in a rat confirmed the efficacy of teicoplanin-loaded nanofibers. It was observed that a significant improvement in wound closure was created, especially with nanofibers containing 4% teicoplanin [136]. The pattern of sustained-release, intensified drug activity, cell compatibility, and significant wound healing activity confirm the potential applications of teicoplanin-rich nanofibers in wound healing and topical antibiotic delivery [137].

2.5 Wound Healing Application

The skin is the largest organ in the body that protects the body from damage caused by external stress [64]. The skin protects our body from microorganisms, helps regulate body temperature, and creates a sense of touch, heat, and cold. As shown in Fig. 2.8, the skin generally consists of three layers: the epidermis, the dermis, and the hypodermis [138]. As the name implies, the epidermis layer is located in the most superficial part of the skin and creates the color of the skin [139]. This layer also provides waterproof insulation of the skin. Below the epidermis is the dermis layer, made up of connective tissue and contains hair follicles and sweat glands. Finally, the last layer, the hypodermis, is the deepest layer of the skin [140]. This layer consists of adipose tissue and connective tissue. Skin pigmentation is caused by special cells called melanocytes that produce melanin pigment [141]. Melanocytes are located in the epidermal layer, so the epidermal layer is responsible for skin color [142].

The process of replacing damaged or destroyed tissue with new tissue is called wound healing. In intact skin, the epidermis and dermis provide a protective barrier against the external environment [144]. Damage to the skin creates a series of biochemical events to repair the damage. Blood clotting (homeostasis), inflammation, tissue growth (cell proliferation), and tissue regeneration (cell maturation and differentiation) are the four stages of wound healing [145]. In the homeostasis stage, which is in the first minutes of injury, blood platelets begin to stick to the affected area [146]. In this phase, the platelets become amorphous to better participate in the structure of the clot, and also in this state, it creates a signal that causes the secretion

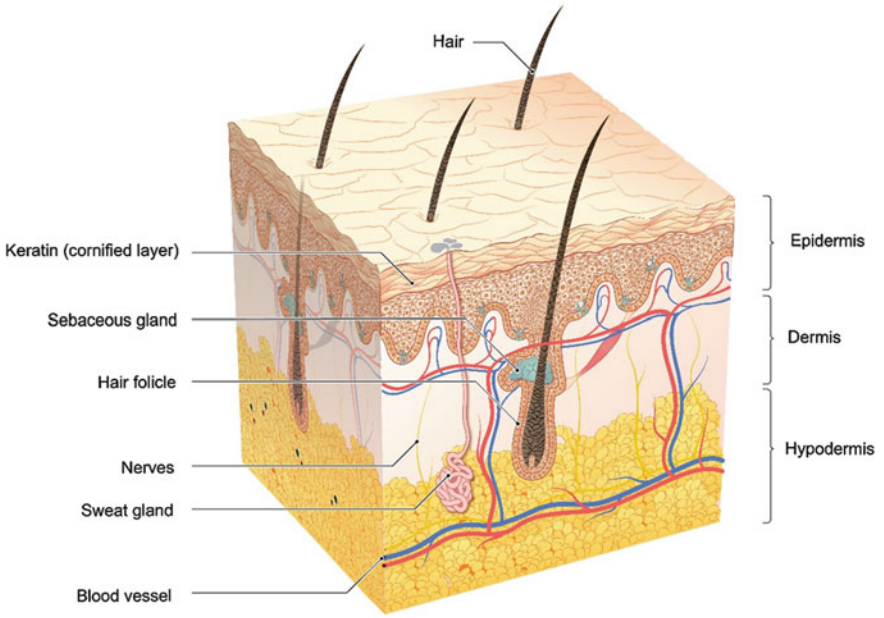

Fig. 2.8 Different layers of skin in a cross section of human skin [143]

of fibrin, and the fibrin acts as an adhesive and strengthens the structure of the clot [147]. The hemostasis phase causes blood clots to form to reduce the severity of the bleeding and stop it [148]. Inflammation stage white blood cells by phagocytosis swallow damaged or dead cells along with bacteria and other pathogens [149]. At the end of this phase, platelet-derived growth factors are released into the wound, causing cells to migrate and divide into the next stage, the proliferative stage [150]. In the reproduction stage, which is the most complex stage, different processes occur together. Angiogenesis or regeneration of blood vessels is the stage that causes the regeneration of new vascular endothelial cells [151]. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new temporary extracellular matrix by secreting collagen and fibronectin [152, 153]. At the same time, epithelial cells proliferate and move to the wound surface to re-epithelialize the epidermis [153]. In the contraction phase, which has a mechanism similar to that of smooth muscle cells, myofibroblasts reduce the size of the wound by gripping the edges of the wound and contracting [154]. Cells that have fulfilled their role suffer from cell death or apoptosis. During puberty and regeneration, which is the final stage of wound healing, collagen is adjusted along the stretch lines, and cells that are no longer needed are removed with programmed cell death or apoptosis [155]. Numerous biopolymers are used today for wound healing, including chitosan and polyethylene glycol. Chitosan, which is known as an antimicrobial polymer, has several other properties in wound healing.

Chitosan activates leukocytes and macrophages for phagocytosis and the production of IL-1, TGF-β, and PDGF [156]. This biopolymer also stimulates cell proliferation, which in turn accelerates wound healing [157]. Stimulation of fibroblast proliferation is another advantage of using chitosan as a dressing [119]. The degree of chitosan acetylation is considered a significant factor in fibroblast stimulation [158]. In order to modify and optimize the properties of chitosan, various copolymers have been used in the production of scaffolding, hydrogels, films, and nanocomposites. The combination of chitosan with other biopolymers, in addition to modifying the properties of this polymer, has shown promising results in wound healing. For example, study on the performance of carboxymethyl chitosan in patients undergoing plastic surgery showed that chitosan promotes better-organized skin tissue and reduces abnormal healing [7]. The chitosan and polyethylene glycol complex also improved chitosan properties by intensifying adhesion, protein adsorption, and stimulating cell growth [159]. Several studies have been performed on diabetic wound healing and burns in mice. Surface binding of growth factors to chitosan and binding of EGF and bFGF to chitosan were observed, improving wound healing by increasing wound contraction and increasing epithelialization, respectively [160]. The polycationic properties of chitosan make it an ideal carrier for encapsulating drug compounds. The addition of curcumin to chitosan increased collagen synthesis in vitro and in vivo [161].

Zinc is an essential trace element in the human body of great importance in times of health and illness [162]. Zn acts as a cofactor in various transcription systems and is also involved in zinc-dependent enzyme systems, including metalloproteinases, which increase the autoimmune degradation and migration of keratinocytes during wound healing [163]. Zinc protects the cell against reactive oxygen species and bacterial toxins through its antioxidant activity [164]. Zinc's antioxidant activity is probably mediated by cysteine-rich metallothioneins, eventually leading to resistance to epithelial apoptosis [165]. It has been observed that delays in wound healing are caused by a lack of zinc in the diet or hereditary reasons [163]. Zinc protects the cell against reactive oxygen species and bacterial toxins through its antioxidant activity [166]. Zinc's antioxidant activity is probably mediated by cysteine-rich metallothioneins, eventually leading to resistance to epithelial apoptosis [167]. It has been observed that delays in wound healing are caused by a lack of zinc in the diet or hereditary reasons [163]. Further studies have shown that topical zinc administration is more effective in reducing infection than oral administration because it strengthens local defense systems, collagenolytic activity, and continuous release of zinc ions, ultimately stimulating wound epithelialization in normoglycemic individuals [18]. The use of zinc oxide in wound dressings reduces skin inflammation and soothes wounds [18].

Metallothioneins (MT) can complex with intracellular zinc up to 20%. MT is a family of proteins attached to minor metals highly protected and rich in cysteine [168]. This protein is considered for zinc homeostasis, protection against oxidative stress, and buffer against toxic heavy metals [169]. MTs regulate gene regulatory molecules and zinc stores within the cell and protect cells from the damaging effects of exposure to high zinc levels [170]. Each MT molecule can bind to seven zinc

molecules [171]. The addition of zinc ion supplementation causes fragmentation in many biochemical and molecular events related to wound healing through over-regulation of MTs and metalloenzymes. Also, defective mRNA encoding of growth factors is usually associated with the expression of finger zinc transcription factors that impair wound healing [18]. Atomic absorption spectroscopy and immunohistochemical techniques can be used to show the quantitative and qualitative distribution of zinc in skin wounds. These techniques can be used to determine zinc-binding proteins such as MT [18].

Differential regulation of the MT gene is performed by a family of proteins called interleukin-1 (IL-1), a group of 11 cytokines. IL-1 creates a complex network of proinflammatory cytokines regulating and initiating inflammatory responses by expressing integrins on leukocytes and endothelial cells [172]. This mechanism may, to some extent, cause a significant increase in zinc in the early inflammatory stage of wounds. An initial increase in zinc levels is associated with high MT in marginal keratinocytes, macrophages, and cutaneous fibroblasts [163]. In later stages, the population of epidermal basal cells increases, while this increase coincides with MT deposits [173]. In the final stages of wound healing, the zinc level in the wound decreases, which is associated with decreased mitotic activity and scar maturation [163]. Demonstrations of zinc metalloenzymes such as alkaline phosphatase, RNA and DNA polymerases, and MMP can be used to demonstrate evidence for the functional role of zinc in repair systems [174]. Alkaline phosphatase, which is present in the early stages of angiogenesis, is a sensitive marker for small cutaneous blood vessels and increases inflammatory activity and connective tissue proliferation [175]. DNA polymerases also act as precise markers of cell proliferation [18].

Matrix metalloproteinases (MMPs), also called matrixes, are calcium-dependent metalloproteinases zinc endopeptidases [176]. MMPs themselves are subsets of a family of proteases called the metzincin superfamily. About 25 MMPs with similar human characteristics have been identified so far, all of which have an *N*-terminal hydrophobic domain, a propeptide domain, and a catalytic zinc-binding domain [176]. The catalytic domain of MMPs consists of a slot containing a catalyst with a tight connection to Zn^{2+} to which the substrate is initially attached before cleavage [177]. This domain also has an additional Zn^{2+} structure [18]. MMPs generally have various proteolytic properties and are capable of destroying all components of the extracellular matrix [178]. MMPs react with a wide range of protein and glycoprotein substrates, including cytokines, cytokine receptors, adhesion molecules, and latent MMPs [179]. MMPs are synthesized under specific conditions and by different cell types in the wound [180]. Keratinocytes in wound margins, macrophages, fibroblasts, and endothelial cells usually synthesize MMPs differently under the influence of extracellular matrix–cell contact and soluble intermediates [181]. MMPs are synthesized as the inactive proenzyme or cysteine residue [182]. The proenzymes form a predomain to cover the catalytic site. Disruption of this predomain exposes zinc ions to catalytic binding to the substrate [177]. Among the MMPs, some are involved in wound healing, including collagenases, stromelysin, gelatinase A, and gelatinase B. Collagenases, including MMP-1, MMP-8, and MMP-13, break down the triple

helix collagen [180]. MMP-2 and MMP-9, which contain gelatinase A and gelatinase B, destroy interstitial and denatured collagen, subcutaneous type IV collagen, and gelatin. Stromelicins, including MMP-3 and MMP-10, contain a wide range of substrates in the wound bed [183].

Zinc is directly involved in regulating the mechanism of action of MMPs in wound destruction, proteolytic characterization, modulation of cell migration, and regeneration of extracellular matrix. The inherent mechanisms of regulation of most endogenous enzymes depend on zinc ions [184]. The integrin genes α2β1, α3β1, α6β4, and αvβ5 are responsible for keratinization and keratinocyte migration in the skin; the expression of these genes requires the presence of zinc ions [185]. Zinc can modulate epithelialization in skin wounds through the expression of integrin in keratinocytes [163]. Zinc supplementation induces the α2, α3, αv, and α6 integrin subunits, which affect the movement of keratinocytes during the recovery phase [186, 187].

Recent studies in rats have shown that topical zinc therapy effectively reduces wound debris and promotes epithelialization in surgical wounds [163]. It was concluded that topical application of zinc and zinc oxide reduces wound residues and necrotic material in wounds caused by various causes by contributing to the activity of MMPs. Conversely, inhibition of MMP due to lack of zinc ions significantly delays wound healing [163, 188, 189].

Further studies in mice showed that reducing zinc ions from the diet delayed wound healing in laboratory mice compared to regular diets [190]. However, this reduction in zinc in the diet did not deplete the zinc stores in the skin unless zinc was wholly removed from the rats' diet for a long time. It was observed that collagen biosynthesis due to the reduction of zinc in the diet of mice occurs at a slower rate [191]. The decrease in zinc ions also disrupted the function of metalloproteinases, which cleave the peptide of procollagen molecules, and affected collagen synthesis [192]. By adding zinc to the mice's diet, the wound healing process returned to normal. It was observed that the use of topical zinc oxide regardless of the status of zinc in the diet of mice healed the wound within 12 days [163]. Improvement of epithelialization by zinc supplementation treatment can be concluded from different perspectives [163]. Involvement of zinc ions in DNA polymerase in mitosis is observed through increased nuclear MT in wound peripheral keratinocytes and active mitotic cells of the basal epidermis [193]. Another reason is the ability of zinc to regulate endogenous growth factors, which mimics the function of growth factors by increasing intracellular mitogenic signaling pathways [187]. For example, zinc ions increase insulin-like growth factor I, which can increase epithelialization [163]. The following reason is probably due to the properties of zinc ions in protecting the cell against oxidative stress and bacterial toxins, which have an anti-apoptotic effect on the epithelium [126]. It was observed that zinc increased locally in keratinocyte proliferation in adult rat wounds by about 30% compared to resident epidermal keratinocytes and localized zinc oxide [163].

Numerous studies have been performed to investigate the effect of zinc ions on non-skin lesions such as gastrointestinal wounds (stomach and intestines) [194]. Increasing the dose of zinc in serum collagen deposition in the gastrointestinal wound,

especially anastomotic wound, should increase significantly. In a similar study, daily zinc intake in rabbits was investigated to affect colon anastomosis healing. It was observed that 2 mg/kg body weight of zinc ion daily could increase fibroblast penetration and increase epithelialization [195]. Regarding gastric wounds in rats, it was observed that zinc deficiency could affect mucosal regeneration, and increasing the amount of zinc ions improved the wound of normal and diabetic rats compared to the control samples [18]. The function of zinc ions in all stages of wound healing is summarized in Fig. 2.9.

Today, zinc is used in various forms to heal various wounds. The standard form of zinc consumption is topical and used in zinc chloride, zinc sulfate, and zinc oxide. Further studies further elucidated the antimicrobial properties of zinc [196]. Topical application of zinc significantly reduced the use of oral antibiotics compared to oral therapy. It was also observed that *S. aureus* could grow in wounds treated with zinc oxide significantly less than untreated samples [197, 198].

Numerous studies have been performed on zinc's antimicrobial properties, and it has been found that this substance has an inhibitory effect against the growth of some microorganisms. The growth ability of gram-negative microorganisms is higher than gram-positive in the presence of zinc ions, but the dose of growth inhibitor is different in different microorganisms [126]. The inhibitory effect of zinc ion on *S. aureus*, *Streptococcus*, *E. coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Enterococcus*, and *P. aeruginosa* was investigated, and it was found that zinc ion can inhibit the

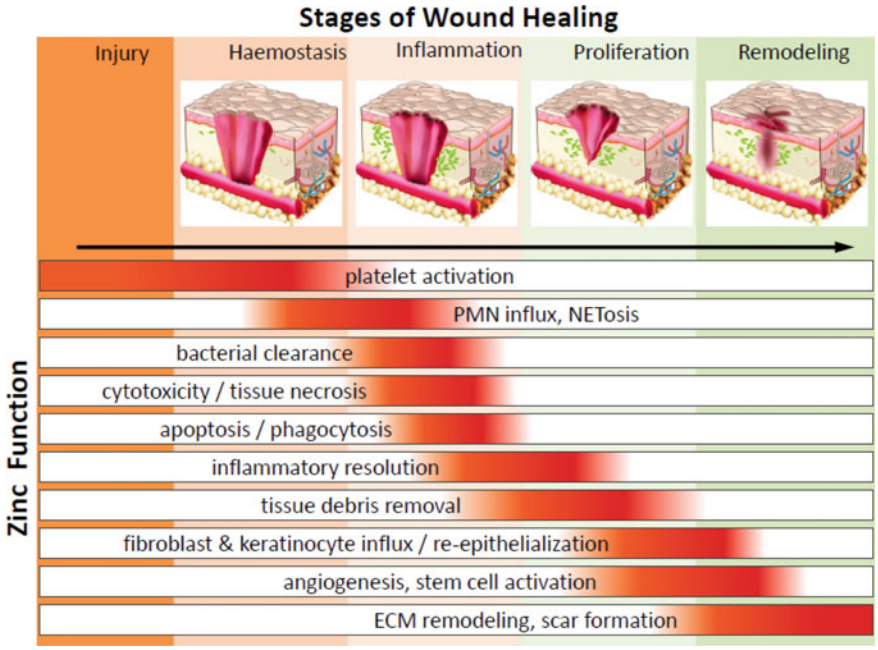

Fig. 2.9 Function of zinc ions in all stages of wound healing [163]

growth of all organisms in sufficient concentration [199, 200]. Zinc oxide also had an inhibitory effect against *S. aureus* and an aerobic and anaerobic endodontic pathogen [201]. Zinc is naturally thought to be an essential regulator of macrophages and multinucleated leukocytes in the bone marrow, thymus, and tissues with high cell turnover [202]. Antioxidant activities of zinc ions against sulfhydryl groups have been reported [167]. The use of different forms of zinc in various dressings such as biofilms, scaffolds, hydrogels, and nanocomposites has shown high effectiveness in wound healing, anti-inflammatory and antimicrobial properties, which makes zinc a suitable option for use in dressings [203]. A significant point about zinc, which causes the widespread use of this compound in skin treatment products, is the rarity of zinc allergy in people using this substance [163, 204].

2.6 Conclusion

All of the above points to the increasing importance of chitosan polymer in designing efficient dressings in wound healing. Properties such as abundant availability, strong antimicrobial properties, biodegradability, and biocompatibility can be seen in chitosan dressings. Among these, polyethylene glycol was proposed as an inexpensive, biocompatible, low toxic, flexible, non-immunogenic properties and available polymer to modify the properties of chitosan, which can improve the solubility of chitosan in water. Also, the mechanical strength of polyethylene glycol is strengthened in combination with chitosan. Increasing the solubility in water in the chitosan/polyethylene glycol composite improves the encapsulation properties of the polymer matrix, so different compounds are easily dissolved and dispersed in this matrix. It has been shown that PEG macromers can bind to growth factors such as epidermal growth factor that deliver this polymer to the wound site, so the application of this polymer in the delivery systems of healing compounds seems to be very useful. The information mentioned above about the effect of zinc ion on wound healing makes this mineral ion a suitable option for use in wound healing. Therefore, chitosan/polyethylene glycol nanocomposite containing zinc oxide nanoparticles is introduced as an ideal option in dressing preparation.

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Chapter 3
Preparation and Applications of Chitosan–Gold Bionanocomposites

Rishabh Anand Omar and Monika Jain

3.1 Introduction

The advancement in new technologies, industrial development and dramatic population explosion has resulted in the environment pollution. As a consequence, a sharp enhancement in the utilization of freshwater has been experienced in the last few decades leading to the generation of huge amount of wastewater. The effluents from various sources (industries, dye houses) are directly discharged into the ambient environment without any treatment. A survey indicated that due to consumption of freshwater, the gross per capita availability of freshwater would decrease in the coming 30 years [1]. Approximately, partial population of the world would face the infirmity and crises caused due to freshwater storages and generation of huge amount of wastewater [2]. The main cause behind the steady downturn of quality of water is the discharge of various heavy metals and dyes which are emitted directly into the environment from industrial productions of various things. Effluent from numerous industries such as paint, leather, textile and paper making is the key source of heavy metals, dyes and other toxic pollutants. Heavy metals and dyes can find their way into human body through food chains and accumulate there. Accumulation of these contaminants is toxic to human brain cells, growing foetus and may also lead to other diseases such as anaemia, gastric dysfunction, brain damage, bone softening and cancer [3]. Therefore, it has become the need of the hour to develop such materials which could remove these harmful contaminants from water before discharging

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into the environment so that the treated water can be recycled and reused for various other purposes.

In the past, several wastewater treatment methods have been developed, viz. advanced oxidation [4], membrane filtration [5], coagulation [6], adsorption [7] and biodegradation [8]. Among them, the adsorption has been contemplated as the most efficient method for treating wastewater due to some remarkable advantages such as cost-effective operations, highly efficient, diverse and simple design [9]. In order to treat heavy metal and dye-laden wastewater, various adsorbents such as porous organic polymers (POPs), carbonaceous materials, clays, biopolymers like chitosan, cellulose and lignin have been successfully fabricated and modified efficiently for their application in wastewater treatment [10].

Recently, chitosan and its derivatives (metal nanoparticle-doped chitosan composite) have been used successfully in heavy metal and dye removal applications as a different class of adsorbents because of their characteristics such as high surface area, surface hydrophilicity, surface electronegativity, cation exchange selectivity and cation exchange capacity (CEC). These properties have developed a keen interest among the researchers to use the metal-chitosan composites in environment remediation applications [11, 12].

Chitosan is a derivative of chitin, formed by deacetylation of chitin (Fig. 3.1). It is mainly composed of two subunits, namely deacylated D—glucosamine and acylated *N*—acetyl—D—glucosamine. These two subunits are joined together by β (1–4) linkages. The beauty of the chitosan lies in the fact that it has abundant amino and hydroxyl groups which forms strong bonds with heavy metals and dyes via co-ordination bond. This amino group of chitosan gets protonated at low pH and interacts with anionic pollutants via electrostatic interactions. In recent years, several studies have been carried out on the removal of heavy metals and dye contaminants from wastewater using chitosan and its derivatives as an efficient adsorbent. Thus, chitosan-based nanocomposites such as foams, films, hollow fibres and hydrosols could be used as an efficient material for the wastewater treatment applications. Chitosan has some unique properties of, such as biocompatibility, excellent film formation and good mechanical strength due to which it is widely used in various applications like wastewater treatment [13].

Removal of heavy metals and dyes using chitosan and metal-doped chitosan nanocomposite has been exhaustively explicated in this chapter. Firstly, a brief introduction to the various studies structure and applications of heavy metals and dyes removal using chitosan and chitosan metal/gold nanocomposite is presented. Then different procedures for synthesis of chitosan and chitosan–gold nanocomposite are given. In continuation with that, various application and mechanism of the chitosan–gold nanocomposites for the removal of heavy metal and dye are explained. This chapter provides brief information on the removal of dyes and heavy metals using the chitosan and chitosan–gold nanocomposite.

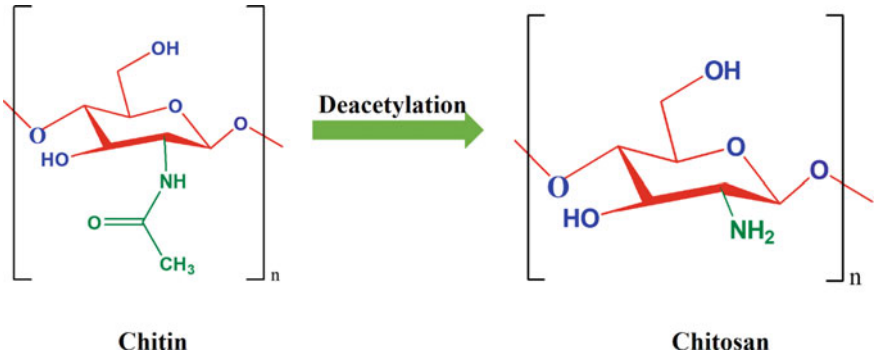

Fig. 3.1 Structures of chitin and chitosan

3.2 Chitosan–Gold Nanocomposite

A functionalized polymer such as chitosan incorporated with any inorganic nanoparticle is called polymer nanocomposite. Polymer nanocomposite represents a tremendous pathway to achieve benefits from two constituents in one entry. Also, sometimes polymer nanocomposite personifies the new features which are not present in its components due to the formation of a new product [14]. These tempting properties of polymer nanocomposites open up a wide variety of implementation in various fields such as waste management, treatment of wastewater, catalysis, energy, drug delivery, biomedical, semiconductor and others [15–21].

In recent studies, nano-sized reduced gold nanoparticles (AuNPs) showed outstanding catalytic activity in mild physical parameters like pressure and temperature, because of their tiny size, which provides high surface-to-volume ratio and high chemical potential [22–25]. AuNPs also showed some drawbacks in past studies, which include mainly (i) In solution, AuNPs encounter several hassles during the actual applications, (ii) AuNPs have high surface energy, which leads to instability and aggregation, resulting in remarkable reduction in their catalytic activity [26, 27], (iii) at last, the AuNPs cannot be recaptured and reused, which makes them cost-inefficient for large industrial applications [28].

Incorporation of these NPs into a solid matrix for a support could be the best solution for these problems. They provide a competent way to remove the NPs from the spent solution for their reusability, which makes the whole process cost-efficient [29]. In addition, the hybrid composite (matrix/AuNPs) gains some unique properties because of combining nanoparticles to solid matrix structures. These properties are not present in individual components of the composite.

In past studies, several solid matrices have been developed by doping the nanoparticles as nanocatalyst, like metal oxides [30], carbonaceous such as carbon beads, nanofibers and nanotubes [31–33], graphene oxides [34, 35], sand [36], silica [37] and polymers [38]. The polymeric materials as a matrix have been widely used due to several reasons including, (i) a variety of their structures which makes the surface

of the material multifunctional, (ii) ease of preparation in different forms such as hydrogel, aerogels, fibres and films, (iii) easy to separate from the spent reaction medium and (iv) at the last, cost efficiency.

3.3 Preparation Strategies for Chitosan–Gold Nanocomposite

Synthesis of chitosan-based nanocomposites is simple and quite successful because it uses certain blending agents such as chemical agents, aqueous extract and some biological reducing agents. In nanocomposites synthesis, sometimes the components of the nanocomposite (NPs or matrix) are synthesized individually and then all the components are mixed to achieve the final product as a nanocomposite. But in others, a final nanocomposite (metal-polymeric product) is achieved by a single step process. This section discusses the different methods of preparation of chitosan–gold nanocomposite. Figure 3.2 briefly describes various methods of synthesis of chitosan–gold nanocomposite.

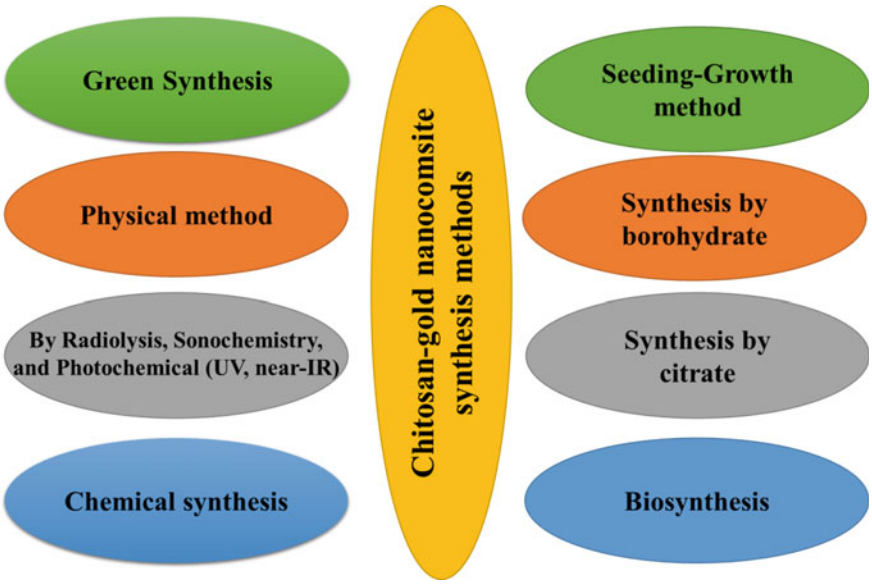

Fig. 3.2 Various methods of synthesis of chitosan–gold nanocomposites

3.3.1 General Synthesis

Generally, the nanocomposites can be synthesized through common dry and wet methods. The polymeric material and the nanoparticles can be used as a nanocoating material that is coating of a nanoscale layer on the solicited substrate to get the specific/desired surface performance [39]. There are many different kinds of physical and chemical strategies, such as electrochemical, thermal decomposition, laser ablation and microwave irradiation for the synthesis of nanocomposites [40, 41]. Among all the synthesis methods, chemical synthesis is widely used because it does not require any harsh conditions such as high temperature and pressure. It also does not require any sophisticated conditions and is environmentally safe, low cost, nontoxic, less energy requirement, therefore also called green synthesis [42–46]. In this kind of synthesis, the metal salts could be reduced by a chemical reducer such as sodium borohydride ($NaBH_4$).

3.3.2 Physical Methods

Another method for the fabrication of polymer-based metal nanocomposite is physical method that is mostly accomplished by combining of two or more polymers, leading to a new product which is a blend of both the materials but with different physical properties [47]. The mechanical, structural, chemical, morphological, biological properties can be optimized in blending method to produce new materials leading to economically favoured products. An additional benefit of blended material is that it develops unique properties which are required in many individual polymers. Also, the process is time-efficient [48]. Various compositions of starting material result in the product with different dynamic properties. There are several physical methods for the synthesis of chitosan–gold nanocomposites [49]. A gelation method has been employed for the fabrication of hydrophobic polymers using ion by Alonso and co-workers (1997). In this method, trivial synthesis array of two aqueous phases has been involved. One phase circumscribes chitosan and polyethyleneoxide and the second one is TPP (polyanion sodium tripolyphosphate). The zeta potential and particle size of nanocomposites of chitosan are found in between +20 to +60 mV and 200–1000 nm, respectively.

3.3.3 Radiolysis, Sonochemistry and Photochemical (UV, Near-IR) Strategies

Another set of methods involves the photochemical synthesis of nanoparticles such as photochemical degradation and light-mediated degradation of a metal salt through radiolysis. The radiolysis can control the size of NPs during the synthesis AuNPs

[50]. Size and shape of Au nanoparticles could be enhanced using UV-irradiation especially when used in combination of micelles [51]. Near-infrared laser irradiation results in the formation of thiol-stabilized Au nanoparticles [52]. Sonochemistry is widely used for the formation of Au nanoparticles inside the silica pores [53]. Laser photolysis can be used for the formation of Au nanoparticles in form of micelles of block copolymer [54]. Small AuNPs can be produced by power ultrasound method. Approximately 5-nm-sized NPs can be synthesized through surface of pre-prepared silica sub-microspheres. In sonochemical reductions, ultrasonic irradiation at room temperature, argon gas is required for the formation of gold nanoparticles [55].

3.3.4 Chemical Synthesis

Metal-containing nanoparticles synthesis of was carried out through various techniques including thermal decomposition, electrochemical irradiation and chemical reduction through green chemistry techniques [56–60]. Huang et al. [39] fabricated a nanocomposite of chitosan and metal in aqueous solution using sodium borohydride ($NaBH_4$). Green synthesis is on priority in solvent system due to its eco-friendly nature and forms a non-toxic preservative at the time of synthesis [61]. In addition, Wei et al. have reduced the silver nitrate ($AgNO_3$) salts using biodegradable and non-toxic chitosan (Cts) in Cts-based silver NCs [62]. Huang et al., have developed a sustainable method to eliminate $NaBH_4$, thus making a "greener" method in comparison with other approaches. Chemical methodologies are pivotal in synthesizing nanocrystals of different materials [63]. For these methods, the primary need is mild conditions. Uniform size and shape is a necessary factor which decides the greatness of synthetic methodologies of synthesized nanoparticles. Various reductants, like hydrogen peroxide sodium hydroxide, sodium citrate, trisodium citrate, ascorbic acid and sodium borohydride, are capable to reduce trivalent Au (III) ions to nascent Au (0) [64–66]. Source of energy for this reduction process is mainly ultrasound irradiation, photoirradiation, or heating in the presence of surfactants, water-soluble polymers and reducing/capping agents. This method provides remarkable uniformity in size and shape of scattered particles. Furthermore, the surface-modifying agents and capping controllers reduce aggregation of nanoparticles and support colloidal stabilization [64]. NPs show improved colloidal stability upon covalent bonding of ligands at the surface of the nanoparticles.

3.3.5 Reduction by Borohydride

Sodium borohydride ($NaBH_4$) is an effective, intensive, low cost and broadly used reducing agent. $NaBH_4$ is widely used for the reduction of silver nitrate by ice-cold to synthesize silver NPs. $NaBH_4$ is needed in a very high amount for the ionic silver reduction for the stability of prepared NPs. Graphene oxides were directly prepared

from graphite using Hummer's method [67, 68]. However, the AuNPs modified graphene synthesis is possible by $NaBH_4$ reduction procedure. Chemical reduction in aqueous organic solvent of metallic ions is the basic need for synthesis of metal NPs [69, 70]. $NaBH_4$ reduction method is also broadly used in the AuNPs synthesis. In another way, some other reducing agents such as superhydride and hexadecylaniline have also been used for Au(III) reduction, in the formation of thiol-stabilized AuNPs [71, 72]. These synthesis methods are efficient but some impurities of boron and difficult to reproduce (mainly in liquid medium) in the final product are still a major issue allied with the reduction of borohydride [73].

3.3.6 Citrate Reduction

In this method, AuNPs are prepared by adding sodium citrate of chloroauric acid solution at its boiling stage [55]. Citrates are generally used to prepare AuNPs with a property of citrate stabilization. However, due to these NPs showed various disadvantageous. Some of the recent studies suggested that reducing solutions of Au/citrate with $NaBH_4$ can maintain the average core size (>10 nm) of nanoparticles. The method is efficient for producing metal NPs, however, having some drawbacks mainly: nanoparticles cannot be stored in solid state because it is difficult to isolate them from the solution. The other one is variation in ionic strength, or pH slightly affects the stability.

3.3.7 Synthesis by Seeding Growth Technique

Seeding growth method is an old technique, used since many years. Approximately, 5 to 40-nm-sized NPs can be prepared using this technique. Size of nanoparticles is manageable by altering the proportion of metal salts and seed [74]. Step-by-step enlargement of particle is more efficient process than one-step-seeding due to the formation of secondary nucleation [75]. Seeding growth technique was successfully used mainly for gold nanorods preparation [76]. The technique involves a growth solution prepared using a feeble reducing agent, a surfactant and Au(III) salt, which is used as a commanding agent that is cherished with pre-prepared "seed" nanoparticles [77].

3.3.8 Biosynthesis Technique

Biosynthesis method uses mainly vitamins, carbohydrates, enzymes, biopolymers and microorganisms, between others. AuNPs have also been synthesized using biological method in some studies [78]. In one-pot synthesis method, achieved

particle size depending on the concentration of reducing agent as in chitosan oligosaccharide, ratio of chitosan and gold should be maintained to synthesize different size particle, chitosan molecular weight also being a dominant factor in size and shape distribution of nanoparticles.

3.4 Applications of Chitosan–Gold Nanocomposites

Chitosan–gold nanocomposite was used in various applications (Fig. 3.3), and some of them are given below .

3.4.1 Textile Industry

For the development of multifunctional fabrics, the enhancement of prevailing property and the synthesis of novel materials with potent features are the prior aims of current textile industries [79]. Due to poor conjugation, the nanoparticle bonding becomes weak on multiple washes. Therefore, to overcome this problem some alternatives are needed. Due to which qualities and properties of fabrics were not stable and durable. Moreover, the emancipation of nanoparticles to the environment from fabric acts as a contaminant. These disadvantages motivated the researchers to focus on polymeric nanocomposites instead of nanoparticles alone due to their stability and durability. The nanocomposites of polymer improve the bonding among the nanoparticles with the textile surfaces due to which the durability of the fabric is increased also, making the environment safe by inhibiting the liberation of weakly bound nanoparticles into the environment [80]. In addition, nanocomposites polymer not only offers the chance to amend the dimensions and

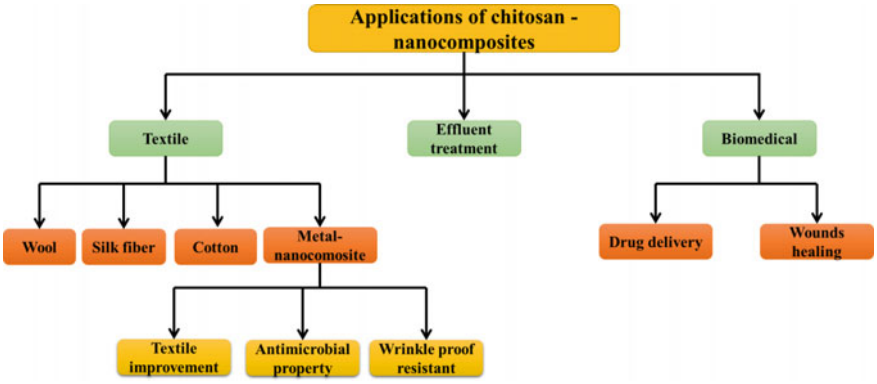

Fig. 3.3 Application of chitosan–gold nanocomposites

chemistry of fabric by changing fillers among them but also permits to add various inorganic and organic substances to prepare the multifunctional fabric. The utilization of multiple hydrophilic/hydrophobic functional matrices of polymer as the dissemination medium for various nanoparticles will provide new characteristics to desired nanocomposites of polymer with an enhanced bonding. This will also convey required wettability with varied functional applications such as microbicidal property, conductivity, resistance to ultraviolet and flame retardancy. These eccentric characteristic properties of different nanocomposites develop a new category of fabrics which is demand of textile industry in nowadays [81]. Chitosan nanocomposites are one of the broadly used nanocomposites in the textile industry nowadays. Chitosan appertains to polysaccharides family formed from β-(1 4)-linkage of *N*-acetyl-D-glucosamine and D-glucosamine biomolecules [82]. The most ordinary source to achieve chitosan is the exoskeleton of marine entities. However, nowadays chitosan is also produced from fungi or insects [83, 84]. Because of various attractive characteristics, such as biocompatibility, biodegradability, magnificent and scope for structural modification at chemical and mechanical levels, have made chitosan a favourite material for research community [85, 86]. Chitosan is amidst the amplest natural polysaccharide found in nature, incorporating numerous utilitarian features that could be accomplished for the advancement of textile such as biodegradability, antimicrobial activity, and biocompatibility and non-toxicity to synthesize higher-grade fabrics [87, 88]. Chitosan nanocomposites are also preferred for the medical use such as bandages, gauze and threads because of hygiene maintenance and high antimicrobial property [89]. The hydrophobic/hydrophilic functional polymers incorporated with nanoparticles enhance reinforcement property of the fabric. Incorporation of chitosan nanocomposites enhances the hydrophobicity and rigidness at the surface of the fabric. Moreover, the chitosan nanocomposites maintain the hysteresis of contact angle that leads to control repellence of water or the absorbance among the fabrics. Maintaining that low contact angle through a change in the structure of the nanocomposites of chitosan allows the industrialist to produce good grade sportswear with a property of moisture management and fabric relay soothing experience to the people. In addition, the chemical stability at high temperature is also enhanced by chitosan nanocomposites [90]. Chitosan nanocomposites incorporated with inorganic UV-blockers like TiO_2, SiO_2, ZnO and Al_2O_3 which improves the property of UV protection in fabrics by controlling Rayleigh's scattering, thus increasing the fabric's stability [91]. Likewise, the bactericidal efficiency of the fabrics is also enhanced by the incorporation of zinc oxide, titanium oxide and nanosilver in nanocomposites of chitosan [92]. Metal chitosan-loaded fabrics are very active and sensitive against the microbial activities because when microbe comes in contact with the fabric, metal nanoparticles abruptly retard the metabolism and inhibit the growth of cells also rupture the cell walls of the microbes [93]. In a study, it was found that the TiO_2 nanoparticle provides protection against microbial strain through photo-catalytic discoloration of microbes [94].

3.4.1.1 Wool Industry

Wool is the paramount category of fibre, which broadly employs in the fabrication of good quality garments. Wool fibre has cuticle structure due to which it has a tendency to shrink, under mechanical action, commonly at the time of washing, still it is one of the most commonly used fabrics. Using nanotechnology and other processes machine washable wool fiver has been also developed [95]. In past time, chlorine–Hercosett process (in this process, chlorine treatment is followed by polyamine resin treatment) was employed to reduce wool fibre felting, which provided finishing to the fibre. The process is efficient but there are two major disadvantages of using this process: the first one is strength loss in wool fabric and yellowing leads to compromise quality and the second one is release of absorptive organohalogens in the effluvium discharged by the process, acting as environment pollutant causing risk to the environment and human health [96]. On considering these problems, an alternative method, i.e. additive treatment, was used in which water-borne polyurethanes were included that offered a substitute which was free from chlorine to eliminate wool fabric felting [97]. However, the polyurethanes postulate strong inter-fibre bonds leading to enhance the strength of fabric. But, due to large polyurethanes dosage, the smoothness of the fabric was lost [98]. In later studies, polyurethanes have been modified with chitosan and investigated. The manipulation in polyurethanes and chitosan showed a breakthrough due to enhanced fabrics mechanical strength also provided non-toxic discharge of the effluent [99]. Basically, at the time of polyurethane synthesis chitosan was added. The use of chitosan and chitosan nanocomposites improved the mechanical strength by amplifying the inter-fibre bonding inside the fabric.

3.4.1.2 Silk Industry

Silk fibre is a biomaterial extracted from *Bombyx mori,* which has always become interested for mankind. This material possesses outstanding mechanical strength, biocompatibility and shows sluggish degradation. These properties have made it a material of choice in textile industry [100]. The fibre is made up of approximately seventeen amino acids having a large porous sponge form which induces the fabrication of b-sheets of protein. It is difficult to achieve the dimensional stability in silk fabric by self [101, 102]. Also, to reduce this problem, various additives have been used but the use of chitosan nanocomposites has made it possible to overcome this problem. The chitosan nanocomposites on silk have two unique features: firstly, the scattering of nanochitosan with different nanoparticles in the matrix of silk fibre is similar and secondly, the fabric's dimensional stability is improved superbly and provides an additional benefit by providing magnificent compression strength [103].

3.4.1.3 Application in Cotton

The antimicrobial fabrics are the need of the hour due to hygienic and detrimental effects of microorganisms [104]. The cotton is considered as one of the comfortable and soothing fabrics. Cotton is a cellulosic fabric anchored with several hydroxyl groups. The dimensional stability and antimicrobial efficiency of cotton could be enhanced by applying multifunctional cross-linking agents. The nanomaterials are widely used nowadays in textile industries for the development of required textile features as per the demand of the people. Chitosan-nanomaterials are one of the highly considered nanoparticles because of their exotic features like biocompatibility and antimicrobial potential. These nanomaterials are having high surface area-to-volume ratio and huge surface energy that provide a better amalgamation of textile substrates could be achieved along with improving its durability, breathability and mechanical strength. In general, chitosan-nanomaterials are used as catalyst and improve recovery also the wrinkle resistance of fabric. During finishing process, chitosan nanomaterial decreases the internal stresses created at the time of spinning and weaving, therefore relaxing the fabric which results in increased durability [105].

3.4.2 Improvement in Textile Functionalities by Chitosan–Gold Nanocomposite

3.4.2.1 Chitosan–Gold Nanocomposites in Enriched Dyeing and Antimicrobial Property

Chitosan is widely known for its microbicidal activity. Therefore, it has gained compelling attention in various research fields. Degree of acetylation and substitution, molecular weight, physical properties and structural compositions of the cell wall of the desired microorganisms are the main parameters to decide the antimicrobial property of chitosan. The chitosan alone cannot provide antimicrobial activity; therefore, a complex of chitosan with other active elements like natural compounds, metals and drugs is an efficient approach to increase its antimicrobial activity. Metal-doped (Ag, Cu, Au, etc.) chitosan nanocomposites possess naturally occurring positive charge which results in peculiar properties like permeability, antimicrobial activity and improvement in mucosal adhesiveness which leads to its possible utilization in different areas [106, 107]. Microorganisms contain negative charge on their cell wall so positive charge on chitosan molecule due to amine groups of can easily attach to the target organism effectively. This results in the modification in cell permeability, thus creating an osmotic deterioration with the effluence of ions and proteins between extracellular space and cytoplasm which inhibits the further microorganism's activity [108]. The bactericidal effect is because of the positive charge and different inherent factors or incorporation of other metals such as Au and Ag. Some other parameters like degree of substitution and deacetylation on

amino groups intrinsically affect positive charge density on chitosan [109]. Degree of deacetylation affects the number of free amino groups on polymers backbone of chitosan nanocomposites giving excellent antibacterial activity [110, 111]. In addition to this, solvent, its pH and molecular weight are also required to be controlled because they could affect the activity of molecule. In a study, it was found that the bactericidal activity of chitosan changes with change in its pH and molecular weight [112]. Also, in the same study, it was suggested that at lower pH value, antibacterial property of chitosan was higher because of higher protonation degree in that particular condition [113]. The antimicrobial property also differs from species to species. In gram positive bacteria, a higher molecular weight chitosan is more preferred in comparison with gram negative bacteria [114]. Chitosan's molecular weight, degree of acetylation and pH are all important for antifungal activity. The efficacy of chitosan can also be improved by changing its structure, i.e. by substituting it with some other functional groups. The properties of fabric can be changed by N-modification on chitosan molecule by quaternarization, alkylation, acylation, metallization and saccharization. Due to these changes, hydrophilic/hydrophobic character of chitosan molecule is manipulated and it provides a new property in the fabric [115]. When the amino group of chitosan molecule is quaternized with aromatic groups or short alkyl chains, it boosts the antimicrobial efficacy of chitosan [116, 117].

Chitosan nanocomposite is also used for the dye removal applications because amino groups of chitosan molecule are cationized easily, resulting in anionic dye adsorption using electrostatic forces in liquid [118].

3.4.2.2 Activity in Wrinkle Resistance

The wrinkle resistance in the fabric can be maintained by several conventional materials. However, the materials have several disadvantages such as decrease in abrasion resistance, fabric tensile strength, water absorbance and dyeing potency. To remove these limitations, the nanomaterials have been used into the matrix of fibre which improves the actual features as per the need [119]. However, instead of using the nanoparticles, some other techniques such as exhaustion and padding have been also used on the fabrics to get the wrinkle resistance property [120]. Moreover, the microwave treatment on fabrics also helps to increase the wrinkle resistance property in fibre same as the oven curing, where it generates higher volumetric heating and frequency to enhance resistance activity which is not good [121].

3.4.3 Effluent Treatment Application

The discharge of wastewater from various industries has become a serious threat nowadays. The wastewater discharged from various industries like leather tanning, fibres, textile, pharmaceutical, wools food technology, paper, plastic produces large amount of contaminants in water [122, 123]. Industrial effluent contains many organic

pollutants, ions of heavy metals and dyes which contaminate the water resources [124]. There are several methods or techniques that have been used to treat industrial wastewater such as osmosis [125], adsorption [126], filtration through activated charcoal [127], electrochemical oxidation [128], incineration [129], organic resin and biodegradable nanocomposites [130]. Adsorption is one of the efficient techniques used for treatment of wastewater. In recent years, the interest in nanotechnology and nanomaterials resulting in fabrication of a number of adsorbents to battle the wastewater hazard. Chitosan–gold nanocomposite is one of the efficient adsorbents because of its environment-friendly nature, biodegradability, non-toxic effect, easy handling cost efficiency and effectiveness in scavenging heavy metals and dyes [131]. In addition, of use of chitosan nanocomposites for wastewater treatment application is advantageous due to insoluble nature of chitosan in H_2O, organic solvent and also in alkaline solutions which is due to hydrogen bonding among the molecules. Solubility of chitosan is only found in acidic medium because of protonation from its amine group. Based on these properties, capability of chitosan-based nanocomposite is increased as compared to other adsorbents in wastewater treatment applications [132, 133]. Changes in degree of acetylation also play a crucial role in pollutants removal efficiency. Chitosan-based absorbents are reported to remove dyes like Reactive Blue, Acid Green, Acid Blue, Direct Blue, Food Yellow effectively [134].

3.4.4 Bioremediation

Environmental pollution has become a matter of serious concern nowadays, and action against it should be taken in priority to inhibit the upcoming environmental disbalance. Various technologies such as ion exchange, adsorption, electroprecipitation, floatation and coagulation–flocculation were developed to solve this problem [135]. Another technique based on nanotechnology, i.e. bionanopolymers, has come into picture which is the emerging and the most promising material in the field of water pollution management. In addition, biopolymers are biodegradable, cost-efficient and eco-friendly and have high surface area [136, 137]. Chitosan nanocomposites have been successful in removing inorganic, organic and xenobiotic compounds, which have developed peculiar interest of researchers in this. In addition, chitosan nanocomposites can work under broad pH range due to the presence of several coordinating moieties in their structure. These properties of chitosan have made it an eligible candidate to be applied in bioremediation applications [138]. Deacetylation and change in the molecular weight could increase the performance of nanocomposites of chitosan as per the requirement. These nanocomposites can be easily functionalized, thus increasing the selectivity of the material to interact with the contaminant [139]. In some studies, chitosan-based materials were already reported for the effective nitrates and phosphates removal from water. In one of the studies, the material was prepared by doping Cu (II) in chitosan nano-matrix ligand exchanger and utilized for the eradication of phosphate. Chitosan nanocomposites were also used to enhance the biodegradation of oil contaminants using osmocote fertilizer

[140, 141]. It has been found that, chitosan-based nanocomposites are the cheapest materials as compared to activated charcoal or other materials in the removal of chromium [142]. Nanocomposites of chitosan can also be used successfully for the eradication of tungsten by altering the net surface charge from negative to positive [143]. A complex of chitosan nanocomposites and green chelating agents was used for the removal of heavy metals like cadmium (Cd), zinc (Zn), copper (Cu) and lead (Pb) xenobiotic compounds and dying chemicals from the industrial effluents [144]. The chitosan nanocomposites can also act as substitute against synthetic polyelectrolytes used as natural coagulant to eradicate turbidity from the drinking water [145]. Chitosan nanocomposites also reduce soil erodibility by improving the cohesive forces of inter-particle in among the soil particles that in turn revitalize the soil [146]. They also improve the mechanical strength and intercept the water-induced degradation of the construction's materials [147]. Another application is their use like an eco-friendly mixture or like an exo-coating agent in construction works [148].

3.4.5 Application in Biomedical Field

3.4.5.1 Drug Delivery Applications

The antimicrobial properties of chitosan lead to inhibition of growth or microorganisms death which are pathogenic. Studies suggested that antimicrobial property of chitosan depending on certain parameters like source and nature of chitosan, chelating capability, stage of cell polymer concentration, solubility, environmental conditions and degree of acetylation [149–152]. Chitosan also responds to outer impetus such as temperature, pH, magnetic and electric field due to its cationic nature [151]. Reported studies concluded that the mechanism behind the antimicrobial property of chitosan is mainly due to two reasons: (i) Increase in the $-NH_3^+$ ions (positive charge) on the polymer leading to binding of chitosan to the bacterial cell walls and (ii) RNA inhibition and disrupted protein synthesis which can be achieved by insertion of chitosan with their oligomers (low molecular weight) inside the cell's nuclear cores [153]. It is reported that chitosan plays a vital role in pathogenicity and cell-to-cell communication by disrupting the formation of HAQs (4-hydroxy-2-alkylquinolines) along allied metabolite [151]. It is still not known that chitosan is responsible for cellular toxicity or inhibits only the growth of microorganism. The antimicrobial efficiency of chitosan can be changed on the basis of side chain functional groups and molecular weight. Relationship between degree of deacetylation and antimicrobial efficiency of chitosan is still unknown and needs to be investigated. Some of the studies have been done on the association between microbial diversity (till strain level) and antimicrobial efficacy of chitosan nanocomposites [154, 155]. Chitosan nanocomposite retards growth of broad range of microorganisms through binding them within liquid form in in vivo and in vitro experiment and act as broad range antibiotic [156]. Both types of bacteria (gram positive and negative) are influenced by quaternized chitosan (QCS) absorbed photo-cross-linked electrospun, portending the

use of chitosan nanocomposites electrospun matrix for dressing material for wounds [157].

3.4.5.2 Wound Healing Applications

Scaffolds Mixed with Chitosan/Synthetic/Natural Used in Wound Healing

Chitin–PAA (polyacrylic acid)-based implantable hydrogel system was prepared for the generation of suitable dressing material for wound healing. On chitin–polyacrylic acid sheath, a regular cellular morphology was observed after 14 days [158]. Chitosan-based hydrogel also promotes some toxicological effects due to hydrogel which is used in rat's fibroblast cells signifying induction of cellular adhesion and proliferation between cells [159]. Cell toxicity was studied on mouse fibroblast (L929) cells. The prepared film was slightly toxic confirming its application in wound dressing [160]. Determined and accurately measured antigen delivery was achieved using chitosan and its porous microspheres derivatives together [161]. Mucoadhesion property in chitosan and some of its cationic derivatives were also observed which is effective in normal pH for drug adsorption capacity. The positively charged *N*-trimethyl chitosan chloride reacts with negative charged cell surface leading to reduction in microbial load [162]. Chemical modification in chitosan biopolymer can change surface characteristics of films like synthesis of stearoyl ingrained chitosan film conveying hydrophobic nature leads to improve adsorption of proteins. However, phthalic/succinic anhydride treatment leads to the formation of hydrophilic films used for lysozyme adsorption [163]. Some of the nanocomposites of PVA-clove extract, PVA-bentonite, PVA-cellulose hydrogel and PVA-Ag NPs were synthesized by Gonzalez et al. A drawback of non-uniform texture and poor spreading was recorded in formulation of PVA-clove; however, considerable moisture carrying capacity and solidity besides enhanced antimicrobial capability was observed in formulation of nanocomposite gel of PVA-bentonite and nanoparticles of PVA-Ag for *E. coli* after inserting fillers (clay and silver nanoparticles) [164]. The movement of growth factor can be promoted by incorporation of heparin which is related to healing on the site of wound leading to enhance rate of wound healing [165].

Wound Healing by Chitosan Graft Scaffoldings Composite

A technique known as electrospinning has been used for the formation of nanofibers of chitosan–sericin, size ranging from 240 to 380 nm with uniform structure. The nanocomposite showed enhanced cellular proliferation and considerable antibacterial activity against gram positive and negative bacteria with adequate dressing efficiency. After 24 h, approximately 90% viability was observed in the cells in all the test concentrations, and after 72 h, approximately 100% viability recorded in the cells when compared with control [166]. Chitosan sulphurylation at o2 and o3 position formed sturdy antiviral agent that leads to efficient inhibition of AIDS

virus [167]. Chitosan with silver sulfadiazine with act as a stable drug, released with an increased cytocompatibility and adequate swelling ability also enhanced antimicrobial effect [168]. Chitosan alginate mixed with silver sulfadiazine provides good durability. It was observed that long duration release of drug happens approximately 50% of alginate concentration proving its dependency on alginate [169]. A combination of alginate and calcium chloride coacervate on chitosan alginate PEC membrane is used for enhancing the property of wound healing. Instant wounds healing accompanying fresh epidermis development were observed in fabricated membrane. It was not harmful to fibroblast cell of human and mouse [170]. Dual-sided dense matrix silver sulfadiazine bandage showed in vivo bactericidal property against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with reduced drug release [171]. A chitosan/CM chitosan membrane showed good homeostatic and bactericidal activity against *E. coli*. CPC found to be an efficient dressing item with a property of non-toxicity and good histocompatibility to skin impeding keloid formation instant healing wounds [172]. A polythiolated chitosan–ciprofloxacin conjugate was utilized for the synthesis of chitosan film having property of heat sensitiveness. The composite was focused to separate easily from wound subject to low temperature [173]. A chitosan–silver–hydroxyapatite (CS-Ag-Hap) biomembrane was prepared on a substrate of anodized titanium encompassing silver, chitosan and hydroxyapatite through electrochemical synthesis procedure. An increased antimicrobial activity and good cell adhesion were noticed in synergistic action of chitosan nanocomposite due to the porous nature of the surface. An enhanced antibacterial and cell adhesiveness reduced the problem of infection concluding their applicability in dressing materials [174]. A silver encapsulated patch of chitosan hydrogel was responsible for high antimicrobial activity. The produced hydrogel manifest improved the swelling ability, blood clotting efficiency and also non-toxicity. Hydrogel showed non-toxic nature in cytocompatibility against Vero cells. It was observed that hydrogel showed a vital role in healing of wounds by retarding *E. coli* and *S. aureus* (gram negative and positive bacterium, respectively) [175, 176]. A composite of CS-γ PGA was noticed to be appropriate with enhanced tensile strength and ability that able to remove easily from injured surface, without harming the injured tissues. CS-γ PGA treated wounds showed rapid healing in comparison with controls. Approximately <50% of re-epithelial cells were observed by using a complex of CS-γ PGA polyelectrolyte [177]. Electrospinning method is also used for the fabrication of chitosan–gelatin blend nanocomposite. Robustness and substantial tensile strength were conveyed to the fabricated fibre miscellany by coherent nanocomposite (Fe_3O_4) dispersed uniformly in overall matrix fibre ensuring the effective liberate from the matrix of chitosan–gelatin. Inhibition of *E. coli* and *S. aureus* has been noted by the use of Fe_3O_4 nanoparticle. Antibacterial activities and physiochemical properties indicated its application in acute wound dressing material [178]. A freeze–thaw technique is also used to prepare stable nanocomposite and to circumscribe, chitosan polyvinyl alcohol is the compatible dressing material. In future prospects, a nanocomposite should have property of swelling nature, enhanced tensile strength and antimicrobial activity which could be applicable as dressing material [179].

Wound Healing by Chitosan–Oil Ingrained Grafts

Zinc oxides chitosan nanoparticle in different concentrations, soaked with castor oil film could be used in the process of solution casting. Increment in the concentration chitosan–ZnO antibacterial property against gram positive and negative (both) strains was also improved. Due to desirable properties of material such as absorption of water, biodegradability, cytocompatibility, antimicrobial activity and property of wound healing, it could be used as potential dressing bandages material [180].

Wound Healing by Plant Extract Entrenched Chitosan Films

Extracts of hydrogel plant can also be utilized in microbicidal activity but they have some drawbacks such as incompetence problem and discharge of less quantity of active materials that directly affect formulation capacity. Plant extract contains some bioactive substances which help in instant wound healing naturally. Gentamycin sulphate-infused hydrogel films along extracts of *Salix alba* and *Juglans regia* plant showed antifungal and antimicrobial properties. These properties are required for the process of wound healing [181–184]. Open (uncovered) wounds have higher chances of contamination from microorganisms such as bacteria, fungi and viruses. So, there is a requirement to develop an efficient material for wound healing with some bioactive compounds to circumvent sepsis and also other contamination from the wound area. Aloevera has anti-inflammatory and moisturizing property due to which it could be a better alternative to be prepared with wound dressing bandage. Conjugating chitosan/aloevera has been reported for highly efficient dressing membrane. Because of high antibacterial activity, huge amount of aloevera is used in aloe/chitosan nanocomposite bandages. A better proliferation and adhesion property of chitosan was also observed. Enhanced adherence property was observed in the spindle-shaped fibroblast and uniform distribution (day 1 to day 7), however, on seventh-day cells attached to membrane on both of the sides. Use of chitosan/aloevera could be a better material for dressing [185]. Some of the chemicals in aloevera like glycoprotein and polysaccharide have more wound healing capability [186]. Acemannan is also liable for retarding growth of microbes and showed high activity of microphages [187]. The antibacterial property in some bioactive materials like cinnamic acid, lupeol, phenols, sulphur and salicylic acid is also observed [188].

Healing of Wound by Chitosan Modified Products

Chitosan and its derivatives with some modifications are broadly used in delivery of drugs because of their improved solubility in acidic medium, which lead to cell definite targeted delivery and enhanced cellular uptake [152, 189]. Chitosan has a drawback of less solubility in physiological pH [190]. Thus, there is a need to produce derivatives of chitosan soluble in aqueous medium like carboxymethyl chitosan, carboxymethyl trimethyl chitosan and trimethyl chitosan for the application

in healing of wounds and drug delivery. Carboxymethyl trimethyl chitosan is also used in cosmetics, to maintain its moisture-capturing efficiency and use it as an essential element [191]. Derivatives of trimethylated have shown outstanding mucosal attachment and capability of loading drug due to strong cationic characteristics of it [192].

3.4.5.3 Toxicological Effects of Trimethyl Chitosan

Methylated trimethyl chitosan has widely been used in drugs absorption. The in vitro evaluation was significantly done using COA-1 and Caco-2 cells [193]. TMC concentration was directly proportional to its cellular toxicity behold in cell line of HeLa cells which leads to complete depredation in concentration >10 mg/mL [194]. By increasing the cationic charge and decreasing molecular weight also increase the toxic effects. Lower toxicity in in vitro cell lines was observed using COS7 (monkey's fibroblast cell line of) and TMC (<55% trimethylation grade) with low molecular weight for MCF7 (human). However, TMC (trimethylation degree = 94%) resulted in less IC50 against COS7 (2.2 mg/mL) and MCF7 (1.4 mg/mL) [195]. Various molecular weighted chitosan (5, 25, 50, 100 and 400 kDa) have been applied on L929 fibroblast cells resulted in IC50 values >1000, 270, 90, 70 and 30 g/ml, respectively, and keep constant degree of methylation [196]. In a study, it is reported that positively charged polyethyleneimine is more toxic than TMC [197]. TMC is cationic in nature. Due to this property, in healing of wounds, TMC plays a vital role, also in various drug delivery applications by complexing with DNA and RNA (anionic).

Wound Healing by Trimethyl Chitosan (TMC)

TMC was synthesized in bilayer through sandwiched with collagen and attached with DNA (which encodes for EGF and VEGF) increasing rejuvenation and angiogenesis of affected area by the resulting product [152]. A complex of TMC and DNA enhances growth factor stimulation, endocytosis, which also defends against the degradation of nuclease [198]. Incision wounds treatment using same technique resulted in increased vascular vessel production and mature RBCs movement because of ferocity of wound obtained close to adjoining cells and blood vessels abutting burn wounds [199].

3.4.5.4 Carboxymethyl and Carboxymethyl Trimethyl Functionalized Chitosan Applications

An increment in biological compatibility and liquid solubility of CMC can be obtained by the adherence of moiety of carboxymethyl to the polymer backbone [200]. CMC synthesis from chitosan by single catalytic reaction in which NaOH, (C_3H_8O) and water (H_2O) mixed slowly with the chitosan-containing reaction mixture, and after that, mixture was treated by chloroacetic ($ClCH_2CO_2H$) acid.

CMC is soluble in a variety of pH range and possesses enormous gelling ability and also has no issue of histocompatibility. CMC increases the rate of wound healing by enhancing fibroblast proliferation, thus showing exceptional ability of binding with drug/ligand and renders uncommon bandage of dressing [201]. Some of the physical parameters such as pH, molecular weight, degree of deacetylation, concentration and charge of cations on the polymer affect antimicrobial activity and capability of CMC [202]. Liu et al. had tested the antibacterial efficiency against *E. coli* in order of *N*, *O*-carboxymethyl chitosan >chitosan >*O*-carboxymethylated chitosan [203]. An increased keloid formation and proliferation of fibroblast were recorded to maximize cytocompatibility. At pH 10.0, a treatment using CH_3COOH and NMP with TMC showed the establishment of *O*-carboxymethyl-*N*, *N*, *N*-trimethyl chitosan [204]. Chitosan beset C3 and C6 as sites for carboxymethylation that might get hindered through *O*-methylation posing hindrance for appended substitutions therefore intact and appropriate monitoring is needed at every step of reaction [205].

Wound Healing by Peptides Conjugates-Chitosan/Derivatives

An exclusive method can speed up the wound healing process. In this method, saturation with biochemical factors is replaced by the peptide functionalization of the scaffolds. Peptide-associated scaffold material can enhance the bonding of dressing material along extracellular materials of skin. In complete and fast traumatic wounds healing, cell differentiation increases with enriched proliferation monitored in L929 fibroblast (mouse). A conjugation between extracellular matrix and RGD (arginine/glycine/aspartate) signalling peptides improves the cell attachment and activates pathway of Rho GTPase resulting in integrin activation which enhances the cellular attachment [184, 185]. The scaffolds of chitosan impregnated with RGD sequence enhances cell attachment and biocompatibility, which was identified by the receptors [206]. So that, the 3D functionalized chitosan scaffold can be used as an ECM accessory [207]. Carboxymethyl trimethyl and tryptophan impregnated chitosan are used in delivery of the genetic material and also for bonding of two or more peptides as well as site-targeted drug delivery [208]. TMC conjugate with CMC by succeeding derivatization results in more optimized and stable product [209]. Polyelectrolyte complex (PEC) was a product produced by addition of Gly–Arg–Gly–Asp–Ser (GRGDS) peptide and carboxymethyl trimethyl chitosan (CMTMC) and has the capability to enhance cell adhesion and migration. Another methods known as ionic complexation procedure were also utilized for nanoparticles formation impregnated with chondroitin sulphate showed wound-healing capability [210]. Active peptide-functionalized scaffolds showed improved adhesion of dermal fibroblast and fibroblast. The previous studies suggested impact of biologically active and modified chitosan and its derivatives in facilitating the tissue engineering.

3.4.5.5 Chitosan Blend in Commercial Dressing Bandages

Naturally originated chitosan and its derivatives are an efficient biopolymer used in form of dressing bandage. Some of the efficient and popular commercial dressing bandages are Chitipack P (Eisai Co.) (Bloated chitin fortified over poly (ethylene terephthalate) and ChitipackS. ChitipackS is capable of promoting early granulation, so that it is used for the traumatic wound and defects of surgical tissue treatment. Marine polymer technologies have been used to develop Syvek–Patch (reinforced chitin fibrils), used to enhance the processes of haemostasis [211]. HemCon (a freeze-dried salt of chitosan) is efficient for prevention of supra-infection and production of haemostatic effect, also at the time of civil medical emergency is used exceedingly [212–214]. Chitosan is efficient to be used as dressing patch (with anti-infective property) of burns and management of wounds and keeps the structural probity, and also at the injury site, the mucosal adhesiveness of chitosan is efficient. Meshed chitosan membrane had shown a remarkable development in tissues structural rearrangement, accelerated wound healing [215]. A reinforced heparin–chitosan membrane has been developed by Kratz et al. to determine the wound healing capability in skin of donors [216]. A novel approach has been developed by Damour et al. to substitute dermis of skin using a dermal substrate [217] used for promoting neo-vascularization also for unambiguous and measured colonization of the fibroblast manifolds [218].

3.5 Future Applications

Metal-doped nanocomposites of chitosan have special properties which make it useful for various applications. Its biological origin and biodegradable nature gained attention in wastewater treatment applications. Nanocomposites of chitosan are hydrophilic, non-toxic, biocompatible, biodegradable and cost-effective in nature. In addition, these are effective in basic and neutral pH also, due to which it can be used for various applications. Modification and renewability in the chitosan as per the requirement enhance the research in the field of bio-polymers. Chitosan nanocomposite synthesis methods and surface functionalization develop the peculiarity of further applications. Coagulation–flocculation efficiency plays a keen role in the activity of chitosan-based nanocomposites which can be leading to improved several applications such as biosensor development, wastewater treatment and bioremediation. People can establish cost-effective techniques for various contaminants and eliminate different pollutants efficiently. The bactericidal efficiency of the fabric could also be increased by differing the functional groups of nanocomposites. The scope of improvement is still left as per the various applications of chitosan composites without harming the environment due to biodegradable nature of chitosan. There is still an economical challenge to introduce commercial viability of sustainable biopolymer in real market as the techniques and innovations executed at industrial scales. However, manufacturing and fabrication of these nanocomposites into functional groups of bioactive component enriched textile is a promising hope.

Further, for industrial realism an insightful investigation of these biopolymers is still needed. These investigations towards acquisition of novel sources, economic-efficient intensive extraction process and execution of innovative techniques would impart substitute to harmful synthetic microbicidal products. Hence, the metals such as Au, Ag-doped chitosan nanocomposite or biopolymer are a potential material for the wastewater application, especially in heavy metals and dyes removal due to its biodegradable, stable, economically efficient nature and easy to synthesize properties. These gold/silver-doped chitosan-based materials could be scaled up for industrial application, after some modification.

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Chapter 4
Environmental Properties and Applications of Cellulose and Chitin-Based Bionanocomposites

Renyan Zhang and Hui Xu

Abstract Bionanocomposites are class of bio-based nanostructured hybrid materials, which exhibit at least one dimension on the nanometer scale. In general, bionanocomposites are comprised of biopolymers and other organic or inorganic sources. Cellulose and chitin are the two most abundant biological polymers in nature. In recent decades, cellulose and chitin-based bionanocomposites have triggered a great deal of attention to understand such composite materials and their applications. Thanks to their renewable, biodegradable, biocompatible, low-cost, low-density, eco-friendly properties, and low energy consumption, cellulose and chitin-based bionanocomposites are excellent green technology materials. Currently, cellulose and chitin-based bionanocomposites are widely used in a variety of fields, such as environmental protection, electronics device industry, biomedicine industry, food industry, and agriculture industry. In this chapter, the sources and properties as well as the extraction and preparation methods of the corresponding cellulose and chitin bionanocomposites are introduced. The environmental characteristics of cellulose and chitin-based bionanocomposites and their applications in various fields are reported.

4.1 Introduction

4.1.1 Cellulose

Cellulose is the most abundant renewable biodegradable polymer on earth, with global production estimated at 10^{10}–10^{11} tons per year [1]. This biopolymer is widely distributed in higher plants, marine animals, algae, fungi, bacteria, and invertebrates

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and even found in protozoa. In 1838, it was discovered and isolated in plant tissues by Anselme Paine, a French chemist.

Cellulose microfibers are the basic structural components of cellulose, consisting of 36 individual cellulose molecules [2]. Microfibrils are then packaged into larger units to form microfibrillated cellulose. Microfibrinolytic cellulose is reassembled into familiar cellulose fibers, as shown in Fig. 4.1. Microfibrillated cellulose (also known as nanofibrillated cellulose) has a diameter of 20–50 nm. The diameter of a single cellulose microfibril ranges from 2 to 20 nm [3, 4], which can be regarded as a flexible hair filament, and the fiber crystals are connected along the microfibrils axis through disordered amorphous structural domains [3]. The ordered regions are wrapped in cellulose chains and stabilized by a strong and complex network of hydrogen bonds [2], similar to nanocrystalline rods. Cellulose is composed of linear oligosaccharides consisting of β-D-glucopyranose units linked together by β-1–4-linkages [5]. The repeating unit structure of cellulose is depicted at bottom panel of Fig. 4.1. It shows a dimer called fibrinolytic sugar in the form of repeated fragments. The monomer, known as the anhydroglucose unit (AGU), has three hydroxyl groups. These groups form strong hydrogen bonds, thus offering cellulose the most important properties, in particular (i) multi-scale micro-fibrosis structures, (ii) graded structures (crystalline and non-crystalline areas), and (iii) high cohesion (vitrification transition temperatures are higher than their degradation temperatures) [1]. In the process of biosynthesis, hydrogen bonds between adjacent hydroxyl and oxygen molecules induce the formation of parallel accumulation of multiple cellulose chains.

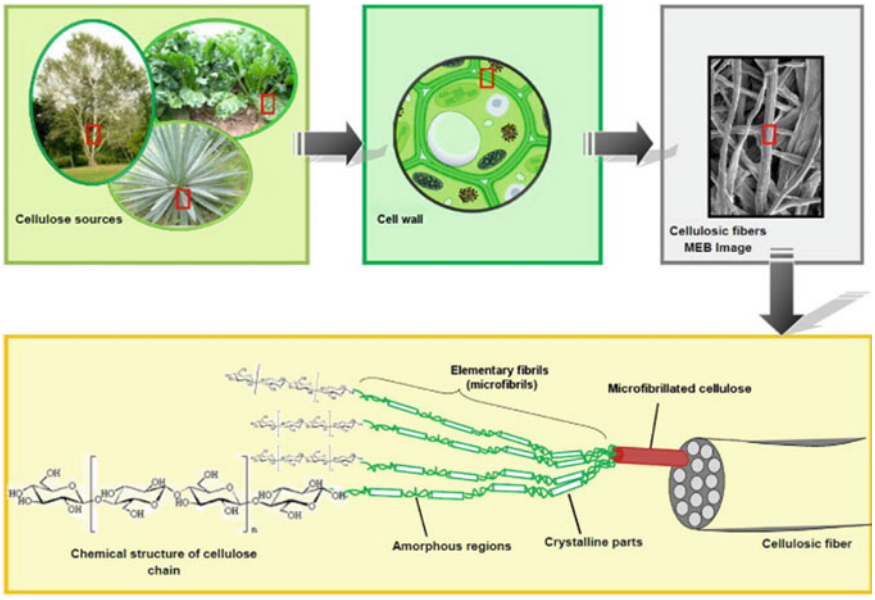

Fig. 4.1 Schematic demonstration of cellulose hierarchical architecture from the cellulose sources present in nature. Reprinted from Ref. [1]. (Copyright (2012) Elsevier Publishing Group)

Cellulose has four different kinds of polymorphs: cellulose I, II, III, and IV. Cellulose I is a form found in nature and occurs in two different forms, I_α and I_β. Attala et al. [6] propose that most natural cellulose is a mixture of the two variant forms. Triclinic I_α is predominant in algal–bacterial cellulose, while monoclinic I_β form is a variant present in typical cellulose of annual plants [7]. Cellulose II is prepared by precipitation in an alkaline solution. These represent the two main polycrystalline forms of cellulose. Main difference is that two kinds of cellulose II with antiparallel accumulation, and chain run in parallel to the direction of cellulose I [8–10]. It is important to note that cellulose II in each glucose residues of additional hydrogen bonds makes this variant the most stable form of thermodynamics [10]. Cellulose III_I and III_{II} are obtained by ammonia treatment of cellulose I and II, and through the modification of cellulose III, eventually produce cellulose IV [1]. Among them, due to its high crystallinity and modulus, the cellulose I is responsible for the mechanical properties, through the tradition of lignocellulose bleaching processing after extraction, used for the preparation of cellulose nanofiller.

Nanoscale cellulose has been found to be very promising enhancement element. There are basically two types of nanocellulose particles: (i) cellulose nanocrystals (CNCs) and (ii) cellulose microfibers (CMFs) [11].

In 1870, Nageli and Schwendener first discovered definite crystalline zones in the amorphous structure of cellulose materials, which is the earliest description of CNCs. In 1947, CNCs were first produced by Nickerson and Harble [12]. In the experiment, it was observed that the degradation of cellulose fibers caused by boiling in acidic solution reached its limit after a certain period of treatment. This is the first literature on the separation of CNCs available. In 1950, Rånby and Ribi produced colloidal cellulose crystals (i.e., CNCs) through hydrolysis of wood and cotton cellulose by sulfuric acid. They further observed for the first time the existence of acicular particle aggregates under transmission electron microscopy (TEM) images [13]. It is also the first time that such crystalline regions have been called "cellulose micelles."

The exploration of CNCs has been going on for a long time. In addition to the discovery process described above, the preparation of CNCs has entered a new stage since the 1990s. And with the progress of the research, a growing number of terms used to refer to CNCs, such as nanocrystalline cellulose, cellulose whiskers, rod-like colloidal particles, cellulose microfibrils, and cellulose microcrystallites. Until 2017, the international organization for standardization issued cellulose standard terms and definitions of nanomaterials, and specified the term "cellulose nanocrystals." One of the breakthroughs was the discovery of the properties of liquid crystals in 1992. Revol et al. [14] reported an in vitro system in which only cellulose exists in the form of fibrous fragments dispersed in water.

In the following years, cellulose nanocrystals received more and more attention and development. In 1995, Favier et al. [15] were inspired by the extracellular high-performance skeleton biological complexes composed of matrix reinforced by fibrous biopolymer synthesized in animals or plants and tried to simulate the biological complexes by mixing cellulose whiskers. They obtained lab-scale nanocomposite structures through enhancement experiments using whiskers extracted from rare cellulose samples. In 1998, Dong et al. [16] found for the first time that chiral

nematic ordering in cellulose microcrystalline suspensions was highly dependent on hydrolysis and preparation conditions. Among them, the particle size, surface charge, and polydispersity of cellulose microcrystals vary with the degree of hydrolysis. Longer hydrolysis time leads to shorter single crystal and higher surface charge. In 2001, Araki et al. [17] successfully prepared spatially stable cellulose microcrystalline suspension by grafting polyethylene glycol (PEG) through carboxylation-amidation process. The grafted cellulose microcrystals are more stable and have the ability to disperse from the lyophilized state to aqueous or non-aqueous solvents. These characteristics are beneficial to the industrial application of microcrystalline cellulose. In 2006, Bondeson et al. [18] employed response surface methodology to optimize the process on the basis of previous studies, aiming to find a faster and higher yield method to obtain water-stabilized colloidal suspensions of CNCs. It was found that the optimized crystal separation required the increase of sulfuric acid concentration and hydrolysis time. In recent years, the development of commercial production of CNCs is still under study [19]. In 2019, Song et al. [20] reported the extraction of CNCs from *Calotropis procera* biomass by classical sulfuric acid hydrolysis (Fig. 4.2).

Microfibrillated celluloses (MFCs), also known as microfibrillar celluloses, cellulose microfibrils, or nanofibrillated celluloses (NFCs), are a relatively new type of cellulose materials. MFCs are getting more favor of the researchers than CNCs. In fact, scientific papers were published almost every two days in 2011. In contrast to straight CNCs, MFCs are long and flexible nanoparticles. They are composed of alternating crystalline and amorphous regions and vary in size, ranging from 10 to 100 nm in diameter and usually in the micron scale in length [4, 21]. In addition,

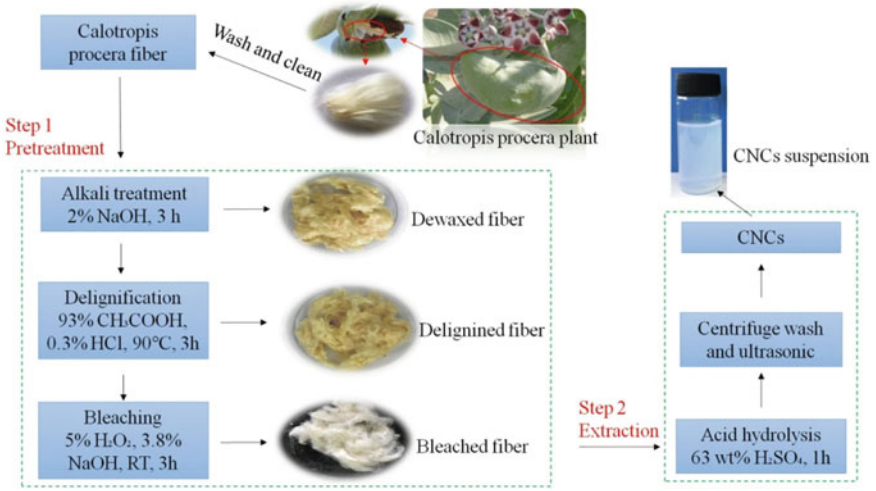

Fig. 4.2 Preparation procedure of CNCs from *Calotropis procera* fiber. Reprinted from Ref. [1]. (Copyright (2019) Springer Nature Publishing Group)

MFCs have a very high L/D ratio, which gives them a very low percolation threshold, and therefore a very good ability to form rigid networks.

MFCs are obtained through the fibrillation process of cellulose fibers. There are several different pathways for the production and preparation of MFCs. Generally, the first step is always to soak the pulp and disperse it in water. The cellulose fibers are then carefully separated into microfibers under high shear forces. To generate this shear force, different intensive mechanical processing methods can be used, such as high-pressure homogenizers [22], grinding [23, 24], low-temperature crushing [25], high-intensity ultrasound, electrospinning [26], and some other methods. Depending on the raw material and degree of processing, necessary chemical treatments, such as enzyme treatments, can also be carried out prior to mechanical fibrillation.

In 1983, Herrick et al. [] and Tubark et al. [22] first produced MFCs by means of a mechanical homogenizer with diluted cellulosic wood pulp-water suspension, in which a large pressure drop was conducive to micro-fibrosis. In 1997, Dufresne et al. [25] conducted a freeze grinding process to generate MFCs from beet pulp. MFCs can also be produced by electrostatic spinning. In 2003, Huang et al. [26] summarized the electrostatic spinning technology and its application in the production of polymer nanofibers. With the achievements in processing and collecting continuous uniaxial nanofibers, their more critical applications as reinforcements for the fabrication of primary loaded nanocomposite elements can be realized. In 2004, Zimmermann et al. [27] proposed a mechanical fibrillation process in the homogenization step using a microfluidizer. It imposes a very high shear rate, resulting in the formation of quite thin MFCs. However, the mechanical refining method did not fully pulverize the pulp fibers. Therefore, a finishing process was added before homogenization. This is essentially internal fibrillation, which loosens the fiber walls and prepares for subsequent homogenization. In 2006, Saito et al. [28, 29] proposed a new method to obtain MFCs through 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation. According to studies, TEMPO-mediated oxidation pretreatment significantly reduces energy consumption compared with repeated cycle energy consumption of high-pressure homogenizer [30]. After the free radical-mediated oxidation of TEMPO, the undried natural cellulose was decomposed into individual microfibers, which were then treated mechanically with homogenization. The combination of oxidation and homogenization has been found to be advantageous, using a much lower energy input to break down the cellulose into microfilaments of smaller width.

Another pretreatment is enzymatic hydrolysis which is usually used in conjunction with mechanical shearing. Henriksson et al. [31] developed an environmentally friendly enzyme-assisted method for the synthesis of MFCs in 2007. It had been found that the pretreatment of pure C-type endoglucanase contributed to the disintegration of cellulose wood fiber pulp into MFCs in more environmentally friendly process without solvent or chemical reactants, compared to the acid hydrolysis method. The resulting MFCs showed high average molar weight and large aspect ratio, which indicated that the enzyme treatment increased the swelling of MFC fibers in water, and thus facilitated the preparation of MFC nanofibers.

4.1.2 Chitin

Chitin, second only to cellulose, is the second most abundant polymer in nature, mainly found in the exoskeletons of shellfish and insects and in the cell walls of mushrooms, and is synthesized by mollusks, crustaceans, insects, fungi, algae, and related organisms at a rate of 10^{10} to 10^{11} tons per year [32, 33]. Chitin is the main component of the shell and is widely distributed in nature. Shrimp and crab shells are the main raw materials for chitin extraction. Braconno, a French scholar, first discovered it in 1811. And it was extracted from crustacean shells by Odier in 1823. Chitin is also structurally similar to cellulose, except that it has an acetamide group at the C2 position. In other words, chitin is composed of (1-4)-linked 2-acetamido-2-deoxy-B-D-glucopyranose unit [33], which is the homopolymer of N-acetylglucosamine (Fig. 4.3).

Due to its linear structure of one acetamide group and two hydroxyl groups, chitin is a biopolymer with highly expanded hydrogen-bonded semicrystalline structure, which usually appears as nanoscale ordered fibrils in living tissues. Chitin can be classified as α, β, or γ-chitin according to species origin and unit arrangement. α-Chitin is commonly found in the shell of shrimps and crabs, and it is the most stable and common form. The repeating units of chitin macromolecules are antiparallel, and they are connected to each other by hydrogen bonds to the maximum extent possible, resulting in a crystallinity of more than 80%. β-Chitin, found in squid, has molecular units arranged in parallel. The fiber can reach 70% crystallinity. γ-Chitin, on the other hand, is formed by the combination of α and β and is alternately assembled by

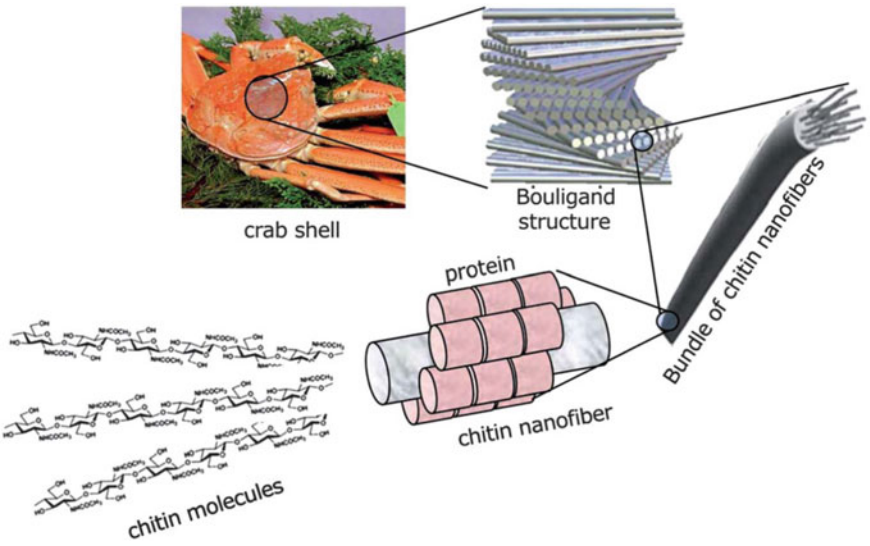

Fig. 4.3 Schematic demonstration of chitin hierarchical architecture from the crab shell. Reprinted from Ref. [32]. (Copyright (2012) RSC Publishing Group)

two parallel chains, one of which is antiparallel. Natural chitin with strong hydrogen bonds in crustacean shells is highly crystalline and arranged in an antiparallel fashion as R-chitin microfilaments. These microfibers consist of nanoscale fibers with a diameter of about 2–5 nm and a length of about 300 nm embedded in the protein matrix [34]. Its structural hierarchy is shown in Fig. 4.3. The chitin with high crystallinity is often referred to as chitin nanocrystals (CTNs), chitin whiskers, or chitin nanofibers.

Because crab shells and shrimp shells have fractionated structures composed of nanofibers, the researchers believe that the separation method of CNCs is also suitable for the preparation of CTNs. The main method is acidic or enzymatic hydrolysis of chitin. However, the specific processing process of chitin varies with its sources, such as crab shells, shrimp shells, and mushrooms. Next, we will introduce the preparation of CTNs from different sources.

CTNs are produced from crab shells through a decomposition process. The usual procedure is to purify the shells by chemical treatment, followed by mechanical treatment, and then by sodium hydroxide aqueous solution and hydrochloric acid treatment to remove proteins and minerals from the shells [35, 36]. Ifuku et al. [34] studied the extraction of natural CTNs with a width of 10–20 nm from crab shells (Fig. 4.4a). It is made by simply grinding dry crab shells under acidic conditions after removing proteins and minerals. Fan et al. [37] successfully prepared dispersed CTNs in water from crab shells by TEMPO-mediated oxidation technology. These methods enable to obtain large quantities of chitin nanocrystals from discarded crab shells, and the resulting CTNs still have their original chemical and crystalline structures.

The method of preparing CTNs from crab shells is suitable for all kinds of shrimp shells because the shells are also composed of the same hierarchical structures. Ifuku et al. [38] prepared CTNs from shrimp shells through a simple grinding process after removing proteins and minerals. The CTNs prepared in an acid-free chemical environment are highly homogeneous and have a diameter of 10–20 nm, which is similar to that of crab shell nanofibers treated in acidic conditions.

CTNs can also be obtained from the cell wall of mushroom [39, 40]. Ifuku et al. [41] isolated CTNs of different widths from the cell walls of five different types of mushrooms by removing dextrans, minerals, and proteins and then simply grinding them under acidic conditions (Fig. 4.4b). Homogenous CTNs produced from mushrooms are important dietary fibers. With the development on mushroom nanofibers, they are now of great interest as functional food ingredient as well as for medical applications.

β-chitin is widely present in squid. Fan and Isogai et al. [42] prepared individualized CTNs with a cross section width of 3–4 nm and a length of at least a few microns from squid pen β-chitin. It was found that N-deacetylation of chitin molecules did not occur during the transformation of nanofibers. Although the crystallinity index was reduced, the β-chitin retained its original crystal structure. The authors suggested that the cationization of C2-amino on the surface of β-chitin of squid pen should be a necessary condition for the preparation of CTNs under acidic conditions.

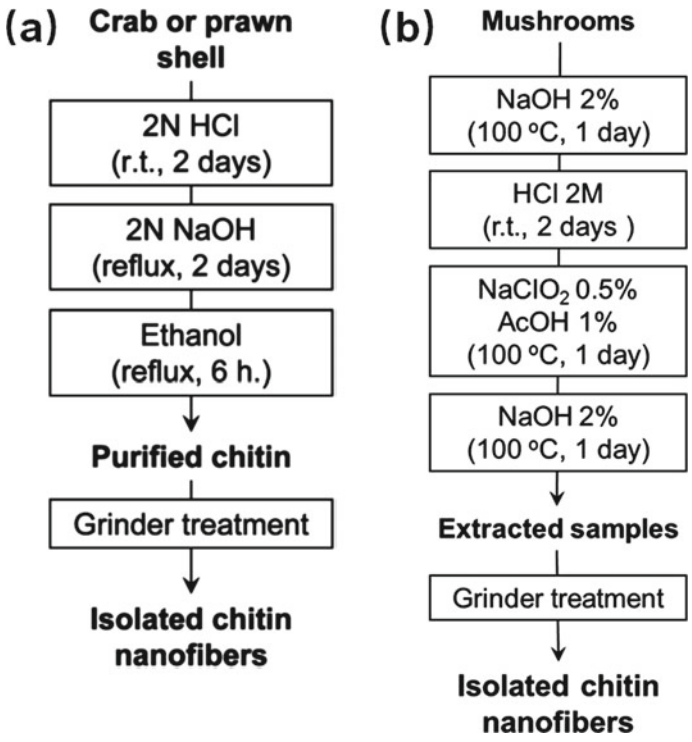

Fig. 4.4 Schematic extraction of CTNs from **a** crab or prawn shells, and **b** mushrooms. Reprinted from Ref. [34, 41]. (Copyright (2009) ACS Publishing Group and Copyright (2011) MDPI Publishing Group)

4.2 Environmental Properties

Environmental properties refer to those physical properties, such as solvent solubility, heat conductivity, mechanical and electrical properties, which relate to the environment. Due to the shortage of natural resources and increased energy needs, renewable, biodegradable, and eco-friendly materials received widespread attention. The accumulation of synthetic plastics used in packaging applications is causing serious environmental problems. This challenge has prompted the researchers to explore the use of polysaccharides such as starch, cellulose, chitin/chitosan, alginate, and animal protein polymers materials, because these biological materials are often renewably sourced and degradable via naturally existing pathways [43, 44]. Cellulose and chitin-based materials are known to be biodegradable. They can degrade in the presence of microorganisms or are susceptible to hydrolysis. Cellulose and chitin-based biomaterials offer opportunities to reduce the utilization of landfills and environmental accumulation of plastics. Therefore, it is not difficult to understand

that the environmental properties of cellulose and chitin-based nanocomposites have been studied more and more.

4.2.1 Mechanical Properties

The large specific surface area and high crystalline arrangement of cellulose and chitin nanofibers lead to impressive mechanical properties, making them potential candidates for improving the mechanical properties of pure substrates. It is generally believed that the nanofibers form percolation networks at critical concentrations, in which the aspect ratio of the fibrils and the high crystalline morphology play an important role, resulting in higher mechanical properties of the composites.

Favier et al. [15] firstly reported the synthesis of CNC-based nanocomposites. The researchers obtained lab-scale nanocomposites by reinforcing the latex with whiskers extracted from cellulose samples. The unusual mechanical property is due to the hydrogen-bonding systems in the composites, which hold the percolating network of the fibers. Wongpanit et al. [45] investigated the effect of CTNs on the dimensional stability of silk fibroin sponge nanofillers. Water suspensions of 4.63wt% CTNs were added to the solution in different proportions. The sponges produced by this method showed a network of interconnecting holes which were clearly observed by scanning electron microscopy (SEM). Compression tests showed that the mechanical properties were significantly improved by hydrogen-bonding interactions between the substrate and the chitosan nanofibers. Choi et al. [46] studied CNCs as fillers of carboxymethyl cellulose to prepare a novel hydrogel composite. The experimental results showed that CNCs significantly improved the strength and stiffness of the composites.

4.2.2 Thermal Properties

The thermal properties of materials are crucial to determine the temperature range of processing and use. With the introduction of cellulose and chitin nanoparticles into the composites, the thermal properties of the composite materials can be enhanced. Lu et al. [47] evaluated the thermal properties of polyvinyl alcohol (PVA) matrix reinforced with MFCs, which was isolated from kraft pulp by a mechanical process. The thermal stability of the MFC–PVA composite films was slightly improved with the addition of MFCs. Hariraksapitak et al. [48] prepared bone scaffolds by freeze-drying α-chitin whiskers in combination of hyaluronan and gelatin. Mechanical and thermal analyses of the scaffolds were characterized. It was confirmed that the mechanical properties of the scaffolds were enhanced by increasing the content of CTNs, and the increase of CTNs also improved the thermal stability.

On the other hand, surface modification of CNCs or CTNs with functional groups may lead to improved thermal properties. Ifuku et al. [49, 50] studied surface maleylation and naphthalylation of CTNs to enhance thermo-responsive properties. The experiment was accomplished by reacting with phthalic anhydride in an aqueous medium, where the phthaloyl group was quantitatively introduced onto the surface of deacetylated CTNs. The unique structure of the nanofiber network was maintained after phthaloacylation. The phthaloylated nanofibers were homogeneously dispersed in aromatic solvents and exhibited reversible thermal responses. Tingaut et al. [51] developed bionanocomposite materials using acetylated MFCs as reinforcing agent in poly(lactic acid) (PLA) matrix. Comparing to the PLA matrix blended with unmodified MFCs, the thermal stability was improved upon acetylation. The grafted acetyl groups reduced the hydrogen-bonding interactions among the MFCs, thus allowing better dispersion in the PLA matrix. And the acetylated MFCs could be stored in dry form, making it possible to achieve large-scale industrial production.

4.2.3 Barrier Properties

Many membranes with high barrier properties may prevent the permeation of gases, vapors, hydrophilic water, and hydrophobic toxic chemicals. Cellulose nanofillers including CNCs and MFCs can be designed into the most effective barrier membranes due to the ease of processability and their good barrier properties. For example, TEMPO-oxidized CNCs extracted from Whatman paper were used as barrier membranes in a PVA matrix. The integration of the oxidized CNCs into the nanocomposite provided a physical barrier through the creation of a tortuous path for the permeating moisture. Hence, the introduced CNCs decreased the water vapor transmission rate of the matrix. The best membrane barrier performance was achieved by the addition of 10 wt% CNCs with 10 wt% PAA.

Surface chemical modification of MFCs provided another route to improving barrier properties from the perspective of packaging applications. For example, Rodionova et al. [52] conducted research on heterogeneous acetylation of MFCs to produce membranes with good barrier properties. Compared with those of common packaging materials, the acetylated MFC films showed a similar oxygen transmission rate.

4.2.4 Biodegradability

Biodegradability is the capacity for biological degradation of materials down to the base substances such as water, methane, carbon dioxide, basic elements, and biomass. Cellulose and chitin-based products are made from natural raw materials, which are biodegradable with the help of microorganisms.

Bras et al. [53] mixed cellulose whiskers with natural rubber to study the effect of CNC content on biodegradation of the nanocomposites. They observed that the biodegradation rate increased sharply with the increase of CNC content compared to pure natural rubber. The rapid degradation of cellulose components leads to increased porosity, void formation, and integrity loss of natural rubber matrix, and thus, the overall decomposition rate of nanocomposite membranes containing CNCs was faster than that of pure rubber membranes. Miller et al. [54] investigated the biodegradation of three PLA thin-film reinforced CTN composites used for packaging applications. The results showed that the biodegradability of all the PLA-CTN composites was faster than that of pure PLA. The faster biodegradation was attributed to the presence of CTN on the surface of the composites. At the same time, the biodegradation was accelerated with the increase of filler content.

4.2.5 Antibacterial and Antifungal Properties

Antibacterial and antifungal properties are another very important properties for cellulose and chitin-based materials. Recently, the antibacterial and antifungal activities of cellulose and chitin and their derivatives have received considerable attention. Ciechańska et al. [55] obtained modified bacterial cellulose in a form of hydrogel from acetic bacterium *Acetobacter xylinum*. The resulting hydrogels had many valuable characteristics, such as good mechanical property, high moisturizing property, and most importantly, good antibacterial bactericidal activity. These features make these modified bacterial cellulose hydrogels good dressing materials for all kinds of wounds, burns, and ulcers. Dutta et al. [56] prepared *N*-halamine CTN films through the reaction of CTNs with sodium hypochlorite solution. The surface *N*-chlorination of chitin nanofiber rendered them antibacterial and antifungal activity. Gram-negative bacteria (i.e., *Escherichia coli*) and gram-positive bacteria (i.e., *Staphylococcus aureus*) as well as two examples of fungi (i.e., *Alternaria alternata* and *Penicillium digitatum*) were investigated to evaluate the antibacterial property and antifungal activity of the chlorinated CTN films, respectively. The *N*-halamine CTN films showed strong efficacies against those above bacteria and fungi.

4.3 Applications of Cellulose-Based Bionanocomposites

Cellulose-based bionanocomposites have attracted extensive attention due to their low density, low cost, non-abrasive, flammable, non-toxic, and biodegradable properties. This section specifically discusses the applications of cellulose-based bionanocomposites in various fields, including electronics device, biomedicine industry, food industry, and environmental protection.

4.3.1 Electronics Device Industry

Cellulose-based biological nanocomposites have been increasingly used in the fields of electronics industry, such as earphone diaphragm, electronic display screen, membrane electronic components, batteries, and so on.

4.3.1.1 Electronic Paper

Transmission and presentation of information have always been an important work in human communication. Among all the existing display information technologies, printed paper using cellulose as raw material has played an extremely important role since ancient times. It is still the most important and accepted media for information display owing to its high reflectivity, high contrast, high flexibility, and low cost. Along with the rapid development of computer and Internet, the traditional form of printing paper display technology clearly wasn't enough to bear the huge amount of information transmission; therefore, the standard of the digital media display technology arises at the historic moment, mainly including liquid–crystal display (LCD), cathode-ray tube (CRT), organic light-emitting diode (OLED), and plasma screen. In recent years, more and more research groups are working to combine the optical properties of paper with traditional digital screens and promote the development of the dynamic display technology of analog paper, commonly known as "electronic paper." Techniques used in so-called electronic paper include electrophoresis, bicolor beads, cholesterol-type liquid crystals, and electrowetting methods [57, 58]. A current research direction is to achieve paper-like properties with real cellulose substrates, thereby directly achieving the desired optical properties of paper.

In the past, the largest source of cellulose used in paper was plant cell walls, and the paper produced by this method was not pure enough to meet the requirements of electronic paper, until 1886 when researchers studied Acetobacter and discovered that this bacterium produces a tough gel film of pure cellulose [59–61]. On the basis of these studies, the Shah et al. [62] the preparation of the size stability has the paper shape as well as the unique microfibrils nanostructures of bacterial cellulose membrane, and through the surrounding sedimentary microfiber ions to provide a conductive path, then electrically induced color dye [63] fixed within the microstructure, thus making cellulose conductive or semiconductive films, and drive circuit, using the standard back or to build using cellulose as a substrate of high-resolution dynamic display device. The device with high reflectivity, flexibility, contrast and pattern biodegradability, and many other advantages has the potential to expand to a variety of applications, such as e-books, tablet computer, electronic newspapers, wallpapers, rewritable maps and learning tools, and even show on textiles, looking forward to providing the world with a highly adaptive and spread the effective medium of communication and education.

4.3.1.2 Organic Light-Emitting Diodes (OLEDs)

Organic light-emitting diodes (OLEDs), also known as organic electromechanical laser display or organic light-emitting semiconductor, have the advantages of self-illumination, high reaction speed, low power consumption, almost infinite high contrast, wide viewing angle, and a variety of emission colors can be selected through the molecular design of organic materials. Commercial OLEDs are made on a glass substrate. In recent years, the rapid development of optoelectronic technology makes flexible display possible, and flexible organic light-emitting display (FOLED) technology based on OLED has attracted wide attention because of its attractive display application characteristics. This technology may make highly portable and folding display technology possible in the future. The use of a suitable biocompatible flexible substrate is essential to the development of such a device. Among them, the bacterial cellulose has received a lot of attention and applications due to its unique properties and high purity [62, 64–70]. More importantly, bacterial cellulose reinforced nanocomposites have a very low coefficient of thermal expansion [65, 70, 71], which can effectively prevent strain and eventual cracking due to the mismatch of thermal expansion coefficients caused by thermal cycling when different materials are deposited on the substrate through heat treatment [72]. It is also worth noting that bacterial cellulose is an excellent enhancer for the design of renewable and biodegradable nanocomposites. Legnani et al. [73] fabricated a flexible substrate for OLEDs using bacterial cellulose film produced by *Gluconacetobacter xylinus*. They used radio frequency magnetron sputtering to deposit the thin film of indium tin oxide onto a dry bacterial cellulose film at room temperature and characterized the structure, optical, and electrical properties of the functionalized substrate. Then, they prepared the small molecule thin film with tri(8-hydroxyquinoline) aluminum (AlQ_3) compound as the luminescent layer. The results clearly showed that functionalized bacterial cellulose membranes that were biodegradable and biocompatible could be used as flexible substrates in FOLED devices. Ummartyotin et al. [74] also successfully prepared transparent flexible nanocomposites composed of bacterial cellulose and polyurethane resin and used them as substrates for OLEDs. The nanocomposite material prepared by thermal evaporation deposition showed good flexible performance. Even when it was bent, it could emit light (Fig. 4.5). This work can also demonstrate that bacterial cellulose-based nanocomposites are a promising candidate for FOLEDs.

4.3.2 Biomedicine Industry

In recent years, rapid progress has been made in the field of biomedical materials, using natural or synthetic polymers for a variety of applications. Due to their unique nanostructure and properties, cellulose-based nanomaterials have proven to be natural candidates used in a very wide range of medical applications, including

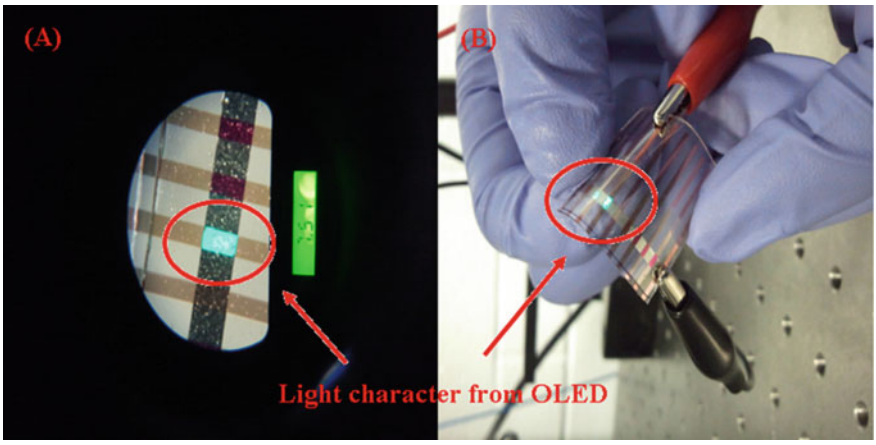

Fig. 4.5 OLEDs on bacterial cellulose nanocomposite. Reprinted from Ref. [77]. (Copyright (2012) Elsevier Publishing Group)

wound healing, vascular grafts, and scaffolds for tissue engineering in vitro or in vivo [75–80].

4.3.2.1 Wound Healing and Artificial Skin

The healing of large areas of skin wounds and even the development of artificial skin are significant medical advances [81]. Wound healing is a complex interaction involving many aspects and a series of processes, in which the healing of chronic wounds, such as ulcers, is particularly important in a series of steps. Various types of wound dressings have been developed and used over a long period of time in order to eliminate adverse environments in chronic wounds and promote proper healing [82–84]. Similarly, burns are an injury that causes extensive and complex damage to skin tissues, and many different wound dressings have been developed [85–90]. Cellulose-based pads, membranes, films have been found to be very effective for improving the healing process by reducing pain and accelerating granulation growth, which is very important for proper wound healing.

Fontana et al. [91] reported that a thick, smooth, and floating cellulose film prepared from *Acetobacter* cultured in a specific medium could be applied to exudate or bleeding tissue as a biological dressing. It is therefore concluded that it is valuable as a temporary skin substitute in the treatment of skin wounds, such as burns and ulcers, as well as an adjunct in the treatment of severe abrasions. Czaja et al. [92] used never-dried microbial cellulose membranes to treat patients with severe second-degree burns and found that the wound healed significantly faster than conventional dressings. Moreover, these cellulose membranes can be made in any shape and size, which is good for treating large wounds and some hard-to-cover areas.

Bacterial cellulose membranes can accelerate the healing process, resulting in a more effective wound dressing material without any change in its beneficial properties. Barud's team [93] developed a biofilm containing bacterial cellulose and standardized propolis, showing a good treatment for burns and chronic wounds. Legeza et al. [94] investigated the effect of a novel bacterial cellulose wound dressing impregnated with superoxide dismutase and PVA on the repair process of deep skin burns in rats. Studies have shown that the healing effect of the burns is significantly accelerated.

4.3.2.2 Tissue Engineering

Tissue engineering looks for new materials and devices that can actively interact with biological tissue as an in vitro basis for cell growth, or for rearranging and developing the tissue to be implanted. Therefore, biocompatible and biodegradable cellulosic materials have come into the attention of scientists and have been employed for in vitro tissue reconstruction or cell scaffolds [95–97]. The chemical surface of cellulose can determine the reaction of cells by interfering with cell adhesion and proliferation, and effectively overcome the mutual repulsion between surface cells, and even be absorbed or biodegradable after a period of time [98–100].

Atherosclerotic vascular diseases continue to be one of the leading causes of death in today's society. Treatment methods and means for cardiovascular diseases have been studied and explored by several generations [101–107]. Surgical treatment of atherosclerosis began in 1952 when Voorhees et al. [101] hypothesized the replacement of diseased vessels with synthetic fibers. However, the small-diameter grafts ultimately failed due to occlusion. Synthetic implants are still found to cause low levels of foreign body reaction and chronic inflammation, and as artificial materials, they increase the risk of microbial infection. Therefore, artificial blood vessel is a biomaterial with characteristics tailored for specific cardiovascular device applications, while taking characteristics such as durability, biocompatibility, prothrombin, and hypocalcification into account [108–112].

Millon et al. [113] developed a PVA-bacterial cellulose nanocomposite with properties similar to heart valve tissue. PVA can be converted into solid hydrogels with good mechanical properties by physical cross-linking using a freeze–thaw cycle [111, 114–120]. It is combined with the bacterial cellulose produced by the bacterium *Acetobacter xylostella* through the fermentation process to form a nanocomposite material showing mechanical properties similar to soft tissue and extensive performance control. Therefore, the PVA-bacterial cellulose nanocomposite is a promising alternative material for cardiovascular soft tissue.

Cellulose nanocomposites such as the above used in wound dressings, artificial skin, and vascular stents are also promising materials as potential scaffolds for cartilage tissue engineering [121–125]. In the past studies, hydroxyapatite-collagen nanocomposites have been used for a large proportion [126–129]. However, in contrast to the high cost and difficult to control cross infection of many factors, such as collagen, the combination of natural polymer composites with hydroxyapatite is

expected to provide high mechanical properties, enough, controllable pore diameter and porosity in situ formability, high water-holding ability, excellent biocompatibility and biodegradability of adjustable and good bone conduction and bone union and other significant features [130–133]. The tight attachment or integration between cartilage tissue and implant surface is a key factor for the successful implantation of biomaterials in orthopedic applications. The key points for the normal functioning of biomaterials are good biocompatibility, bioactivity conducive to bone attachment, and sufficient mechanical properties [134–140].

Normal cartilage can be considered as a multifunctional hydrogel, so the use of hydrogels to develop cartilage substitutes is also a potential direction for the development of artificial cartilage [141–147]. Yasuda et al. [148] developed a cellulose/polydimethylacrylamide (PDAAM) gel composed of bacterial cellulose and PDAAM and confirmed the wear properties of the novel double-network hydrogels using a plain wear test. The studies indicated that this unique gel material has great potential as an artificial cartilage.

Applications of tissue engineering are also seen in the field of ophthalmology [149], nasal reconstruction [150], and tooth tissue regeneration [151–155]. Jia et al. [149] explored the potential of nanocellulose as a tissue-engineered corneal scaffold. They studied the growth of human corneal stromal cells on nanocellulose. The results indicated the potential of the biomaterial as a tissue engineering scaffold for corneal prosthesis. Amorim et al. [150] studied the tissue response of rabbit nasal dorsum to cellulose. After six months, the histological evaluation of the treatments revealed that the cellulose blanket of *Acetobacter xylostacter* had good biocompatibility and remained stable throughout the study. So it could be used as good materials for nasal dorsal enhancement. Novaes et al. [152] reported that a nanocellulose called Gengiflex was used in the dental tissue regeneration. Gengiflex was composed of inner microbial cellulose layer and outer chemically modified alkaline cellulose layer. The inner layer offered rigidity to the membrane. The synthetic hydroxyapatite was utilized as grafting material in the dental cavities, and the Gengiflex membrane was employed to cover the implant. After 6-month reentry, a complete restoration of the defect was observed (Fig. 4.6).

4.3.2.3 Drug Delivery

Cellulose materials have excellent compaction performance when mixed with other pharmaceutical excipients, which makes the tablet carrier form a dense matrix suitable for oral administration [156–158]. On the other hand, nanocellulose has potential advantages as an excipient for drug delivery. Its relatively large surface area and negative charge give it a high payload and the potential for optimal dose control. The rate of tablet disintegration and drug release can be controlled by particle inclusion, excipient delamination, or tablet coating. For example, Baumann et al. [159] reported the development of a series of physical hydrogel mixtures composed of hyaluronic acid (HA) and methylcellulose (MC), which are planned for independent delivery of one or more drugs and ultimately for spinal cord injury repair (Fig. 4.7). In a similar

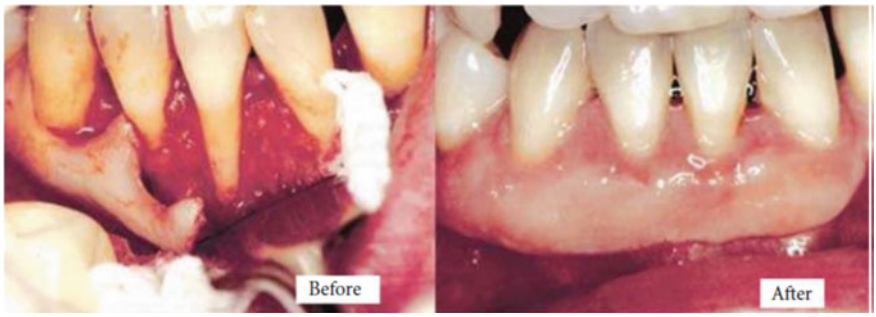

Fig. 4.6 A nanocellulose called Gengiflex used in dental tissue regeneration. Reprinted from Ref. [96]. (Copyright (2011) Hindawi Publishing Corporation)

manner, Jeganathan's team [160] prepared pH-dependent and modified release tablets using anion hypromellose acetate succinate polymers and cationic Eudragit E polymers to deliver DS in the lower GI tract via an LBL adsorption method. They found that the release of the drug from the polymer coating is pH-dependent and found that the addition of citric acid helps to change the microenvironmental pH of the drug preparation, thus controlling the release of the drug from the drug preparation, such as non-ionized and hydrophobic drugs.

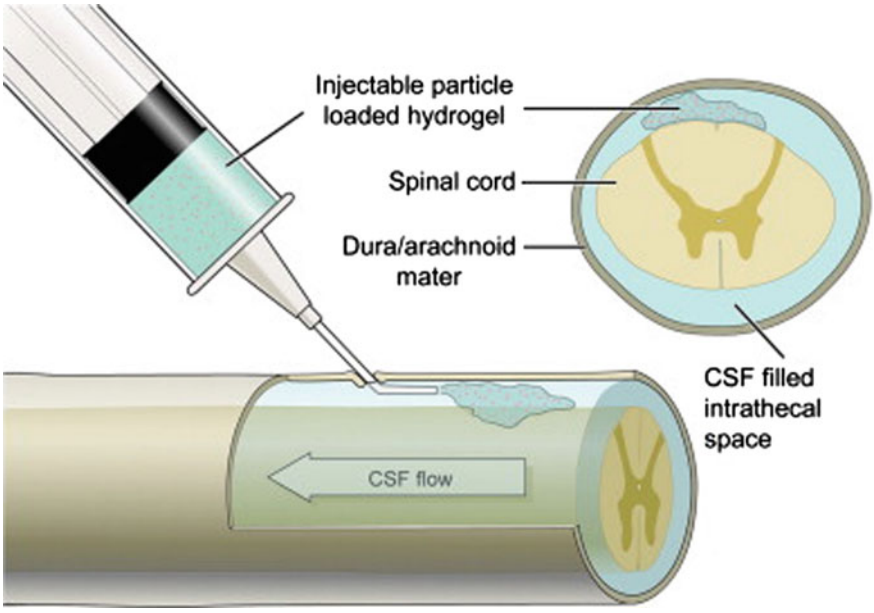

Fig. 4.7 An intrathecal drug delivery system by HA/MC composite hydrogel. Reprinted from Ref. [159]. (Copyright (2009) Elsevier Publishing Group)

4.3.3 Food Industry

Nanocellulose and its derivatives have developed their applications in the food industry because of their high surface area, length–diameter ratio, rheological properties, hydroscopicity, and non-cytotoxicity and genotoxicity. This part mainly introduces three different applications: (1) as food additives, (2) as functional food ingredients, and (3) in food packaging applications.

4.3.3.1 Food Additives

Since Turbak and his colleagues [161] first considered nanocellulose as a food additive in 1983, nanocellulose and its derivatives have become increasingly widely used in food additives. It has been found that nanocellulose tends to stabilize oil-in-water emulsions because it is wetted by water more easily than oil. It is not only an excellent suspension medium for other solids, but also an emulsifying substrate for organic liquids. Therefore, nanocellulose and its derivatives can be used to stabilize oil or fat in food and are widely used in the preparation of cake icing, salad dressings and sauces, as well as stabilizers in cream, etc. [162–166].

In the preparation of surimi-based product, such as traditional Chinese fish balls, lard is usually added to make the heated product smoother mouthfeel. However, Lard is considered to be less healthy because it has about half as much saturated fat as butter. Considering both health and taste requirements, the scientists tried to find lard alternatives. Yoon et al. [167] used vegetable gum and cellulose gel instead of lard to enhance gel intensity and cooking tolerance. Later, Lin et al. [168] added a highly absorbent bacterial cellulose to Dolphin-Fish Surimi to evaluate the properties of the composite gel. The results showed that alkali treatment changed the structure of bacterial cellulose and formed a dense porous network. This alkali-treated bacterial cellulose showed high water retention. Therefore, it is speculated that the use of alkali-treated bacterial cellulose as a fat substitute and additional dietary fiber source in processed fish surimi products is feasible. Chen et al. [169] modified the surface of nanocrystalline cellulose with food-grade octenyl succinic acid to improve its surface hydrophobicity and significantly improve its emulsification performance, which is conducive to the preparation of Pickering high internal phase emulsion. Stable and gelatinous Pickering high internal phase emulsions with fine droplets can be easily prepared using nanocrystalline cellulose modified by this method, even at very low particle concentrations in the aqueous phase. This will promote a wide application of nanocrystalline cellulose in emulsion formulation in food fields.

4.3.3.2 Food Ingredients

Turbak and his colleagues also discovered the potential of nanocellulose in preparing fat-reducing formulations, demonstrating that nanocellulose could replace oil in the

production of low-calorie salad dressings [163]. In addition, Dell Chemical Industries has also developed the invention of using nanocellulose as a functional food ingredient that uses nanocellulose and water-soluble sugars to treat intestinal diseases [170].

At the same time, nanocellulose is a dietary fiber that has a beneficial effect on the overall health of adults, helping to decrease the risk of chronic diseases as well as promote beneficial physiological effects, such as lowering blood cholesterol and blood sugar [171–179]. Nanocellulose can be considered as a potential functional cellulose with dietary fiber properties for the treatment of intestinal diseases.

Nanocellulose can be used to produce low-calorie foods to treat abnormal weight [180–182]. Nanocrystalline cellulose can be used as non-nutritive fillers and high-calorie materials such as sugar and fat substitutes in functional foods. Nanocrystalline cellulose has good emulsification and can be treated in a specific way to obtain a greasy feeling similar to fat, so it can be used as a fat substitute [166].

4.3.3.3 Food Packaging

The current food packaging market requires high performance, biodegradable films with good mechanical properties, optical transparency, thermal stability, and high gas resistance [183]. And the barrier property is critical for evaluating and predicting the shelf life of packaged products. Because of their positive impact on mechanical properties and low permeability to oxygen, cellulose nanoparticles have been used as fillers for pure thin films to produce sustainable packaging [184–191].

Ashori et al. [192] chemically modified cellulose nanofibers (CNFs) with acetic anhydride using pyridine as catalyst to change their surface properties. Contact angle measurements confirmed that the surface properties of acetylated CNFs changed from hydrophilic to hydrophobic. Through such chemical treatment, the water barrier performance of the CNFs was improved. Rodionova et al. [52] used acetic anhydride to heterogeneous acetylation of MFCs, which reacted with hydroxyl groups on the cellulose molecules, thus making the hydrophilic surface change into hydrophobic. The surface acetylation of the MFCs appears to be a promising method to develop MFC films with good barrier performance for liquid water and excellent resistance to oxygen. These surface modification methods can improve the barrier performance of the cellulose materials, which is very important for sustainable food packaging.

4.3.4 Environmental Protection

With the consumption of primary energy, the concept of green chemistry of chemical fiber products is gradually moving away. Therefore, the development and utilization of cellulose-based bionanocomposites as the main body are also in line with the goal of sustainable development and environmental friendliness. Therefore, the application prospect of cellulose modification technology is broad, and it is mostly used for

the adsorption treatment of sewage, such as the adsorption treatment of N, P, As, Cr in water. At the same time, the research and application in the field of air purification are also increasing gradually.

4.3.4.1 Water Purification

Nowadays, the world is faced with the severe problem of water shortage. In addition, the production of a large amount of wastewater also brings great pressure to human beings. Traditional wastewater treatment technologies such as coagulation, oxidation, electroprecipitation, ion exchange, membrane separation, floating evaporation are not enough to deal with the increasingly serious problem of water pollution. Therefore, the development of new wastewater treatment technologies has become an urgent problem to be solved. It is well known that adsorption is one of the best technologies for water purification. Although activated carbon filtration is a commonly used technology based on the adsorption of contaminants onto its surface, it does not save money or energy. Therefore, adsorption using low-cost adsorbents has become a new research direction.

New functional materials based on biopolymer have come into people's sight. Cellulose is an attractive choice because it is rich in sources, renewable, non-toxic to the environment, low-cost, biodegradable, and biocompatible. With the progress of nanoscience, it is possible to develop cellulose-based materials with nanoscale size. Cellulose-based nanocomposites have been widely used in the adsorption of heavy metal ions in wastewater to achieve the purpose of removal, enrichment, and recovery. Compared with the general heavy metal treatment methods, the modified cellulose adsorbent for adsorption, separation, and extraction of heavy metal ions in wastewater has the advantages of large adsorption capacity, fast adsorption speed, low cost, simple operation, and no secondary pollution [193–203]. Gouda et al. [204] successfully synthesized cellulose-graft-polyacrylonitrile (cellulose-*g*-PAN) nanofibers. The cellulose-*g*-PAN nanofibers loaded with silver nanoparticles (AgNPs) have excellent antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi,* and *Escherichia coli*. So the cellulose-*g*-PAN/AgNPs bionanocomposites can be used for water disinfection. Liu et al. [205] studied the introduction of phosphate groups on nanocellulose as biological adsorbents aimed at removing metal ions (e.g., Ag^{+}, Cu^{2+}, and Fe^{3+}) from industrial wastewater (Fig. 4.8). Studies have shown that phosphorylated nanocellulose was a highly effective biomaterial for simultaneous removal of various metal ions from industrial wastewater.

At present, clays such as montmorillonite are of particular research interest as dye adsorbents, but they have proved ineffective in the treatment of anionic dyes [206, 207]. Zhao et al. [208] prepared carboxymethyl cellulose/montmorillonite nanocomposites by solution intercalation. It was found that the higher temperature and acidic conditions are favorable for the adsorption of chromium on carboxymethyl cellulose/montmorillonite nanocomposites.

The introduction of magnetic particles into cellulose matrix has been explored as a promising modification method for removing arsenate. Nata et al. [209] prepared

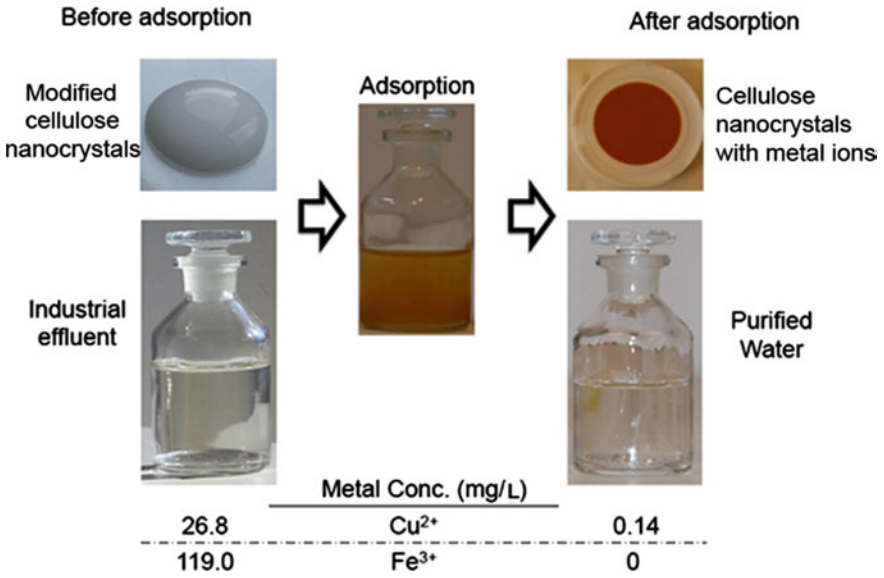

Fig. 4.8 Adsorption of metal ions Ag^{+}, Cu^{2+}, and Fe^{3+} (≈100%) from industrial wastewater by phosphorylated nanocellulose. Reprinted from Ref. [205]. (Copyright (2015) Elsevier Publishing Group)

surface-functionalized bacterial cellulose nanofibers with aminated magnetite nanoparticles by one-pot solvothermal reaction of 1,6-hexanediamine, $FeCl_3 \cdot 6H_2O$ and bacterial cellulose, significantly improved the thermal and mechanical properties of nanostructured bacterial cellulose films. The amine-rich magnetic cellulose nanocomposite can be used as an efficient recyclable absorbent for the removal of arsenate.

Printing and dyeing wastewater is a kind of important pollutants that cause serious environmental problems and harmful to humans. Therefore, the removal of pollutant dyes from wastewater is critical to the environment. Cellulose-based nanocomposites containing polyhedral oligomeric silsesquioxane can be used as new biological adsorbents for organic dyes. Xie et al. [210] investigated the adsorption properties of nanocellulose hybrid materials for reactive dyes in aqueous solution. It was found that the removal ability of the nanocomposites was much higher than that of the control cellulose. Therefore, it is concluded that nano-cellulose hybrid material as a biological adsorbent has potential application value in low concentration printing and dyeing wastewater.

4.3.4.2 Air Purification

In addition to water purification, in the field of air purification, activated carbon particles or fibers have been used as air adsorption filtration materials for a long

time. Although activated carbon has the characteristics of a wide range of application, because its adsorption process is physical behavior, so it is not suitable for high temperature, high humidity conditions. At the same time, its adsorption of some polar gas molecules (such as SO_2, NH_3, and H_2S) is often completed after impregnation with a variety of chemical catalysts, so the reproductivity is very poor, usually belongs to the disposable non-renewable materials. In contrast, the natural fiber-modified ion exchanger is a reversible chemical reaction to complete the separation and enrichment of various polar molecules. Moreover, it can be prepared into the appropriate fabric shape, so that it can provide a considerable filtration area in a small volume operating unit, giving it excellent permeability stability, low resistance to airflow characteristics. Therefore, it can be used in air purification units or gas masks and masks in the form of packed exchange columns or woven fabrics to achieve the purpose of air purification.

4.4 Applications of Chitin-Based Bionanocomposites

Chitin shows significant similarities to cellulose. So besides cellulose, the application of chitin-based nanocomposites is also attracting attention. This section specifically discusses the applications of chitin-based bionanocomposites in various fields, including biomedicine industry, environmental protection, food industry, agriculture, and cosmetics.

4.4.1 Biomedicine Industry

Due to their non-toxicity, biocompatibility, bioabsorbability, low antigenicity, and good antimicrobial property, chitin and its derivatives are proved to have extensive applications in wound healing and dressing, scaffolds for tissue engineering as well as hydrogels for drug delivery systems in biomedicine industry [211–218].

4.4.1.1 Wound Healing and Dressing

Chitin has special biochemical significance, especially it can accelerate the migration of macrophages and the proliferation of fibroblasts, and promote the formation of granulation and blood vessels. When the chitin salt and chitin nanofibers acted synergistically, the wound healing ability was significantly enhanced. Muzzarelli et al. [219] prepared three forms of wound medicaments (i.e., spray, gel, and gauze) by combination of chitin nanofibrils, chitosan glycolate, and chlorhexidine. The gauze was found to be the most effective in all the dressings. It could induce better epithelial differentiation and keratinization as well as better reorganization of the basal lamina in healing injuries or ulcers (Fig. 4.9).

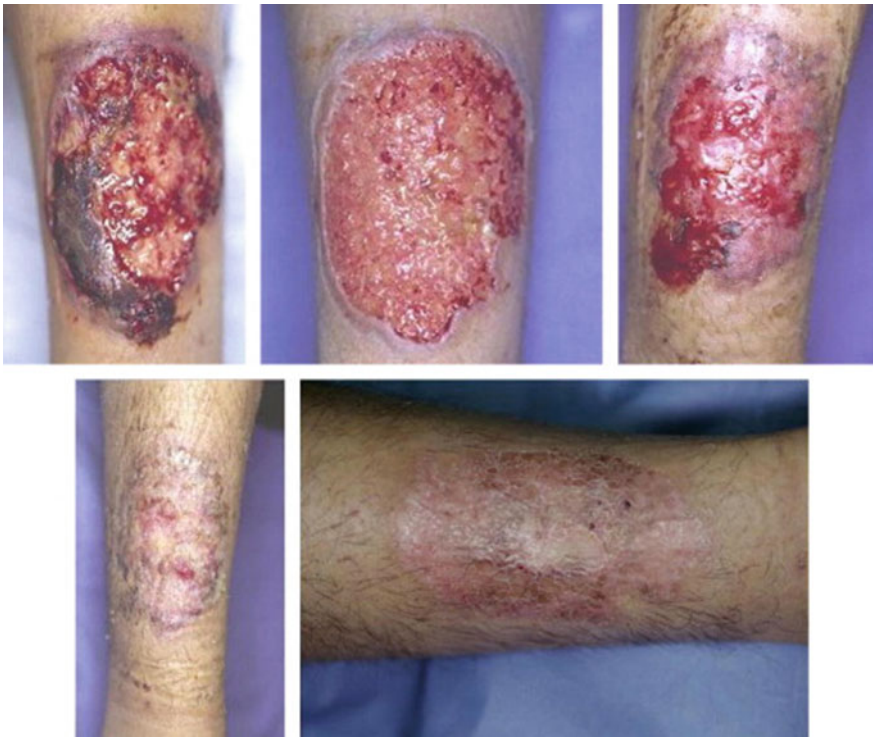

Fig. 4.9 Gangrenous pyoderma on the surface of the tibia healed within 40 days treated by the gauze made from composite of chitin nanofibrils, chitosan glycolate, and chlorhexidine. Reprinted from Ref. [219]. (Copyright (2007) Elsevier Publishing Group)

Naseri et al. [217] successfully prepared electrospun chitosan-based random-oriented fiber containing 50 wt% chitin nanocrystals as reinforcers. The results show that due to the uniform dispersion of chitin nanocrystals in the chitosan matrix, there are no defects in the prepared electrospinning porous random felt, which indicates that the matrix has good chemical compatibility with chitin. At the same time, the addition of chitin nanocrystals improved the water stability of primary felt and promoted the water-mediated cross-linking process. This nanocomposite with improved mechanical properties and flexibility, combined with its biocompatibility, was considered as potential candidates for wound dressing applications.

4.4.1.2 Tissue Scaffolds

Tissue scaffolds made from natural polymers exhibit poor mechanical properties, which may limit their practical application in some demanding areas. In essence, chitin is a kind of scaffold material that is beneficial to cell adhesion and growth. Therefore, chitin nanofibers, the product of acid treatment, can be used as both

enhanced nanofillers and bioactive reagents to prepare scaffolds in tissue engineering. That means the addition of chitin nanofibers or whiskers to the scaffolds can improve their mechanical properties, thermal stability, and biodegradability. Along this line of thought, Hariraksapitak et al. [48] successfully prepared continuously enhanced hyaluronic acid gel nanocomposite scaffolds by freeze-drying method. It was found that the properties of the composite scaffolds could be adjusted by changing the amount of chitin whiskers, so as to achieve an optimal balance between their physical, chemical, mechanical, and biological properties. It was finally proved that the high proportion of chitin whiskers added enhanced the thermal stability and biodegradation resistance of the scaffolds, while the relatively low proportion of cellulose whiskers enhanced the tensile strength and increased the biocompatibility of adhesion and proliferation of human osteosarcoma cells, showing a promising prospect as a bone cell culture medium.

4.4.1.3 Drug Delivery and Release Control

Zhang et al. [220] were the first to incorporate cellulose and chitin nanocrystals into supramolecular hydrogels combined with cyclodextrin/polymer inclusion complexes. The data showed that the elastic modulus of these composite hydrogels was increased to 50 times than that of natural hydrogels. The addition of polysaccharide nanocrystals has shown many advantages, such as accelerated gelation, enhanced mechanical strength, improved corrosion resistance of the solution, and promoted long-term sustained drug release. In addition, the cell viability assessment confirmed that the addition of polysaccharide nanocrystals to supramolecular nanocomposite hydrogels does not induce additional cytotoxicity, which is the basis for biomedical application. Therefore, the supramolecular nanocomposite hydrogels doped with polysaccharide nanocrystals seem to be good candidates for drug delivery and release control systems with injection and implantation functions.

4.4.2 Environmental Protection

Environmental protection has become an increasingly important global issue, and all industries are concerned about the development of technologies to solve environmental problems. Chitin-based bionanocomposites for environmental protection have attracted more and more considerable attention. This part mainly introduces the applications of chitin-based bionanocomposites in the removal of dyes, organic and inorganic pollutants, and the remediation of metal pollution.

4.4.2.1 Removal of Organic Pollutants

Chitin as organic pollutant adsorbents can also be used to treat colored wastewater by adsorption. However, the use of chitin for dye removal has extensively been studied because of its low surface area, high porosity, and high crystallinity. Therefore, it is very necessary to find a technology that can change the physical structure of chitin to expand its application as dye adsorbents [221–228].

Tang et al. [229] successfully prepared a chitin-based hydrogel from a concentration of 3% chitin solution dissolved in 8% sodium hydroxide and 4% urea aqueous solution as raw material and then cross-linked with 5% epichlorohydrin at low temperature. The experimental results showed that the chitin-based hydrogel had a high removal capacity on malachite green dye in aqueous solution because of its microporous structure, large surface area, and strong affinity to the dye (Fig. 4.10).

Wang et al. [230] prepared a solar photocatalyst for the in situ synthesis of cuprous oxide in a regenerated chitin/graphene oxide composite membrane using porous chitin membrane as a microreactor. Cuprous oxide in the matrix excited and generated free photoelectrons and electron holes, leading to the degradation of dyes. The graphene oxide sheets promoted the transfer of photoelectrons, leading to a significant increase in photocatalytic activity under sunlight. The results show that the porous chitin film can support cuprous oxide and graphene at the same time and make the photocatalyst easy to recover and reuse. This portable solar photocatalyst has the advantages of high efficiency and easy recovery and is expected to be used in water treatment.

In addition, organic contaminants such as melanoids, which are widely distributed in food and beverage, also need to be addressed. Dolphen's team [231] studied the adsorption of synthetic melanoids by CTNs prepared from shrimp shell waste, and the results showed that chitin nanofibers have a promising application in the adsorption of melanoids. The results of Fourier transform infrared spectroscopy and elution studies

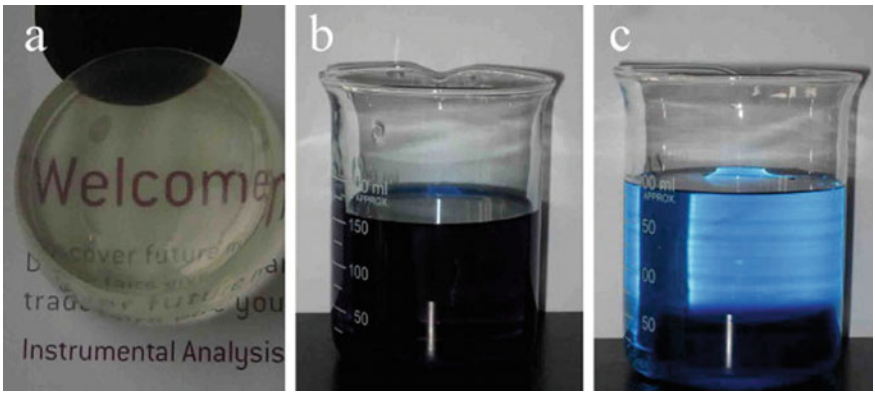

Fig. 4.10 Adsorption of malachite green in wastewater by a chitin-based hydrogel. Reprinted from Ref. [229]. (Copyright (2012) Elsevier Publishing Group)

also confirm that the interaction between melanoid and chitin nanofibers includes electrostatic adsorption and chemical adsorption. The authors also suggested that chitin nanofibers could be used to adsorb melanoids and other pigments in syrup in sugar industry.

4.4.2.2 Removal of Inorganic Pollutants

Metal is the main inorganic pollutant in the world. Removing toxic metals from water is very important in water treatment. Trace toxic metal ions are difficult to remove from aqueous solution. The use of low-cost adsorbents attracts considerable attention [232–242]. Biosorption of biogenic materials such as chitin-based nanocomposites is also considered an emerging technology for treating water containing heavy metals.

Gandhi et al. prepared a polymer composite composed of hydroxyapatite (HAP) and chitin and studied its ability to remove Cu^{2+} from aqueous solution. The adsorption capacities of N-HAPC and N-HAP/chitin composites were 4.7 and 5.4, respectively, indicating that the adsorption capacity of N-HAPC composites was relatively higher than that of HAP. Kousalya et al. [243] modified chitin appropriately, such as protonated chitin, carboxylated chitin, and grafted chitin, to improve the metal adsorption capacity and investigated their adsorption properties for copper and iron ions. The results show that these adsorbents can effectively remove copper and iron. Saravanan [244] conducted a batch adsorption study of hexavalent chromium in aqueous solution using a chitin complex. The results show that chitin complex can be used as an efficient biological adsorbent with good metal binding ability to remove chromium ions from aqueous solution. It is also suggested that this adsorbent is not only suitable for the adsorption of chromium ions, but also suitable for other heavy metal ions in wastewater. Karthik et al. in situ synthesized polypyrrole functionalized chitin for its application in the removal of hexavalent chromium from aqueous solutions. The results showed that the polypyrrole functionalized chitin had a medium adsorption capacity to remove chromium ions from aqueous solution. Hanh [245] grafted acrylonitrile onto deacetylated chitin by radiation-induced polymerization method to produce PAN-grafted chitin bionanocomposites. The nitrile groups on the surface of chitin were further converted into amidoxime which greatly enhanced the adsorption capacity of metal ions. These PAN-grafted chitin bionanocomposites could also be used to treat arsenic contamination in groundwater or drinking water.

4.4.3 Food Industry

Chitin and its derivatives occupy an important position in the application of food industry. Previous studies have fully proved that chitin and its derivatives are nontoxic. They are natural macromolecular compounds with special amino and hydroxyl functional groups on the molecular chains. Therefore, compared with many synthetic macromolecular compounds, they are more suitable for use in food industry.

Chitin can stabilize the oil–water interface because of its amphiphilic structure of sugar ring and thus can be used as food additive to adjust the texture of food. High internal phase emulsions (HIPEs) have become an ideal choice not only for food, but also for porous material templates, multiphase soft materials, tissue engineering, and other fields [246–249]. Emulsions can be stabilized by Pickering stabilization of colloidal particles, which effectively prevents droplet coalescence. Compared with surfactant stabilized emulsions, Pickering emulsions provide superior stability at a relatively low particle dosage [250, 251]. Chitin stands out as food grade, biodegradable, biocompatible, and non-toxic. For example, chitin nanocrystals prepared by hydrochloric acid hydrolysis can be used as a Pickering stabilizer for cetane water coating [252]. Zhu et al. [253] successfully prepared a stable oil-in-water high internal phase Pickering emulsion using a simple two-step strategy. The results show that chitin nanocrystalline stable HIPEs can meet the requirements of food and green material cleaning labeling.

Ge et al. [254] reported that CTNs as a reinforcing nanofiller were integrated into gelatin to the enhancement of mechanical properties and gelling ability of the gelatin. The resulting gelatin/CTN composite hydrogels had more compact network structure because of strong hydrogen bonding and electrostatic interaction between chitin nanocrystals and gelatin. Thus, the nanocomposite hydrogels showed better stability than pure gelatin hydrogel. The authors assume that these improved gelatin/CTN composite hydrogels will be widely used in the food industry. Yuan et al. [255] prepared stable positively charged CTN suspensions by a simple microfluidization method without changing the chemical structure. A novel soybean protein gel with adjustable texture properties was obtained by cross-linking with glutamine transaminase, which is attributed to the high mechanical properties of CTNs and their strong interaction with soybean protein. On the other hand, studies have found that after moderate deacetylation of CTNs, more amino group exposure will lead to stronger antibacterial activity. Therefore, partial deacetylation of nano-chitin as material reinforcement agent can also give certain antibacterial properties to the composites, which has a great application prospect in the food industry [256].

Chitin and its derivatives can also be used in functional foods. Hyperlipidemia is a major cause of coronary atherosclerosis and subsequent associated cardiovascular disease, which has been associated to some extent with obesity. The traditional treatment for hyperlipidemia is lipid-lowering drugs. These synthetic drugs are effective, but they can also cause adverse reactions. Therefore, the lipid-lowering activity of many bioactive ingredients extracted from natural materials such as polysaccharides and dietary fiber has been explored [257–260]. Studies have shown that chitin and its derivatives can reduce plasma cholesterol, which plays a crucial role in the prevention and treatment of cardiovascular diseases. Ye et al. [261] prepared a partially deacetylated α-chitin nanofiber/nanowhisker mixture (DEChNs) using 35% sodium hydroxide and followed by hydrolysis at pH 3–4. To study the hypolipidemic effects of different doses of DEChNs on male Kunming mice. Histopathological examination of liver cells showed that DEChNs effectively reduced the accumulation of lipids in the liver and prevented the development of fatty liver. The results showed

that DEChNs reduced the absorption of dietary fat and cholesterol and effectively reduced hypercholesterolemia in mice.

4.4.4 Agriculture

Chitin coming from a wide variety of sources is safe and non-toxic and has a wide range of use in agriculture. Chitin-based bionanocomposites can be employed as plant growth regulator, soil improver, plant disease inducer, seed coat agent, drought-resistant agent or water-retaining agent, fruit and vegetable preservative, feed additive, pesticide carrier, degradable mulching film, and so on.

As early as 1963, Michele et al. directly added lobster shells or chitin to the soil, and they found that doing so could effectively reduce plant diseases caused by pathogenic bacteria in the soil. The chitin-breaking bacteria in the soil that the researchers analyzed produce the enzyme chitin, which not only reduces the biological activity of some fungi but also kills nematode eggs. And microbes can not only break down chitin to provide nutrients for plants, but also use it to improve their systems. Once the microbial flora is improved, soil aggregates can also be improved. Chitin and chitosan can be used as seed coating materials because of their excellent film-forming properties. Researchers at the University of Washington coated wheat seeds with chitosan and found that the treatment not only protected them from damage by soil fungi during the winter, but also prevented them from a disease that rots the roots of wheat.

4.4.5 Cosmetics

Chitin derivatives, especially chitin-based bionanocomposites, have been used in the field of cosmetics because of their safety, non-toxicity, good biocompatibility, film forming permeability, anti-static, and moisturizing properties. Many countries have used chitin and its derivatives as matrix to produce cosmetics, and more than 70 kinds of products have been sold. They are mainly used for shampoo, hair lotion, hair gel, skin care night cream, sunscreen, advanced antiseptic bath liquid, advanced soap, lipstick, skin disease health cream, and so on. For example, CTNs functionalized with quaternary ammonium salt are integrated into shampoo to obtain the characteristics of anti-static, dustproof, easy to comb, and promoting hair growth. Baths, hand cleaners, and face cleaners prepared with 2–15% CTNs have the effect of preventing skin diseases such as rough skin and prickly heat. Moreover, they are also very effective in the removal of body odor, antipruritic, antibacterial, and other aspects.

Shervani et al. [262] explored possible application of CTNs loaded with gold nanoparticles (AuNPs) in different fields including cosmetics. As CTNs and AuNPs both have antibacterial properties, the obtained organic–inorganic hybrid CTN-AuNPs composites may double dose effect when CTNs are conjugated with the

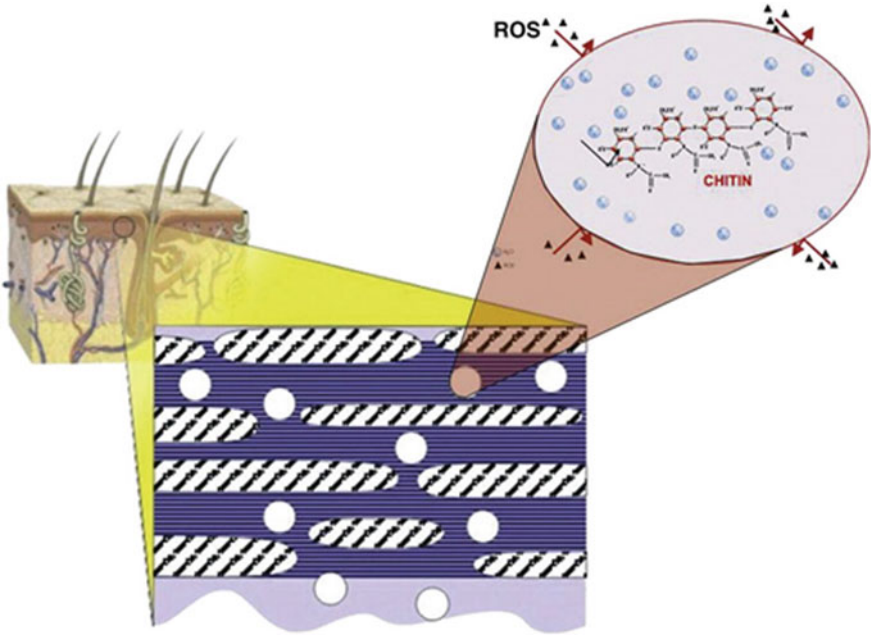

Fig. 4.11 Chitin nanofibrils for advanced cosmeceuticals by maintaining cutaneous homeostasis. Reprinted from Ref. [263]. (Copyright (2008) Elsevier Publishing Group)

metallic nanoparticles. The composites molded in thin film would be as suitable for use in cosmetics. Morganti et al. [263] also studied CTNs for advanced cosmeceuticals in terms of promoting health and beauty. They suggested that CTNs appear to help maintain the stability of the skin's internal environment and the activity of neutralizing free radicals and represented a natural carrier conducive to transdermal penetration of many active ingredients (Fig. 4.11).

4.5 Conclusion

The growing demands for new functional materials have encouraged the use of bio-based materials. Due to environmental factors and their biocompatibility, biodegradability, and renewable source, biopolymers, in particular cellulose and chitin may serve as attractive biomaterials for many applications. The ease of physical processing and chemical modification of these biopolymers attracts more and more attention to engineer new biomaterials and their based bionanocomposites. A wide variety of studies focused on improvement strategies to enhance the properties and functions of the bionanocomposites. The main advantage is that the nanocomposite materials always exhibit stronger mechanical, physical, and chemical properties than

their constituent materials. The development of new biomimetic composites from cellulose and chitin can be foreseen in the future.

Although cellulose- and chitin-based materials have been extensively investigated and performed well in numerous applications, the research of their based bionanocomposites has not yet made significant discoveries and breakthroughs, and thus, further explorations are still needed. Moreover, there are still many challenges regarding control of mechanical and physical properties of these bionanocomposites. For further applications, potential risk of acute and chronic toxic effects is the foremost challenge to face.

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Chapter 5
Polylactic Acid/Halloysite Nanotube Bionanocomposite Films for Food Packaging

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5.1 Introduction

The average production of agricultural products globally has reached 23.7 million tons of food per day in recent years, while the destruction and waste of food annually account for a significant amount of this reform [1]. The Food and Agriculture Organization (FAO) estimates that about 1.3 billion tons of food is wasted annually, or about 15% of total annual production [2]. This number is equivalent to 33% of the average daily consumption of an adult. The U.S. Department of Agriculture (USDA) says a significant portion of this food spoilage occurs at the retail level, most related to vegetables and fruits [3]. Smarter use of food packaging can significantly reduce the waste of various foods [4].

Today, a wide range of polymers are used to produce food packaging films [5]. Among these materials, synthetic polymers (of petroleum origin) are among the most popular materials used in the food industry for packaging various types of food [6, 7]. These polymers include polyethylene terephthalate (PET), low- and high-density polyethylene (LDPE and HDPE, respectively), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) [8]. Synthetic polymers have advantages such as good strength, optimal flexibility, chemical inertness, and resistance to all types of chemical degradation [9]. In addition to these advantages, petroleum polymers have disadvantages such as using toxic substances for preparation, high migration of packing monomers, and very low degradability, leading to environmental pollution [10].

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On the other side are biopolymers that, unlike the previous group, are of non-petroleum origin [11]. These polymers can either exist naturally or be synthesized from organic materials [12]. Naturally occurring organic polymers include carbohydrates (homopolysaccharides, such as starch, cellulose, and glycogen, and heteropolysaccharides such as gums) [13], proteins (proteins of animal origin such as whey, casein, collagen, and gelatin and plant-based proteins such as gluten and zein) [14], and fats (such as waxes and fats) [15]. Another class of organic polymers is not found naturally in nature and is made from materials found in nature [16]. These polymers may be synthesized chemically, such as polylactic acid (PLA), or they may be synthesized by microorganisms such as polyhydroxyalkanoate (PHA) and polyhydroxybutyrate (PHB) [17].

A significant issue about polymers is the packaging of the degradability of these polymers in nature [18]. The very positive point of biopolymers compared to petroleum polymers is their short biodegradability, making these polymers attractive for use in food packaging [19]. Not all biopolymers have biodegradability (Fig. 5.1), and the same is valid for petroleum polymers, meaning that some of these polymers have biodegradability properties [12]. Biodegradable polymers of bio-origin include PLA and PHA, and petroleum-derived polymers include polybutylene adipate terephthalate (PBAT) and polybutylene succinate (PBS) [20]. On the other hand, polymers such as bio-derived polyethylene, polyamide (PA), and polytrimethylene terephthalate (PTT) are bio-originating but lack biodegradability. PP, PS, and PET are also standard petroleum and non-biodegradable polymers [21].

Among the proposed polymers, polylactic acid (PLA) has received considerable attention in recent decades [23]. PLA is an aliphatic polyester that is generally obtained through the synthesis of lactic acid [24]. Corn, starch, sugar, or other renewable sources can produce lactic acid [25]. PLA is a biodegradable thermoplastic polyester with a unique property, so this polymer has high potential in various applications such as surgical and medical applications, paper coatings, fibers, films, and packaging [26]. The unique properties of polylactic acid led the Food and Drug Administration (PDA) to FDA-approved PLA in 2008 as a Generally Recognized as Safe (GRAS) [27]. Modifying the properties of polymers to cover the weaknesses of a polymer and strengthening them to produce ideal packaging films are one of the standard measures in optimizing packaging films [28]. Polymers can be modified by chemical modification of the polymer structure or by using various physical methods in their preparation [29]. Mixing polymers with different properties to produce composites that have intermediate properties of primary polymers is one of the most common ways to improve the properties of packaging films [20, 30]. The addition of nanoscale reinforcements such as different types of nanoparticles can significantly affect the properties of packaging nanocomposites compared to the control film [31].

Regarding PLA polymer, the results indicate the weakness of this polymer in oxygen permeability, which is a major weakness for use in food packaging polymers because the contact of oxygen causes oxidation in food [32]. Numerous studies have been performed to improve the barrier properties of PLA in permeability to various gases [33]. Halloysite is a clay mineral composed of aluminum silicate and exists in

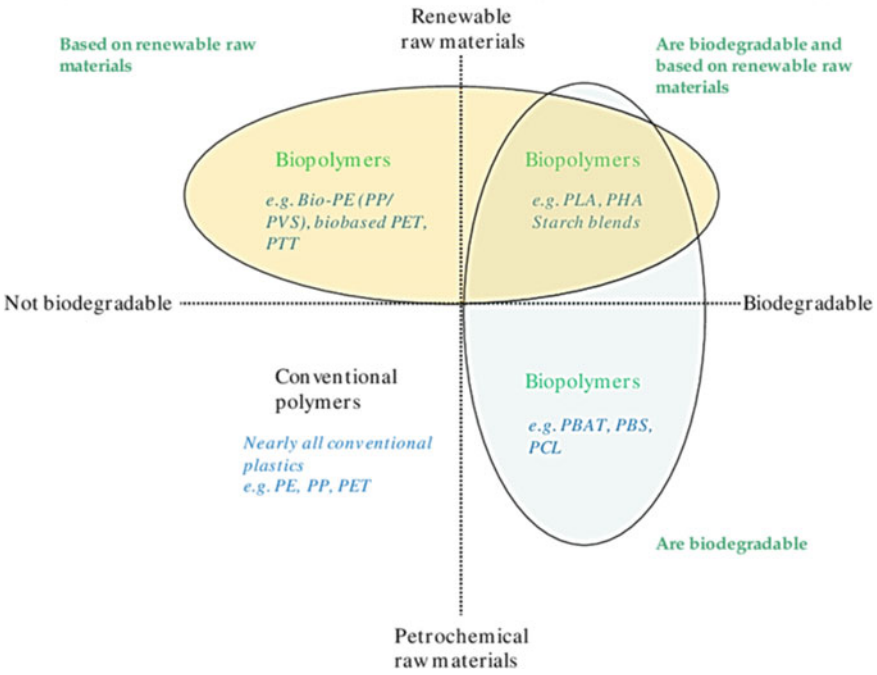

Fig. 5.1 Types of bioplastics, both biodegradable and non-biodegradable [22]

the form of mostly hollow nanotubes [34]. $Al_2Si_2O_5(OH)_4$ is the chemical formula of halloysite, composed of oxygen, silicon, aluminum, and hydrogen in ratios of 55.78, 21.76, 20.90, and 1.56% [35]. It has been observed that this mineral has a high potential in improving the properties of packaging films. Strengthening the barrier properties and increasing the mechanical strength are among the advantages of using halloysite nanotubes to prepare packaging nanocomposites [36]. Therefore, we have tried to introduce polylactic acid as an ideal polymer in food packaging in this study and also show the potentials of halloysite nanotubes in modifying the undesirable physicochemical properties of this polymer.

5.2 Polylactic Acid

Polylactic acid (PLA), formed from the polymerization of lactic acid, is a biodegradable, biocompatible, and renewable thermoplastic polyester [26]. The lactic acid used in the preparation of this polymer is mainly obtained from the bacterial fermentation of corn, sugarcane, potato, and other biomass [37]. Good mechanical properties and good processing capability are convenient features of PLA, which makes this polymer a suitable candidate for replacement with petroleum polymers [23].

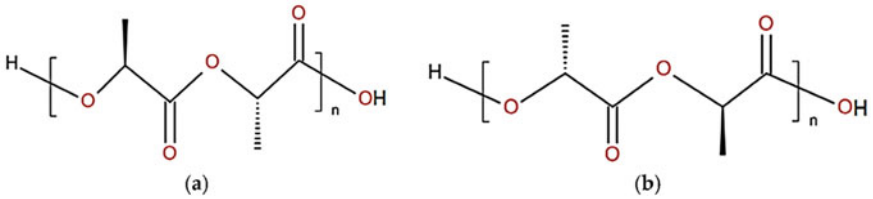

Fig. 5.2 Chemical structure of **a** PLLA and **b** PDLA

In addition to the appropriate properties, this biopolymer has weaknesses such as high hydrophobicity, high oxygen permeability, poor strength, slow degradation rate, reactive side chain groups, and low thermal stability [38]. However, easy access to polylactic acid monomers has resulted in a lower cost than other biodegradable polymers [39]. As shown in Fig. 5.2, PLA has two forms, PLLA and PDLA. Lactic acid (2-hydroxypropanoic acid) has two enantiomers L and D [40]. Lactic acid L is a common compound in human metabolism, but lactic acid D is produced by some microorganism strains or some less related metabolic pathways [41]. Lactic acid L is an endogenous compound, but lactic acid D is a harmful enantiomer [41]. L-lactide is produced if the main acid is L-lactic acid, and if the primary acid is D-lactic acid, D-lactide is produced.

The difference in these two enantiomers determines the final form of PLA [42]. PLA properties are highly dependent on the ratio between forms L and D. L-PLA or PLLA shows higher crystallization, which can lead to increased melting temperature and brittleness [43]. Pure PLA in either L or D form has a melting point of 180 °C and a glass transition temperature of 60 °C [44]. It has been observed that the crystallization of PLA can be reduced entirely after combining 15% D-lactide in poly L-lactide [42]. Copolymerization of L and D forms leads to forming an amorphous structure in the final polymer [45]. High molecular weight PLA is usually obtained by open-loop polymerization of lactide monomer or bacterial fermentation of cornstarch or sugar beet [46]. In order to better integrate the polymer, PLA with a longer chain is needed, and the introduction of a branch in the PLA structure can be a good option for this [47]. Numerous studies introduce polylactic acid properties as intermediate properties required for fully stable polymers' product stability and essential properties [42]. Also, high research activity is dedicated to overcoming common PLA weaknesses such as low impact resistance, low heat distortion temperature, and creating user-friendly PLA grades for use in specific applications [48].

Lactic acid and lactide (cyclic diester) are the most common monomers in PLA synthesis [49]. There are several ways to synthesize PLA, not all of which are cost-effective. The most common ways to obtain PLA are cyclic polymerization of lactide with various metal catalysts (usually tin octave) in solution (or as a suspension) and direct condensation of lactic acid monomers [40]. In the direct condensation method, the reaction temperature must be below 200 °C to prevent the formation of the entropically favored lactide monomer [40]. During the polymerization of lactic acid or lactide, a molecule of water is produced in the reaction, leading to disruption of the

reaction, so it is necessary to remove water from the environment [42]. Using vacuum systems to remove water is very efficient. The most important goal in polymerization is to obtain polymers with long chains and high molecular weight [50]. It has been observed that the removal of water molecules in the PLA polymerization process increases the molecular weight of the final product [42]. Accurate crystallization of the polymer from the melt also directly affects increasing the molecular weight but prolongs the processing time [51, 52]. Different enantiomers in polymer synthesis also have a significant effect on the final chains [40]. Using this variable, PLA can be converted to different degrees of crystalline and amorphous, which can be selected based on the purpose of polymer production [42]. Other industrial methods in the chemical synthesis of this polymer include lactonitrile, acrylonitrile, and propionic acid [42]. The price of raw materials is considered the main factor for choosing between different polylactic acid production methods [42]. For example, the propionic acid method is less used in industries due to the high cost of raw materials [42, 53].

By controlling the degree of crystallinity and amorphous, polylactic acid can be changed from a completely amorphous glass polymer to a semi-crystalline, the highly crystalline polymer [54]. This change in the chemical structure of PLA causes this polymer to have a glass transition temperature between 60 and 65 °C, a melting temperature between 130 and 180 °C, and a tensile modulus between 2.7 and 16 GPa [55]. For example, by combining different L and D enantiomer ratios, the PLLA melting point can be increased by 60 °C and brought closer to the PDLA melting point [56]. The combination of these two polymers causes the formation of a regular structure and increases crystallization [57]. PLDA acts as a nuclear agent and increases the rate of crystallization [58]. The most suitable structure is observed in the ratio of 1:1 of polymers. Studies have shown that the mechanical properties of PLA are intermediate to the properties of polyethylene terephthalate and polystyrene polymers [59]. Many solvents are used to dissolve PLA. The solubility of PLA is generally related to its degree of crystallization [60]. The higher the crystalline structure in PLA, the harder it is to solve and the higher the temperature required [60].

Conversely, increasing the amorphous structure results in better solubility of this polymer [61]. This polymer's hydrophobic and organic structure makes many organic solvents capable of dissolving PLA [61]. Chloroform, dichloromethane, ethyl acetate, and propylene carbonate are the most common organic solvents used to dissolve PLA [62]. Ethyl acetate is more popular due to its ease of access and low risk of use. Ethylbenzene, toluene, acetone, and tetrahydrofuran can also partially dissolve PLA [63]. Increasing production of packaging waste prepared with petroleum polymers has led to the use of biodegradable polymers obtained from renewable sources to be prioritized [64]. Thermodynamic properties, mechanical strength, barrier, and biodegradability are critical points in the packaging industry. Polylactic acid has been proposed as a qualified substance due to its suitable properties [65]. In 1932, DuPont first developed PLA by a chemist named Wallace Carothers. This product was not cost-effective due to its low molecular weight. The company was able to produce this high molecular weight polymer in 1954, which is the beginning of PLA in the world [20, 42]. In the '60s, after various studies on PLA, this polymer was

introduced as a suitable raw material for making supplies and raw materials for the medical industry [42]. Finally, after FDA approval in 2008, polylactic acid became a common and widely used polymer in the food and medical industries [46].

The unique physical properties of polylactic acid make it suitable for use in various plastic products for food packaging, such as packaging films and food boxes [66]. Suitable tensile strength and ductility make it suitable for various processing tools such as melt extrusion mold, injection mold, blown film mold, foam mold, and vacuum mold [67]. In addition, the excellent biocompatibility of PLA has led to its widespread use in biochemical medicine [67]. Studies on the degradation of polylactic acid have shown that the degree of crystallization affects the rate of PLA degradation [68]. Various factors can affect the degree of crystallization of PLA, the most important of which is the molecular weight of this polymer [69]. Highly crystalline polymers last for several months, and metabolism takes place only after a few years, while polymers with low crystallinity and amorphous structure can decompose in a few weeks [42]. The following property that can affect the performance of materials according to the type of polymer processing is the glass transition temperature or Tg [70]. This factor, unlike biodegradability, is not related to the degree of crystallization of the polymer [69]. Due to its molecular weight, PLA has a glass transition temperature between 55 and 60 °C and a melting temperature between 130 and 180 °C [71]. The study of mechanical properties has shown that the tensile strength of PLA can increase up to 50 MPa by increasing the degree of crystallization and molecular weight [72]. PLA undergoes hydrolysis at temperatures above 200 °C, lactide recombination, major oxidative chain cleavage, and intermolecular or transmolecular transesterification [38, 59]. This series of reactions cause the PLA to have sufficient thermal stability to reduce degradation and maintain molecular weight and function [42].

The biodegradation of PLA is related to several factors, including time, temperature, low molecular weight impurities, and catalyst concentration [39]. Further research has shown that modification and purification of this polymer can be effective in its biodegradation [42]. Hydroxyl terminal acetylation increases the decomposition temperature by about 26 °C by reducing the molecular weight of PLA [42]. A theory about the biodegradation phenomenon of PLA introduces a simple proton-catalyzed hydrolysis chain as an influential factor in PLA biodegradation [73]. This reaction is reversible, so the purity of the polymer can be affected by explaining the degradation kinetics of PLA on the degradation process during the synthesis reaction process [42]. Another factor determining the degree of autocatalysis and biodegradation is the degree of crystallization of the polymer [74]. Enzymes also play a role in the degradation of PLA, but the enzyme's exact mechanism is already unknown to us [75]. This fact means that it cannot be said with certainty that enzymes directly catalyze the degradation process of the polymer or indirectly contribute to the reaction by removing the by-products. However, assuming that PLA is mainly degraded by hydrolysis, its degradation is divided into two main stages: (1) non-enzymatic melting of ester groups and (2) random cutting of low molecular weight polymers by microorganisms to produce Co_2 and H_2O [42].

Apart from the attractive properties of polylactic acid, this polymer is not free of defects. The high hydrophobic properties make this polymer unsuitable as a carrier for various compounds such as bioactive compounds and antioxidants in delivery systems [76]. Low mechanical strength compared to other polymers and brittleness of PLA limit the use of this polymer [77]. The oxygen permeability of PLA is known as one of the most significant disadvantages of this polymer [78]. Many efforts have been made to improve the weaknesses of PLA, and many findings have been published in recent years [79, 80]. Various methods have been proposed to increase the efficiency of PLA, some of which are mentioned below. The preparation of nanocomposites from this polymer has had positive results; for example, the addition of different nanoparticles during the synthesis of PLA or during its processing can significantly modify and improve the properties of this polymer [81]. The use of different copolymers had the same results, so that the use of low Tg polymers in combination with PLA can improve the flexibility properties of this polymer and make it possible to use this polymer at lower temperatures [42]. In one study, the properties of polylactic acid/polyglycolic acid copolymers were investigated [78, 82]. Since PGA has a high melting point of about 228 °C and Tg of about 38 °C, PLA/PGA polymer composite has a low Tg point and amorphous structure, which has higher flexibility than pure PLA [82]. In addition to thermodynamic properties, PGA has higher hydrophilicity, which in addition to making this polymer suitable for delivery systems also increases the rate of biodegradation because it has a higher hydrolysis rate than PLA [78]. Poly (ε-caprolactone) and polylactic acid composites were evaluated in another study [83]. The Tg point in poly (ε-caprolactone) is about −60 °C, and its melting point is 59.5 °C. The composite has high flexibility and crystallization with a high melting point. It was observed that this polymer has variable mechanical properties [83, 84].

The use of additives such as low molecular weight citric acid, succinic acid, tartaric acid, and oxalate to PLA as a plasticizer can partially alter this polymer's mechanical and thermodynamic properties [85]. There are also reports of a positive effect of montmorillonite on the strength and flexibility properties of PLA [86]. Plasticizers increase flexibility and reduce brittleness by reducing the Tg in the polymer [87]. In PLA, the addition of a plasticizer can reduce the Tg by up to 26 °C [88]. On the other hand, the addition of montmorillonite and polyethylene glycol could make the PLA structure more agglomerated and keep the elongation below 5% [89–91]. Adding starch to PLA is the simplest and cheapest way to change this polymer's physicochemical properties and dramatically increases the biocompatibility of this polymer [42]. Combining starch with PLA reduces tensile strength by increasing water absorption [92]. This feature has many advantages, but it also has disadvantages, such as increased fragility, which can be slightly eliminated using plasticizers. Polyether and PLA copolymers also recorded good results in modifying the degree of hydrophilicity, degradability, biocompatibility, and flexibility [93]. The mechanical properties of PLA/polycaprolactone and PLA/polyethylene oxide composites did not show a significant difference, but the high hydrophilic properties of polyethylene oxide increase the degree of hydrophilicity in the composite, which accelerates biodegradation [93, 94]. Recent studies have shown that the use of

polyethylene-polypropylene copolymer produces longer polymer chains than PEO [95, 96]. Other methods of modifying polylactic acid include the use of crosslinkers. These compounds work by altering the rheological and thermodynamic properties of the material by creating crosslinks between the polymer chains [38]. Crosslinking compounds often act as an intermediate compound that binds two polymer chains together [97]. Oxepane or 5,5′-bis(oxepane-2-one) is a common crosslinker known in the polymer industry [42]. The use of this material for crosslinking has been reported in PLA, which has also yielded positive results. Other common crosslinkers used in PLA polymers include silane, dicumyl peroxide, and triallyl isocyanurate [98]. This method is known as an efficient and relatively simple method that, unlike previous methods, does not require the addition of copolymers or nanoparticles [99].

High compatibility and good biodegradation are the prominent features of this polymer. Also, PLA metabolism in the human body does not cause the formation of toxic compounds, which leads to the widespread use of this polymer in the food and medical industries [42]. Polylactic acid is used today in various fields such as tissue engineering, drug delivery systems, encapsulation of bioactive compounds, controlled release, and active food packaging [91]. The low price of raw materials in the production of this polymer has made the use of PLA in the food industry cost-effective [64]. On the other hand, ideal properties such as thermoplastic properties and suitable ductility make it possible to use this polymer differently. High quality and high compatibility are other suitable properties of this polymer, but another important point is the prevention of PLA against the passage of ultraviolet light, which can reduce the adverse effect of food absorption by ultraviolet light [100]. All of these properties make PLA an ideal polymer in food packaging. PLA classification in the GRAS group makes this polymer suitable for packaging in direct contact with food. Also, the thermoplastic properties of PLA are very similar to common polymers such as PET, so the use of PLA in the form of thermoforming looks pretty appropriate. Other forms of PLA food packaging products that have been used in recent years include extruded containers, oriented and flexible films, and cast films. These methods are used to prepare typical packagings such as food and beverage containers, bottles, glasses, packaging films, and coated paper and boards [42].

5.3 Polylactic Acid/Halloysite Nanotube Bionanocomposite

Polylactic acid polymer and many properties also have weaknesses, some of which were mentioned above. Numerous studies have been performed in recent years to correct the weaknesses of this polymer and strengthen its favorable properties. Polymer composites have always received more attention than other polymer modification methods due to their simplicity in preparation [101]. The use of nanoscale additives to PLA will lead to much better results [102]. The very small size of the nanomaterials makes it easy to fit between the polymer chains and perform better due to the high surface-to-volume ratio. The placement of nanomaterials between the polymer chains creates crosslinks between the chains and can affect the film's

mechanical, barrier, and even solubility properties [86]. In this section, the properties of PLA bionanocomposites containing halloysite nanotubes are investigated.

Halloysite is known as a derivative of kaolin [103]. The name of this substance is derived from the name of the Belgian geologist Jean Baptiste Julien d'Omalius d'Halloy, who discovered it in 1826 [104]. As seen in Fig. 5.3, the natural form of this rare material is in the form of nanotubes. These nanotubes are formed by weathering plate-like kaolin particles [105]. In this way, plate-like kaolin particles roll due to weathering, and in the meantime, the impurities in kaolin are leached out to produce high purity nanotubes [106]. Halloysite nanotubes have a length of about 1–15 microns, and the inner diameter of the nanotubes varies from 10 to 150 nm [107]. Halloysite properties are a combination of excellent mechanical properties, tubular microstructure, high biocompatibility, flame retardancy, and diverse surface chemistry [108]. The use of this material in combination with various polymers and the preparation of polymer nanocomposites to modify the polymer properties are widely used [31]. Today, halloysite is used in various polymer matrices such as thermoplastics, thermosets, and elastomers to make various nanocomposites [109]. Halloysite nanoparticles effectively modify various properties of polymer nanocomposites such as thermodynamic, mechanical, permeability, solubility and surface properties, degree of swelling, biodegradability, and other properties [110]. Other suitable properties of halloysite include the abundance of this substance and the easy solubility in water and other organic solvents, which causes the proper dispersion of this substance and simplifies its use [111]. Every year, various studies are published on the different effects of halloysite on the properties of polymer packaging films [112]. In general, the two factors of halloysite dispersion and surface interactions between halloysite and polymer matrices are known as the main factors in the impact of this clay mineral [113]. Unlike layered silicates such as nano-silica, halloysites have more hydrophobic properties due to the lower density of surface hydroxyl groups but are generally hydrophilic in nature [109]. This degree of hydrophobicity provides a better dispersion for halloysite nanotubes in polymer matrices [114]. Various studies have shown that halloysite nanotubes can be covalently bonded with hydrophilic polymers such as polyethylene alcohol and chitosan, increasing surface adhesion and improving the interaction between the polymer and the nanotube [108]. On the other hand, due to the hydrophilic nature of halloysite nanotubes, any interaction between these materials and non-polar polymers is not done properly [115]. Various strategies have been proposed to overcome this challenge, including the use of various surfactants to form covalent bonds, the use of polymers with lower hydrophobicity, and the use of intermediates such as polar macromolecules [116, 117].

In addition to the mentioned features, halloysite nanotubes have other applications in the preparation of active packaging. One of their most critical applications is carrying bioactive compounds in food packaging films [119]. Food packaging plays a vital role in maintaining food quality. Maintaining food quality is difficult despite environmental phenomena such as oxidation, rancidification, and the growth of microorganisms [100, 120]. By using bioactive compounds such as antimicrobial and antioxidant compounds, there is the ability to control these undesirable

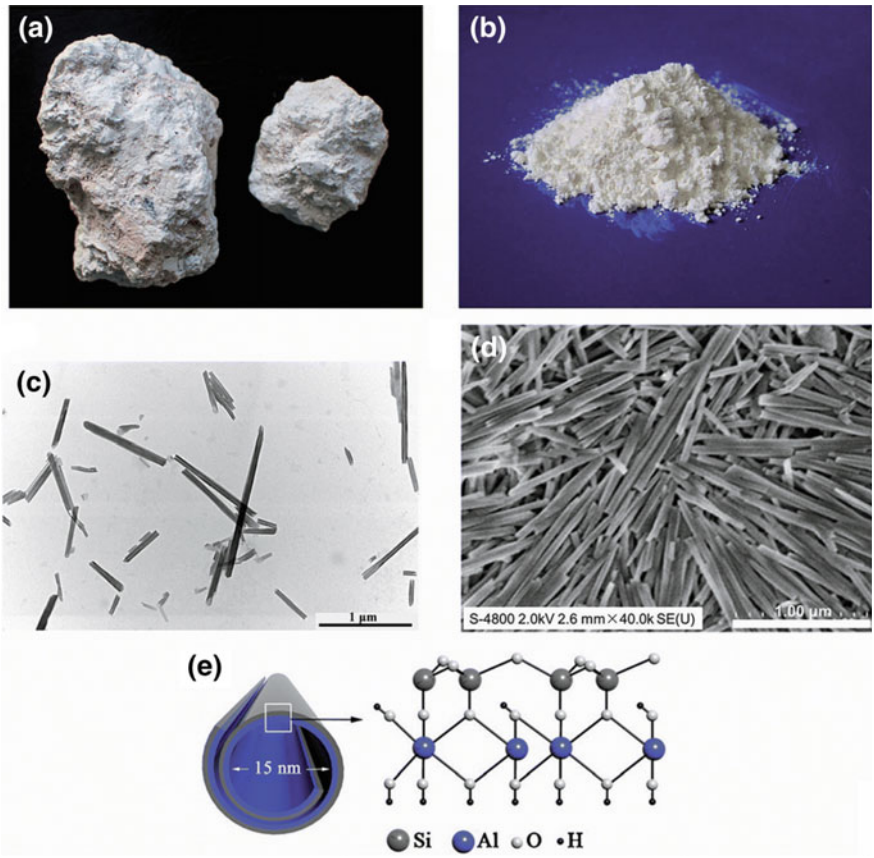

Fig. 5.3 **a** The raw halloysite and **b** ground halloysite; **c** TEM and **d** SEM photos of HNTs mined from Hunan Province, China; **e** schematic illustration of the crystalline structure of HNTs [118]

phenomena in food [100]. Direct use of bioactive substances increases the consumption dose and increases food packaging costs [121]. Also, due to the high sensitivity of bioactive compounds to environmental factors such as light, temperature, and oxygen, a significant part of these materials are degraded and lose their effectiveness quickly [122]. Encapsulation of bioactive compounds greatly reduces the required dose of these substances and protects them from degradation by various environmental factors [122]. Halloysite nanotubes have high potential in bioactive compounds' carriers due to their hydrophobicity and geometric properties [123]. Numerous studies have been performed on the effectiveness of halloysite nanotubes in encapsulating various compounds to be used in food packaging, all of which indicate the positive effects of this clay mineral [119]. A noteworthy point about active packaging is controlling the release rate of bioactive materials encapsulated in the polymer matrix [91, 124]. With this information, the release pattern of bioactive substances can be identified and the best shelf life of food (the best time for effective

packaging) can be obtained. With the help of this information, it is possible to design food packaging according to the expiration date of the product. Using the release rate control feature, food packaging can be designed to store food in short periods or very long intervals [125]. In this way, the food is kept in the best quality conditions during the storage period and has the maximum quality at consumption.

The use of nanotechnology is an excellent way to improve the properties and cover the weaknesses of biopolymers [10]. In general, nanotechnology is the use of scales of 1–100 nm in engineering designs. In the case of food packaging composites, the use of nanomaterials with at least one of their dimensions at the scale of 1–100 nm creates packaging nanocomposites [126]. Composites consist of a polymer matrix or a continuous phase and a discontinuous phase or filler, which in the case of nanocomposites are fillers at the nanoscale [127]. High differentiation by nanotechnology in food packaging has made nanocomposites a viable alternative to conventional packaging. Biopolymers are sensitive to the passage of water vapor due to their hydrophilic nature [128]. Oxygen and carbon dioxide permeability is also higher in biopolymers than in petroleum polymers [129]. Undoubtedly, this defect is one of the most limiting factors for biopolymers in the food packaging industry. Modifying and enhancing the barrier properties of packaging films with the help of nanotechnology have shown promising results in the last decade [130]. Other ways to improve the barrier properties of packaging films include the use of polymer blends [131], high barrier coatings [132], and multilayer films containing a high barrier film [133]. However, various studies indicate a higher impact of nanocomposites than composites; on the other hand, using several techniques simultaneously will bring much better results. There are concerns about the toxic properties of nanoparticles, and further studies have shown that if the nanoparticles are smaller than a specific size, they can cross the cell barrier into the bloodstream without endocytosis [134, 135]. The nanoparticles pass through the cell barrier due to their small size without a specific mechanism and through the cells of the small intestine (villi) [136]. Passage through villus cells can interfere with the main mechanism of material transport that is diffuse (Active Transport, Facilitated Diffusion, Osmosis, and Simple Diffusion) [137].

Today, due to the increasing demand for food with minimal processing and long shelf life, it has created a large market for food packaging [138]. To gain more market share, creativity in the production of packaging materials is of great importance. Nanocomposites undoubtedly have a large share of this market. The use of such packaging enhances the inhibitory properties of packaging films and prevents the migration of oxygen, carbon dioxide, water vapor, and flavoring compounds [139]. Reinforcement barrier properties ultimately lead to more excellent retention of freshness and quality of packaged food, impacting its shelf life [140]. As mentioned above, the main disadvantage of biopolymers is water vapor permeability due to their hydrophilic nature. Reduction in water vapor permeability rate is of particular importance in biopolymers. Studies have shown that with the help of nanocomposite technology, this inherent defect of polymer-based packaging materials can be largely covered [141]. In many cases, it has been reported that the barrier properties can be improved by up to 50% compared to the properties of the control polymer film [142].

Part of this reduction in permeability rate is increased tortuosity, which increases the motion of gas molecules, thus reducing permeability and increasing barrier properties [143].

Another noteworthy point is the adverse effect of ultraviolet rays on food quality and even the content of bioactive compounds in food. Polylactic acid is known as a protective polymer against ultraviolet light [144]. So far, many studies have shown the positive effect of PLA in reducing the adverse effects of ultraviolet light [100]. On the other hand, the use of nanomaterials in the preparation of nanocomposites increasingly gives the ability to repel the properties of ultraviolet light to food packaging [145]. Halloysite nanotubes also can refract ultraviolet light and prevent the direct contact of this harmful radiation on the surface of food [115]. The combined use of PLA and halloysite nanotubes can be highly effective in protecting from ultraviolet light. The last important point is the thermal resistance of polymers.

The thermal resistance factor is mostly used in the production of active packaging films [139]. Bioactive compounds such as antioxidants and antimicrobial compounds are degraded due to their high sensitivity to process temperatures in the preparation of packaging films (in thermoforming methods) during the packaging production process [125]. Degradation of these bioactive substances in active packaging causes the quality of the packaged food to be adequately maintained during storage in the packaging [125]. Encapsulation of bioactive compounds can significantly affect the preservation of these compounds during the packaging preparation process [146]. Halloysite nanotubes can be loaded with bioactive compounds due to their spatial structure. This phenomenon increases the thermal resistance of these temperature-sensitive compounds and prevents their thermal degradation during the process [147]. In addition, the increase in thermal resistance in the packaging polymer is also increased by the use of nanoparticles. Numerous studies concerning Differential Scanning Calorimetry (DSC) have shown that the thermal degradation temperature of the packaging polymer shifts to a higher temperature zone when using different nanoparticles, especially mineral nanoparticles [148, 149]. In this section, we tried to express some of the positive points of nanocomposites simply compared to composites. Improving the properties of packaging films in various aspects, some of which were mentioned above, has made nanocomposites one of the most widely used fields of research and study in recent years.

5.4 Food Packaging Application of Polylactic Acid/Halloysite Nanotube Bionanocomposite

In recent years, various studies have been published focusing on nano-biocomposites. Each, in turn, has examined the characteristics of packaging films from a different perspective. In this section, an attempt has been made to introduce the strengths and weaknesses of packaging nanocomposites with the help of this research. However, the main focus is on polylactic acid polymer and halloysite nanotubes. A study conducted

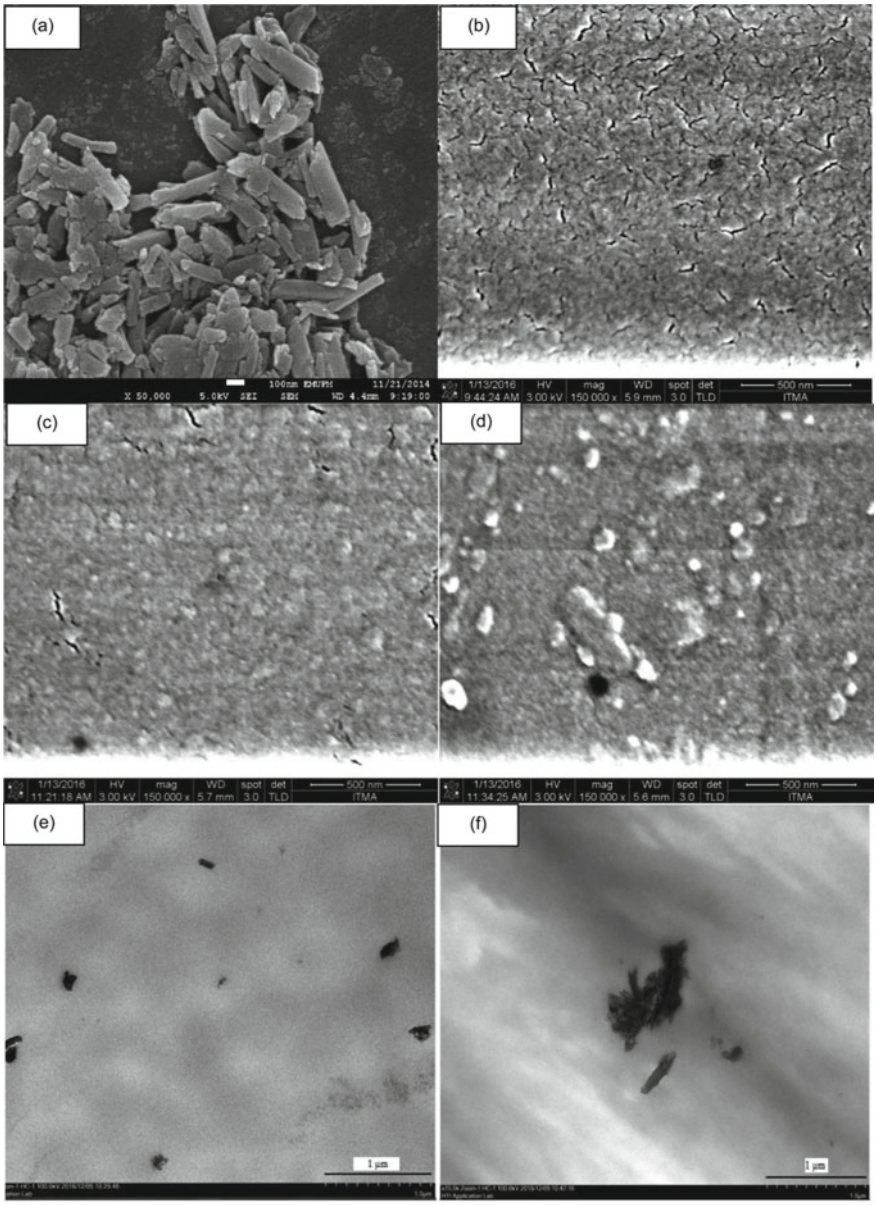

Fig. 5.4 FESEM images of **a** halloysite nanotubes (HNTs), **b** neat polylactic acid film and polylactic acid/HNT bionanocomposite film incorporated with **c** 3 wt% and **d** 6 wt% HNTs and TEM micrographs showing the distribution of HNTs in PLA matrix for **e** 3.0 wt% and **f** 6.0 wt% HNTs with 15,000× magnification [150]

last year by Risyon et al. attempted to modify limiting factors such as poor mechanical properties, low thermal stability, and low barrier properties in biopolymers [150]. The author proposed using nanoscale fillers and the preparation of nano-biocomposites to solve the problem of these biopolymers. In this regard, polylactic acid/halloysite nanotube nano-biocomposite prepared using the casting method was introduced. Doses of 1.5–6.0 wt% were used to evaluate the effect of different concentrations of halloysite (Fig. 5.4). The results of various experiments were evaluated with a halloysite-free PLA control film. The effects of different halloysite concentrations on the dispersion, chemical bonding, and average molecular weight of bionanocomposite films were investigated to observe changes in their mechanical, thermal, and barrier properties [150]. Field emission scanning electron microscopy (FESEM) imaging showed good dispersion for a concentration of 3.0 wt% of halloysite in the PLA polymer matrix. This suitable dispersion is probably due to the good interaction between PLA and halloysite due to hydrogen bonds between PLA hydroxyl groups and halloysite siloxane groups [151]. As shown in Fig. 5.5, the carboxyl group in the PLA structure can form a hydrogen bond with the hydrogen atoms of the hydroxyl group in the halloysite due to the negative charge [152]. On the other hand, hydrogen bonds can be formed by the negative charges of oxygen atoms on the halloysite surface, attracting the positive charges of hydrogen atoms in the PLA matrix [153]. These bonds indicate a good interaction between PLA and halloysite, which can be seen by Fourier-transform infrared spectroscopy (FT-IR). Further magnification in FESEM showed that halloysite nanotubes fill the pores in the structure of PLA film. Thus, it can increase thermal stability, barrier reinforcement, and increasing mechanical strength in nanocomposites [150]. It was observed that at higher doses of about 6.0 wt% halloysite, nanotubes tend to form aggregated structures in the polymer

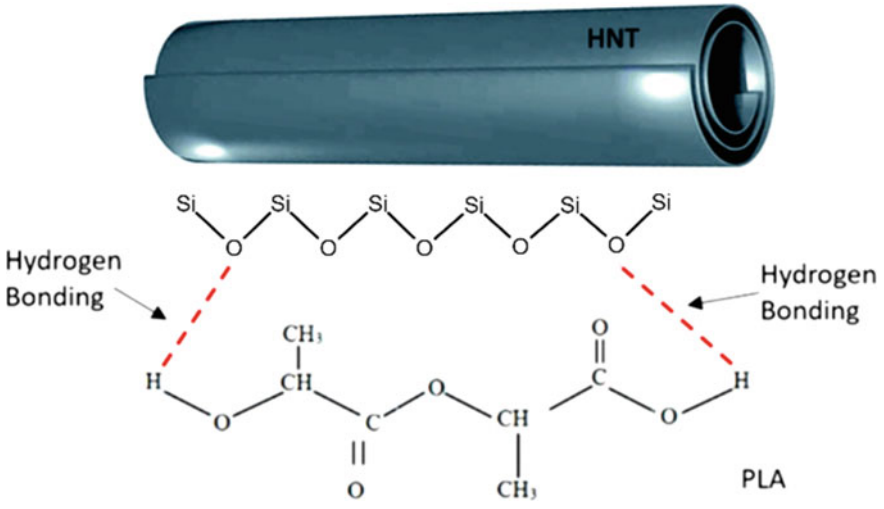

Fig. 5.5 Schematic diagram of the interaction mechanism between PLA and halloysite nanotube [166]

matrix. Probably at high concentrations due to more collisions between molecules, the adhesion between halloysite nanotubes increases due to van der Waals interactions, which lead to the formation of aggregated structures [154]. Thus, a dose of 3.0% by weight to achieve the best dispersion in halloysite was introduced as the optimal dose. The study of the film's mechanical properties showed that the addition of halloysite at a concentration of 3.0% could lead to an increase in tensile strength and Young's modulus while decreasing elongation at break [150]. Increasing the dose of halloysite to 4.5 and 6.0 wt% reduces the tensile strength and Young's modulus while increasing the elongation at break. Weakening of tensile strength due to increasing halloysite dose may be due to the formation of aggregated structures at high concentrations. At low doses of about 1.5–3.0 wt%, dispersion is well-formed, and a large hydrogen bond is formed between the halloysite nanotube and PLA, thus enhancing mechanical properties such as tensile strength and Young's modulus. The results showed that the concentration of halloysite greatly affects the properties of the nanocomposite. Increasing the density of the hydrogen bond reduces the mobility of the PLA polymer chains, which in turn hardens and reduces the elasticity of the packaged film and reduces the elongation at break [155]. This reduction in elongation at break can also be achieved with the help of FT-IR at the peak of 1050–1250 cm^{-1}, which is related to the C–O–C band [156]. It was observed that increasing the halloysite concentration leads to a decrease at the peak of 1050–1250 cm^{-1}, which is consistent with a decrease in elongation at break [150]. The results of the thermal stability test showed that the concentration of 3.0 wt% could be effective in increasing the thermal stability of nanocomposites due to proper dispersion in the PLA matrix and the formation of hydrogen bridges. However, higher doses such as 6.0 wt% have lower thermal stability due to the formation of aggregated forms of halloysite nanotubes and the presence of free hydroxyl groups in PLA. The inhibitory properties of PLA films with different ratios of halloysite nanotubes to the permeability of water vapor and oxygen were evaluated [157]. It was observed that at doses below 3.0 wt%, the effectiveness of nanotubes in reducing the rate of water vapor permeability is not significant. On the other hand, doses of 3.0–4.0% wt% could significantly reduce the rate of water vapor permeability. The results showed that at lower doses, due to the inadequacy of nanotubes in filling the PLA film structure's pores and the lack of hydrogen bonds, water vapor permeability rate reduction is non-significant. At doses of 3.0 and 4.0 wt% of nanotubes, due to the proper dispersion in the polymer matrix, which leads to the formation of tortuous paths, the formation of high hydrogen bonds, and the filling of microscopic cavities, the rate of water vapor permeability is significantly reduced [158, 159]. Lack of proper dispersion in the polymer matrix at doses of 6.0 wt% and the formation of an aggregate form increase the rate of water vapor permeability. Insufficient hydrogen bonds and non-filling of microscopic cavities are some of the factors that can explain this phenomenon. On the other hand, the lack of proper dispersion in the polymer matrix prevents the formation of tortuous paths, which is the main factor in reducing the rate of water vapor permeability. Halloysite nanotubes have a negative charge due to the presence of hydroxyl groups on the surface and are naturally hydrophilic in nature. However, the placement of the halloysite nanotube in the PLA matrix results

in the establishment of a negatively charged hydrogen bond between the oxygen at the nanotube surface and the hydrogen in the PLA hydroxyl group [150]. In this way, the desire for water permeability through nanotubes is not increased and also water vapor permeability in nanocomposites is not increased. It can be assumed that the formation of tortuous pathways is formed by the establishment of hydrogen bridges between the halloysite nanotubes and the PLA polymer, so the greater the hydrogen interactions between the two materials, the greater the expectation of a reduction in the rate of water vapor permeability [150]. As previously mentioned, halloysite nanotubes are hydrophilic in nature, so increasing the dose of this material increases the tendency of the nanocomposite to absorb water and increases its water vapor permeability. A dose of 3.0 wt% of the nanotube was selected as the optimal nanocomposite sample due to its mechanical and barrier properties, and its oxygen permeability was evaluated. It was observed that the nanocomposite has a 33% lower rate of oxygen permeability than the control PLA film. As before, the formation of tortuous paths can be effective in penetrating other gases such as oxygen [143]. Therefore, the obtained results are expected to be consistent with the results of water vapor permeability experiment. In other similar studies, titanium oxide and montmorillonite nanoparticles were used to modify the barrier properties of PLA film and similar results were obtained [33, 160]. Therefore, it was concluded that increasing the winding paths has a great impact on reducing the permeability to various gases. The presence of oxygen in food packaging can lead to food spoilage such as loss of nutrients, discoloration, flavor, and microbial growth. This study showed that the use of nanomaterials can greatly improve the barrier properties of PLA films to the extent that it is suitable for use as food packaging. In order to show the potential of the prepared films (PLA bionanocomposite film/halloysite nanotube with a concentration of 3.0% wt% with control PLA film), these films were used for packing cherries to evaluate the shelf life of packaged food [150]. Weight loss and firmness of tomatoes were assessed for nine days. The results showed that the firmness of tomato tissue packed in bionanocomposite decreased by 16% during nine days, while this decrease was calculated up to 25% in the control PLA film. Tomato weight loss during nine days due to water vapor permeability in bionanocomposite was measured at about 1.9% and in control PLA film 2.8%. Thus, it can be concluded that the use of PLA bionanocomposite containing 3.0 wt% of halloysite nanotubes can reduce the loss of agricultural products during storage in packaging [150].

In a similar study by Therias et al., halloysite nanotubes were used as fillers up to 12.0 wt% in PLA film [161]. Filler nanomaterials were added to the PLA film in both melt-compounded and dry-mixed forms. Key properties of nanocomposites, such as mechanical properties and nanotube distribution in the polymer matrix, were evaluated. Morphological analysis was performed by transmission electron microscopy (TEM) through nanocomposite samples containing 6.0 and 12.0 wt% of nanofillers. Adequate distribution in the dispersion of nanofillers was observed in both melt-compounded and dry-mixed forms at a dose of 6.0 wt% but at a dose of 12.0 wt% showed signs of aggregates of nanofillers [161]. The formation of aggregated structures due to van der Waals interactions is due to high collisions between nanofillers at high concentrations [162]. Studies on mechanical properties

have shown that the tensile strength and Young's modulus change under the influence of nanofiller content. The maximum tensile strength for different samples was estimated to be almost constant at about 60 MPa, but Young's modulus increased with increasing nanoparticle dose [161]. According to Hooke's law, an increase in Young's modulus means a decrease in elasticity, and more force must be applied to the body to create the deformation, so nanocomposites with a higher load percentage than nanofillers have a harder texture [163]. Weaknesses in PLA include sensitivity to photooxidation [59]. PLA can protect food from the sun's ultraviolet rays at the cost of degradation and photooxidation of the polymer itself [59, 164]. In this study, the author investigated the effect of photooxidation on nanocomposites compared to PLA control film without nanofillers. To evaluate photooxidation, IR absorption in the range of 1845 cm^{-1} can be used for different nanocomposite samples, and the peak intensity is related to the oxidation intensity, because the absorption range of 1845 cm^{-1} is related to the products of photooxidation and reducing the intensity at this peak means reducing photooxidation [165]. It was observed that the rate of PLA oxidation in the presence of halloysite nanotubes after 240 h is significantly higher than the control PLA film. Also, the intensity of adsorption at the peak of 1845 cm^{-1} was not significantly different between samples with different doses of nanofillers, which means that the effects were the same in samples with different concentrations of nanofillers. Undoubtedly, halloysite nanotubes have a destructive effect on PLA photooxidation, which can be related to chromophore impurities, which can have an induced effect on the mechanism of radical oxidation of PLA [161]. Another reason could be due to the presence of iron impurities, as reported in natural clays, which cause higher degradation of nanocomposites. Higher photooxidative degradation can increase the biodegradation rate of this polymer and also cause it to absorb more of the harmful energy of sunlight during use as food packaging and prevent it from coming into contact with food [161].

PLA/halloysite nanotube films were fabricated using the soluble casting method to investigate their properties for packaging applications by De Silva et al. [151]. Concentrations of 2.5 and 10 wt% of halloysite were used, and their effect on mechanical properties such as tensile strength and tensile strength was evaluated. The results were compared with the control film (PLA without halloysite tubules). The results showed that the addition of 5.0% by weight of nanotubes could enhance the tensile strength of the nanocomposite. Infrared spectra showed that the hydroxyl groups of PLA have chemically interacted through hydrogen bonding with the surface siloxane groups of halloysite. The contact angle value of the water droplet does not change significantly with the addition of the halloysite, so it does not absorb more moisture in the PLA film. TGA results showed that thermal stability is significantly increased by adding halloysite due to char residue and lumens of halloysite. The author introduces PLA/halloysite nanotube nanocomposite as a suitable alternative to petroleum packaging [151].

5.5 Conclusion

This chapter tried to introduce the advantages and problems of polylactic acid as a biodegradable biopolymer in food packaging. Excessive use of petroleum polymers leads to bio-pollution, so the use of biodegradable polymers is a priority. On the other hand, the destruction of a significant portion of food during storage encourages us to use more efficient packaging. Direct use of PLA biopolymers is limited due to weaknesses such as brittleness, high hydrophobicity, poor mechanical properties, high permeability to oxygen and water vapor, and high cost compared to petroleum polymers. Various solutions such as chemical and physical modification were proposed to improve the properties of PLA. The use of nanomaterials due to the high surface-to-volume ratio showed a much higher effect. Halloysite nanotubes are used as fillers in PLA structures. Halloysite, as a mineral, has a tubular structure and a polar nature. Surface hydroxyl groups in the halloysite structure can form hydrogen bonds with the hydroxyl group in PLA. The establishment of abundant hydrogen bonds increases the nano-biocomposite's mechanical strength and reduces the permeability of the nano-biocomposite to oxygen and water vapor by reducing the mobility of the chains. It was observed that concentrations less than 5.0 wt% of halloysite were not effective enough, and concentrations higher than 5.0 wt% were not effective due to the formation of aggregated structures, so a concentration of 5.0 wt% halloysite was introduced as the optimal concentration. In addition to the mentioned advantages, the use of nanotubes increases the loading capacity of bioactive compounds in the packaging film because PLA, due to its high hydrophobicity, is not able to encapsulate polar compounds properly. The polar nature of halloysite improves the encapsulation properties of nanocomposites.

On the other hand, studying the release pattern and controlling the release of encapsulated bioactive compounds can be effective for preparing active packaging to maximize effectiveness during shelf life. In this chapter, an attempt was made to introduce PLA/halloysite nanotube nano-biocomposites as an alternative to petroleum polymers. Solutions have also been introduced to address the challenges facing it. Undoubtedly, the desirable properties of this bionanocomposite will be more welcomed in the future for the preparation of various food packaging.

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Chapter 6
Preparation of ZnO/Chitosan Nanocomposite and Its Applications to Durable Antibacterial, UV-Blocking, and Textile Properties

Tanmoy Dutta, Abdul Ashik Khan, Nabajyoti Baildya, Palas Mondal, and Narendra Nath Ghosh

6.1 Introduction

During the last few decades, scientists are engaged to develop cost-effective and eco-friendly synthetic natural therapeutic materials. Despite remarkable development of pharmaceutical and medical technology, harmful multidrug-resistant bacteria are a great threat and day-by-day bacterial infection attracts the attention of researchers due to increasing the number of multidrug-resistant bacteria [1]. Large pharmaceutical industries are showing less interest to find out new antibiotics as it is time-consuming, expensive, and risky [2]. On the other hand, the rise of multidrug-resistant bacteria decreases the rate of approval of the antibacterial agent [3]. Hence, resources and attention both are devoted to finding out smart solutions which will be effective and inexpensive [2]. The demand for antibacterial finishes on textile goods as consumers are more aware nowadays of the potential utilization of these materials [4]. Various types of toxic chemicals are used in textile processes, but these are not easily degradable in the environment [4]. There is a huge demand for non-toxic, eco-friendly,

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antibacterial materials as a replacement for toxic textile chemicals. Sun protection creams and textiles are two common choices for the protection against UV radiation [5]. There are organic and inorganic UV-blockers. The organic blockers generally absorb the UV rays, and the inorganic blockers (e.g., ZnO, TiO_2) efficiently scatter both UV-A and UV-B radiations, mainly responsible for skin cancer. Compared to organic UV absorbers, inorganic UV-blockers are more preferable due to their chemical stability under UV illumination [6–8]. Due to harmful multidrug-resistant bacteria, an increasing demand has been observed to the food industries for the production of high-performance packing materials. Synthetic packaging materials are a big headache for the food industry as they left a huge amount of toxic waste materials in the environment. Nowadays, biopolymers are being explored as environment-friendly packaging materials [9]. There is a huge demand for antimicrobial packaging, achieved by the utilization of antimicrobial polymeric materials or adding antimicrobial ingredients to the packaging materials [9]. Generally, fouling on biofilms is formed by the colonization on pipelines, ship hulls, medical devices, drinking water treatment. Frequently formed fouling in membrane technologies results a decrease in permeate waterflux with an increase in energy consumption. It also decreases the extent of the membrane life period [10]. Therefore, novel strategies are required to decrease the microorganism growth on membranes. A better remedy for biofouling is the use of antimicrobial membranes [10].

In the year 1859, chitosan was discovered and first time discussed by Rouget [11]. In the early 1990s, chitosan entered the pharmaceuticals industries, which motivated industrialists and researchers to create a more effective therapeutic system based on it. The presence of active amino groups makes chitosan applicable in versatile fields. Structurally, it is composed of (copolymer) D-glucosamine and *N*-acetyl-D-glucosamine units with two hydroxyl (–OH) groups and one amino ($–NH_2$) group as shown in Fig. 6.1 [12].

Chitosan (Fig. 6.2a), the most abundant polysaccharide, is produced commercially through the deacetylation of chitin [1]. Chitosan remains as a polycationic species at low pH, because of the protonation in the amino group (Fig. 6.2b) with an increased solubility property. Initially, chitosan was used in medical applications like wound dressing, tissue engineering, and slimming. However, with time, it debuted as a prominent candidate for the drug delivery system [13]. Chitosan is

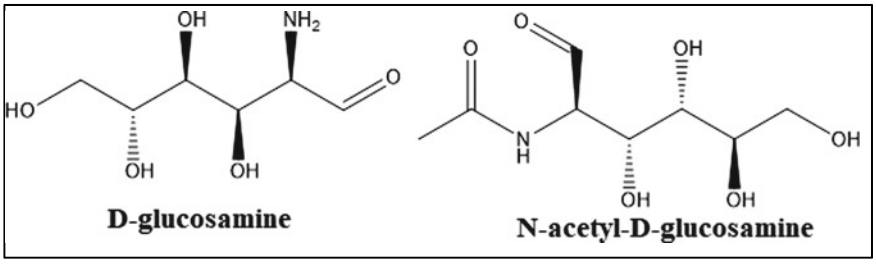

Fig. 6.1 Structure of D-glucosamine and *N*-acetyl-D-glucosamine

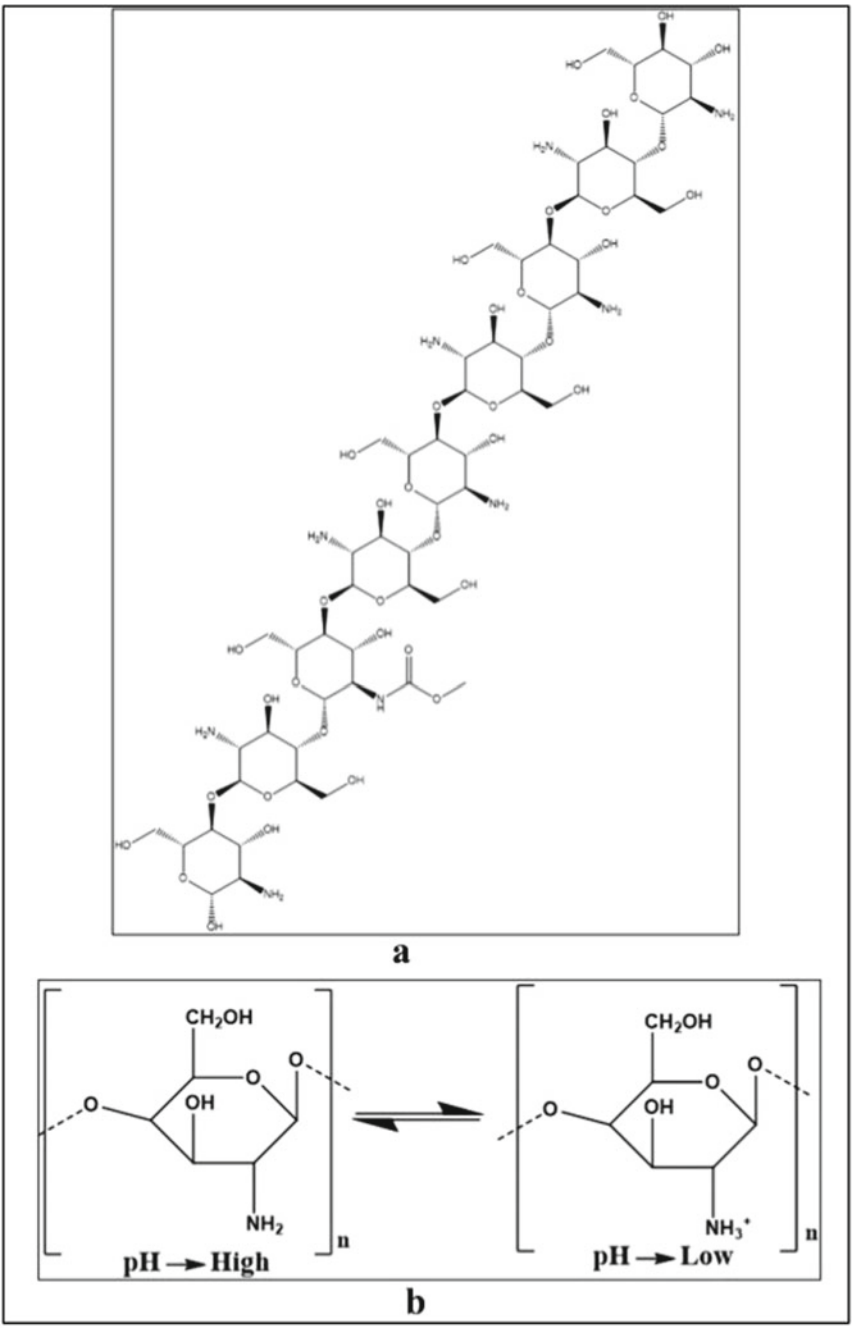

Fig. 6.2 **a** Structure of chitosan; **b** structure of chitosan at different pH

widely used in industries starting from foods to textiles, water treatment, agriculture, cosmetics, pharmaceuticals. Due to its unique biological characteristics, like non-toxicity, biodegradability, etc. [14]. However, the poor solubility of chitosan above pH 6.5 limits its practical applications [2]. On the other hand, antibacterial properties of chitosan decreases with decreasing pH of the medium [15]. Another strategy to retain the antibacterial property of chitosan even in low pH is its combination with metal (oxide) nanoparticles (NPs) [16].

Nanotechnology, a research hot spot of modern materials science can provide miscellaneous applications in different fields like food processing, textile industry, UV-blocker, fabric compounds, agricultural production, medicinal techniques [17]. It is a new horizon of research that deals with the synthesis, characterization, and applications of nanometer (nm) scaled (1–100 nm) materials. In this area, the pertinent materials show new and considerably enhanced physicochemical properties with a distinct phenomenon due to nanoscale size [18]. NPs generally offer larger surface areas compared to the macro-sized particles [19]. NPs are basically manipulated particles at the atomic level (1–100 nm). Metal NPs gained more attention due to their specificity and higher chemical reactivity as compared to their bulk state. The large surface-to-volume ratio of NPs makes nanoscale materials attractive for a large number of applications [20]. NPs provide extremely active centers, but they oxidize in air or agglomerates easily due to their high energy and extra-large surface area which leads to inactivation of both reactive and adsorption sites of NPs [20]. It reduces the contact between metal NPs and target molecules [21]. Over the past decades, scientists have adopted several approaches to achieve this goal. Metal NPs capping with biopolymers or natural compounds are the most common process to stabilize it for long-term utilization [22–26]. Nowadays, nanocomposite term is most common due to its versatile applications and long-term stability. The term nanocomposite indicates dispersed NPs in filled polymers [22]. Nowadays, ZnO/chitosan nanocomposite has attracted the attention of researchers due to its versatile application in various fields.

Zinc oxide (ZnO) presents in the earth's crust as a mineral zincite, while most of the ZnO used commercially is produced through different types of synthetic methods [27]. It is non-toxic and compatible with human skin with creating an additive for textile surfaces that are in touch with the flesh [27]. In comparison to bulk, zinc oxide nanoparticles (ZnO-NPs) have unique properties [28]. ZnO-NPs are portrayed as strategic, functional, versatile, promising, and inorganic materials with a wide range of applications [29]. The synthesis of ZnO-NPs has led to the exploration of its use as an antibacterial agent. With its unique antibacterial properties, ZnO-NPs also possess high optical absorption in the UV-A (315–400 nm) and UV-B (280–315 nm) regions and for that reason, it is used as a UV-blocker in cosmetics [30]. ZnO-NPs seem to powerfully resist microorganisms, and several reports showed its sizeable antibacterial drug activities that are attributed to reactive oxygen species (ROS) generation on the surface of these oxides [31]. Antibacterial activities of ZnO-NPs are also used in textile industries [32]. ZnO-NPs work on biocidal effects on bacterial species and exhibit marked antibacterial activity at low concentrations in neutral pH region (~7) [33]. To enhance the different activities (e.g., antibacterial, UV-blocker), biocompatibility, and stability of ZnO-NPs, recent studies have focused on the effective coating

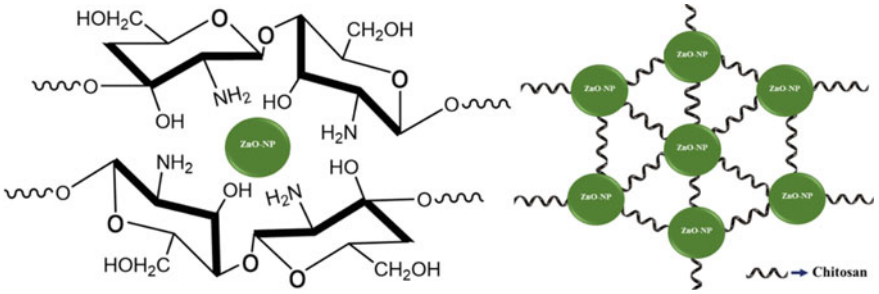

Fig. 6.3 Chitosan-capped ZnO-NPs

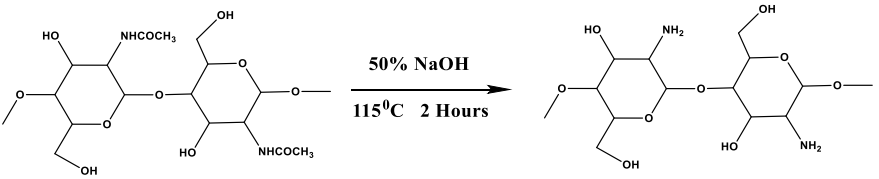

Fig. 6.4 Deacetylation of chitin to chitosan

of ZnO-NPs with biopolymers [34]. Chitosan is the suitable one for the effective coating of the ZnO-NPs. Due to the presence of active functional groups (i.e., amine, hydroxyl), chitosan can bind ZnO-NPs to form stable ZnO/chitosan nanocomposite which allows us to formulate new environment-friendly composite-based biomaterial in a cost-effective manner using natural resources[1, 20, 35]. This organic–inorganic hybrid materials (Fig. 6.3) show excellent antibacterial and UV-blocking properties which increase its application in textile industries [34].

6.2 Preparation of ZnO/Chitosan Nanocomposite

Saad et al. followed a common route for the preparation of ZnO/chitosan nanocomposite by using extracted chitosan [36]. The fresh shells of shrimp are washed thoroughly with double distilled water and dry in a vacuum. Powder form is produced by grinding in a mortar, and then, it is soaked for 24 h in 1 M NaOH solution. After that, it is again washed and dried. Then, the powder is deproteinized, demineralized, discolored using 1 M NaOH, 1 M HCl, and acetone, respectively. Then, deacetylation of the obtained chitin is followed using 50% NaOH at 115 °C for 2 h. For a higher degree of deacetylation, the process is repeated (Fig. 6.4). The resulting chitosan is then rinsed with distilled water followed by filtered and dried for 24 h.

$Zn(NO_3)_2{\cdot}6H_2O$, ethanol, $(NH_4)_2CO_3$, and deionized water are used as the starting materials to prepare ZnO-NPs. At first, $(NH_4)_2CO_3$ and zinc nitrate are dissolved in deionized water to form solutions. Zinc nitrate solution is slowly added into the

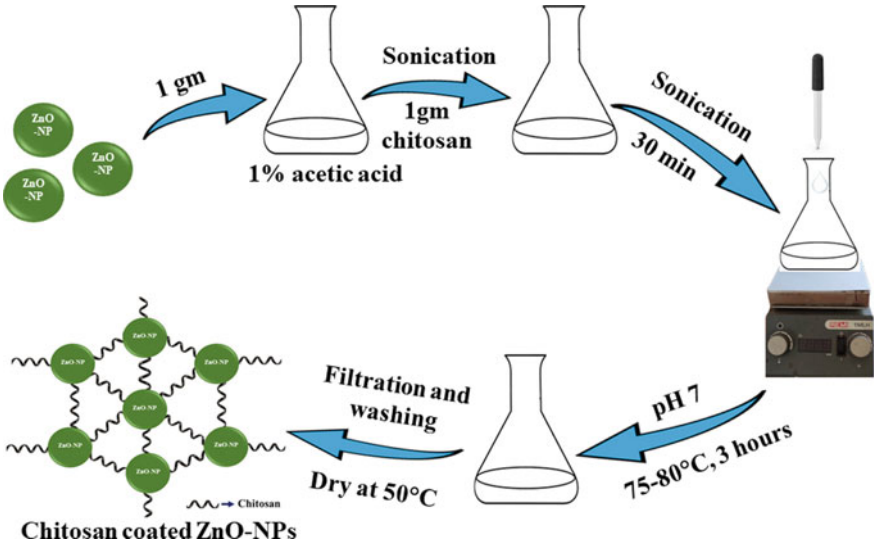

Fig. 6.5 Preparation of ZnO/chitosan nanocomposite by precipitation method

$(NH_4)_2CO_3$ solution with continuous stirring at 40 °C for 60 min. The precipitates are collected by filtration with a micrometer filter paper and rinsed with deionized water followed by ethanol. Precipitates are dried at 80 °C and calcined at 550 °C temperature for 2 h in the muffle furnace to get white ZnO-NPs. One gm of ZnO-NPs powder is added in acetic acid (1%, 100 mL) solution followed by the addition of chitosan (1.0 gm). NaOH solution (0.10 M) is added after keeping the solution in a sonicator bath for 30 min to maintain the pH 7. After that, the resulting solution is filtered and washed with distilled water then dried at 50 °C to get ZnO/chitosan nanocomposite (Fig. 6.5).

X-ray powder diffraction (XRD) study of the ZnO-NPs (Fig. 6.6b) reflects nine characteristic peaks ($2\theta = 68.97°$, 67.90°, 66.38°, 62.720°, 56.54°, 47.47°, 36.24°, 34.44°, and 31.74°). Li et al. also support this kind of observations [37]. The synthesized ZnO/chitosan nanocomposite (Fig. 6.1a) is amorphous in nature and exert of strong diffraction peaks at $2\theta = 47.470°$, 34.440°, and 31.740° reveal that the ZnO-NPs are well coated by chitosan with peaks between 2θ of 20° and 10° and it is well supported by the studies of Sivaraj et al. [38]. Formation of globular morphology of ZnO/chitosan nanocomposite is well supported by the field emission scanning electron microscope (FESEM) and bright-field (BF) scanning transmission electron microscopy (STEM) studies (Fig. 6.6c). Transmission electron microscope (TEM) study also shows the spherical nature of the particles with an average size of 58 nm (Fig. 6.6d).

Fourier Transform infrared spectroscopy (FTIR) study describes that hydroxyl and amine groups are mainly responsible for the stabilization of ZnO/chitosan nanocomposite [1].

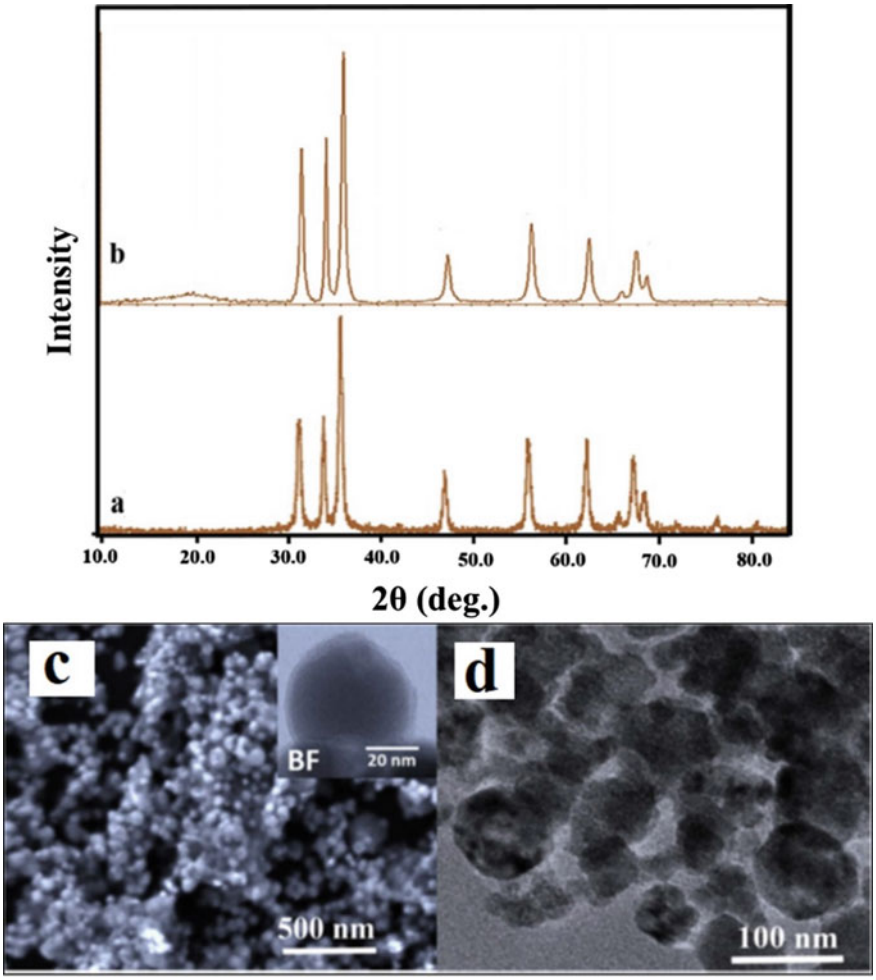

Fig. 6.6 XRD pattern of **a** ZnO/chitosan nanocomposite, **b** ZnO-NPs; **c** FESEM and bright-field STEM images and **d** TEM image of ZnO/chitosan nanocomposite [36]

In another study, Saeed et al. synthesized ZnO/chitosan nanocomposite in a different manner [35]. Different amounts of ZnO are dissolved into acetic acid followed by the addition of high-molecular-weight chitosan. Acetic acid is added for increasing the volume of the solution. A continue stirring is very much needed to dissolve the chitosan flakes. 1*N* NaOH may be added to maintain the pH 10 of the solution. After heating in a water bath and cooling at room temperature, the mixture is filtered. The obtained white precipitate is washed with distilled water and dried at 60 °C for getting ZnO/chitosan nanocomposite. TEM study suggests that synthesized ZnO/chitosan nanocomposite is in the range of 20 nm.

Dananjaya et al. [34] also followed almost the same process for the synthesis of ZnO-NPs as followed by Saad et al. In this case to get ZnO/chitosan nanocomposite, the mixture (synthesized ZnO-NPs, acetic acid, and chitosan) is sonicated with the addition of NaOH. Two sets of diffraction peaks (for ZnO and chitosan) confirm the successful formation of ZnO/chitosan nanocomposite. The purity of the synthesized ZnO/chitosan nanocomposite can be confirmed by the absence of peaks due to the impurities like Zn or $Zn(OH)_2$. The mean crystalline size of the synthesized ZnO/chitosan nanocomposite can be estimated by using Scherrer's formula [39], and it is found in the region of 22 nm. Spherical-shaped ZnO-NPs can be determined from the FESEM image (Fig. 6.7a). The inset TEM image (Fig. 6.7a) also reflects good dispersion of the ZnO-NPs. Zn and oxygen dominate the energy-dispersive X-ray spectroscopy (EDS) profile (Fig. 6.7b) indicating the absence of elemental impurities present. If carbon tape is used for the sample loading, then there is a chance of getting a carbon peak in the EDS profile. A cluster-like morphology is generally observed in the FESEM image (Fig. 6.7c) of ZnO/chitosan nanocomposite. The inset TEM image (Fig. 6.7c) of ZnO/chitosan nanocomposite reflects irregular shape of clusters having randomly aggregated NPs. The EDS profile (Fig. 6.7d) of ZnO/chitosan nanocomposite further confirms the formation of ZnO/chitosan nanocomposite. The presence of nitrogen in Fig. 6.7d is probably due to the presence of amine (–NH2) group of chitosan.

The UV–Vis absorption spectroscopic (UV–Vis) study (Fig. 6.8) of ZnO-NPs and ZnO/chitosan nanocomposite displays a similar pattern of absorption spectra with a slight variation of absorption maxima. ZnO-NPs exhibits an absorption maxima around 365 nm, but it shifts to 355 nm in ZnO/chitosan nanocomposite due to the presence of the interaction between chitosan and ZnO-NPs (Fig. 6.8).

6.2.1 General Mechanism of the Formation of ZnO/Chitosan Nanocomposite

In acidic medium (pH: 6–9), at first ZnO converts to Zn^{2+} followed by the formation of $Zn(OH)_2$. As the $–NH_2$ and –OH groups of chitosan can form a co-ordination bond with metal ions [40], with increasing the pH of the solution (generally by the addition of NaOH), a stable complex of ZnO/chitosan nanocomposite is formed [5].

6.3 Antibacterial Activity of ZnO/Chitosan Nanocomposite

ZnO-NPs are quite attractive due to their significant antibacterial properties. Biocompatibility of the ZnO-NPs increases when it is capped by chitosan [41]. For this reason, the scope of versatile biomedical application of ZnO/chitosan nanocomposite increases. Furthermore, the antibacterial action ZnO/chitosan nanocomposite

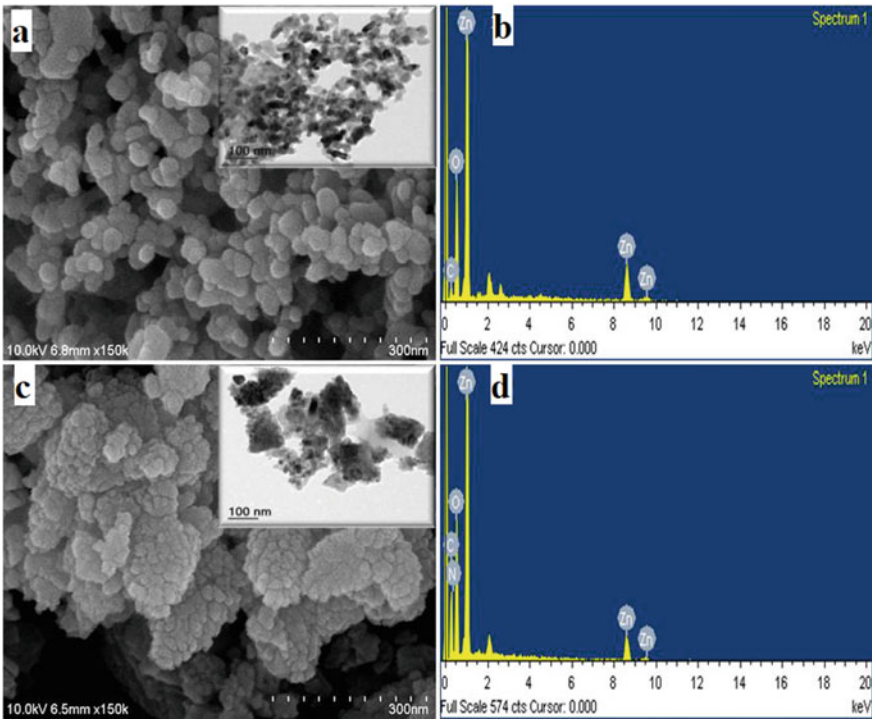

Fig. 6.7 **a** FESEM image (inset TEM image) and **b** EDS profile of ZnO-NP; **c** FESEM image (inset TEM image) and **d** EDS profile of ZnO/chitosan nanocomposite [34]

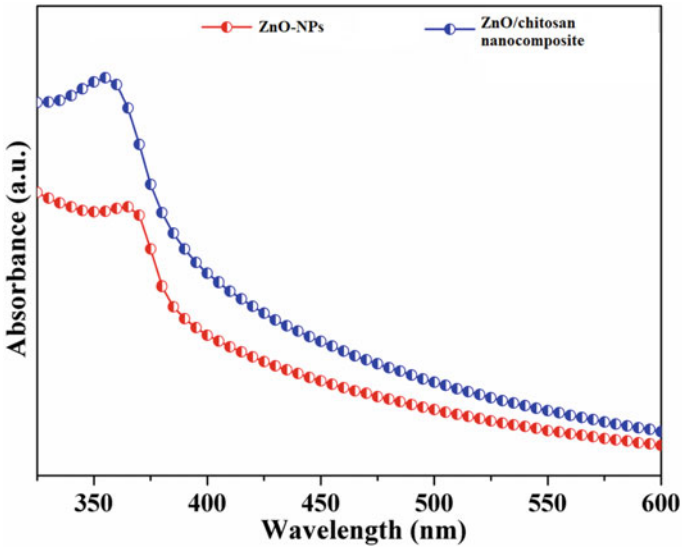

Fig. 6.8 UV–Vis study of ZnO-NPs and ZnO/chitosan nanocomposite

improves significantly with respect to its reference material (only ZnO-NPs or chitosan) [10, 42–44]. Theodoridou et al. made an antibacterial assay of ZnO/chitosan nanocomposite against *Escherichia coli (E. coli)* BL21(DE3), *Corynebacterium glutamicum (C. glutamicum)* ATCC 21,253, and *Brevibacterium lactofermentum (B. lactofermentum)* ATCC 21799 [41]. This study reflects that the antibacterial activity of ZnO/chitosan nanocomposite is high against *B.lactofermentum*, moderate against *E. coli* and almost absent against *C.glutamicum.* The observed differences may be related to the compositional and structural differences in cell membrane of each bacterium [10]. Rahman et al. prepared ZnO/chitosan nanocomposite film to increase its utility in the food industry as a food packaging material [45]. For this reason, they checked the antibacterial effect of the film against *Staphylococcus Aureus* (*S. aureus*, MTCC 737) and *Escherichia Coli* (*E. coli*, MTCC 1687) bacteria by colony-forming units (CFU/gm) method. As per this observation, the composite film shows significant antibacterial activity with respect to the control chitosan. ZnO/chitosan nanocomposite film showed higher antibacterial activity against *E. coli* than *S. aureus.* This investigation also reveals that antibacterial activity is directly related to the amount of ZnO-NPs particles in the composite films. The decreased activity of composite film toward *S. aureus* bacteria is due to the presence of thick peptide glycan layer in its cell wall [46]. Another research work also reflected same kind of results (Table 6.1) at different concentrations of ZnO/chitosan nanocomposite against *E. coli* and *S. aureus* [5].

Vaseeharan et al. also reported the antibacterial activity of ZnO/chitosan nanocomposite against Gram-positive *Bacillus lechiniformis* (*B. lechiniformis;* accession No-HQ693275) and Gram-negative *Vibrio parahaemolyticus* (*V. parahaemolyticus;* accession No-HQ235407) isolated from aquatic environments by broth culture and zone inhibition methods in the pH range of 6–7 [47]. In that study, the growth curves of the *B. lechiniformis* and *V. parahaemolyticus* without ZnO/chitosan nanocomposite showed increased level of bacterial growth up to 150 min. On the other hand, *V. parahaemolyticus* and *B. lechiniformis* treated with ZnO/chitosan nanocomposite show a significant reduction in growth (Fig. 6.9). Furthermore, growth of the tested bacteria decreases after treating with ZnO/chitosan nanocomposite from 30 to 150 min. Zone inhibition test also shows that there is no zone of inhibition against the tested bacteria.

Inhibitory activity of ZnO/chitosan nanocomposite may vary with the pH of the medium and the percentage of the $-NH_2$ functional group on the chitosan surface [47].

Table 6.1 Antibacterial activity of ZnO/chitosan nanocomposite at different concentrations [5]

Concentration of ZnO/chitosan nanocomposite (%)	Inhibition zone (mm)	
	S. aureus	*E. coli*
0.5	5	7
1	9	10
2	11	12
4	11	13

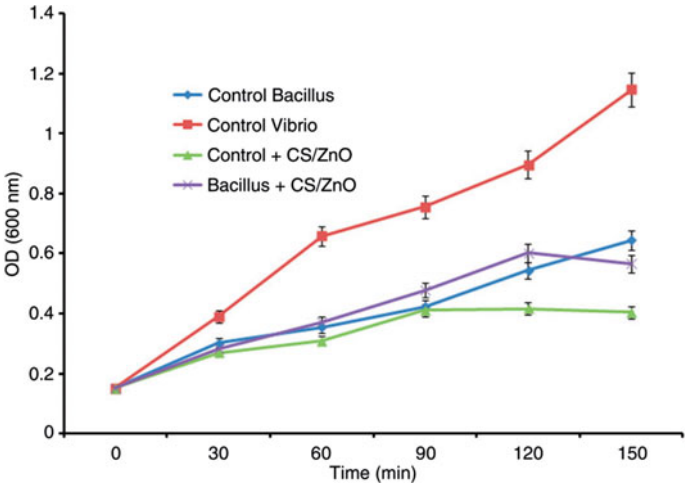

Fig. 6.9 Growth curves of *B. lechiniformis* and *V. parahaemolyticus* in the presence of ZnO/chitosan nanocomposite and without ZnO/chitosan nanocomposite. (The error bars indicated the standard deviation, and CS/ZnO represents ZnO/chitosan nanocomposite) [47]

Vaseeharan et al. also studied the antibiofilm efficiency of ZnO/chitosan nanocomposite [47]. Light microscopy (Fig. 6.10) and confocal laser scanning microscopic (CLSM) images (Fig. 6.11) showed the bacterial growth inhibition with disruption of biofilm growth after 24 h of treatment with ZnO/chitosan nanocomposite. ZnO/chitosan nanocomposite inhibits the biofilm formation of *B. lechiniformis* and *V. parahaemolyticus* at the concentrations of 20–60 μg/ml. Antibiofilm efficacy results show higher colonization in control *V. parahaemolyticus* and *B. lechiniformis* biofilm growth, whereas in the presence of ZnO/chitosan nanocomposite, a greater reduction of colony and a gradual decrease in the biofilm growth are observed (Fig. 6.10a and b).

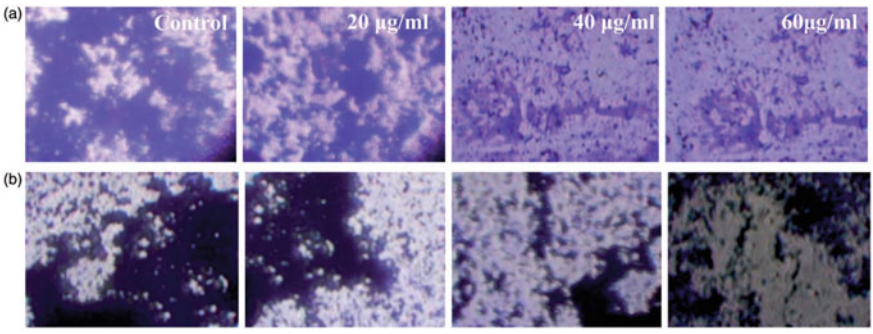

Fig. 6.10 Light microscopic images (×40); antibiofilm efficacy of different concentrations (20–60 μg/ml) of ZnO/chitosan nanocomposite against **a** *B. lechiniformis* **b** *V. parahaemolyticus* [47]

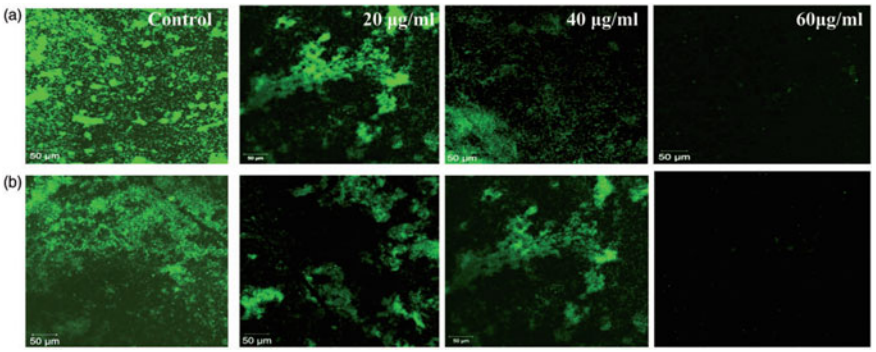

Fig. 6.11 CLSM images; antibiofilm efficacy of different concentrations (20–60 µg/ml) of ZnO/chitosan nanocomposite against **a** *B. lechiniformis* and **b** *V. parahaemolyticus* [47]

The thickness of the biofilm is reflected in the CLSM images (Fig. 6.11). CLSM studies establish that *V. parahaemolyticus* and *B. lechiniformis* biofilm growth is completely arrested at a concentration of 40 mg/ml and 60 mg/ml, respectively (Fig. 6.11 a and b).

6.3.1 Probable Mechanism of the Antibacterial Activity of ZnO/Chitosan Nanocomposite

The suggested mode of antibacterial action of the ZnO-NPs includes (a) the dissolution of ZnO-NPs followed by the release of Zn^{2+} antibacterial ions, (b) penetration of nanosized particles with cell membrane damaging, and (c) reactive oxygen species formation on the surface of the ZnO-NPs which destroys bacterial cells [29, 48, 49]. The antibacterial activity of ZnO-NPs depends on the size due to the internalization and accumulation of NPs inside the cells [29]. Rahman et al. also suggest a probable mechanism of antibacterial activity, similar to the previous study [45]. ZnO-NPs release ROS. The ROS and Zn^{2+} ions attack negatively charged cell wall which causes cell leakage and ultimately the death of bacteria [50]. On the other hand, ZnO-NPs enhance the positive charge on the $–NH_2$ group of chitosan which accelerates the interaction with the anionic components on the bacterial cell wall [50].

6.4 Applications of ZnO/Chitosan Nanocomposite in Textiles

Nowadays, there is a huge demand for antibacterial finishes on textiles as the textiles are considered as the ideal environment for bacterial growth. Significant antibacterial activities and UV-blocking properties of ZnO/chitosan nanocomposite increase its demand in textile industries. Farouk et al. selected cellulosic fabrics to check the antibacterial activity of ZnO/chitosan nanocomposite against *E. coli*, and *M. lutues* and they observed that, in case of both *M. lutues* and *E. coli* there was nearly 100% reduction of the colonies after 3 h in case of cotton 100% treated fabrics with ZnO/chitosan nanocomposite against *M. lutues* and *E. coli* bacteria [51].

SEM micrographs (Fig. 6.12) reflect that the surface of the treated fabric becomes smoother compared to the untreated fabrics due to the homogeneous distribution of ZnO/chitosan nanocomposite within the coating layer. Cotton/polyester fabric treated with ZnO/chitosan nanocomposite shows a lower absorbance value of formazan with respect to the formazan absorbance value of untreated cotton/polyester fabric. The tensile strength of fabric samples treated with ZnO/chitosan nanocomposite is significantly increased. This increase may be attributed to penetration of the chitosan molecule which makes some kind of sizing to the treated fabrics and improves the tensile strength. Air permeability is considered an important factor in the performance of textile materials. Coating to the fabrics by ZnO/chitosan nanocomposite does not cause decreasing of air permeability.

Arafat et al. used ZnO/chitosan nanocomposite as an adsorbent to remove reactive dyes (Black HN and Magenta HB) from textile dyeing industry effluent [52]. As per their observation, ZnO/chitosan nanocomposite has a dye removal efficiency of 95–99%. They also claim that color adsorption ability increases with

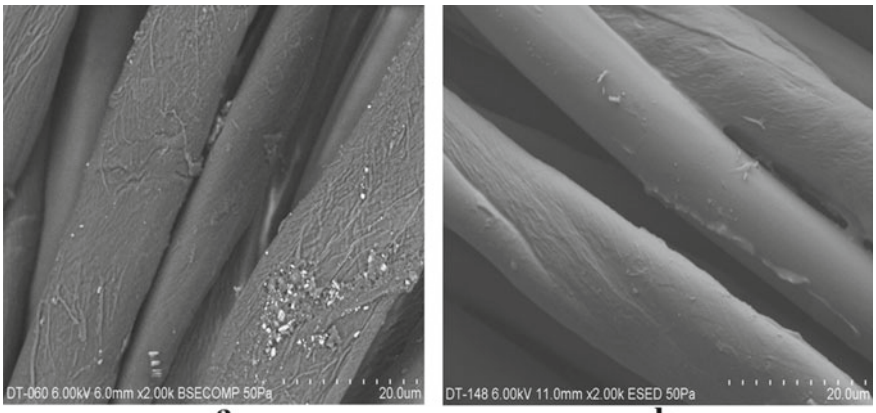

Fig. 6.12 Scanning electron microscope (SEM) images of the **a** untreated cotton/polyester (65/35%) fabric and **b** ZnO/chitosan nanocomposite-treated cotton/polyester (65/35%) fabric [51]

increasing the dosage of ZnO/chitosan nanocomposite. This study also reflects 2 gm of ZnO/chitosan nanocomposite per liter of effluent at an ambient temperature (50 °C) and 60 min of contact; it is possible to remove approximately 99% of the original color of the effluent. Petkova et al. also used ZnO/chitosan nanocomposite for sonochemical coating of textiles to get rid of microorganisms [53]. This coating improves the durability, biocompatibility, and antibacterial activity (against *S. aureus and E. coli*) of the textile.

6.5 Applications of ZnO/Chitosan Nanocomposite as a UV-Blocker

Due to the depletion of the ozone layer in the earth's atmosphere, the effect of UV radiation increases on human skin. This UV irradiation has a cytotoxic effect on the skin cells (keratinocytes and fibroblasts) and causes of skin cancer with the damage of the epidermis [54, 55]. Therefore, researchers are interested to focus on the development of textiles with UV protection functionality [56]. With sunscreen, UV-absorbing compounds are also in other personal care products, like lipstick, hair spray, shampoo, toilet soap, body wash, and insect repellent [57]. UV is the part of invisible light which are categorized with three different part according to their wavelength: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm) [58]. Chemically, two types of UV-blockers are available in the most recent decades—inorganic and organic. The mechanistic pathway of UV-blockers is either absorbing or scattering the UV radiation [57, 59]. Especially, organic materials (known as chemical filters) act as an absorber, mainly UV-B radiation, whereas inorganic materials (known as physical UV filters) follow the scattering and reflection pathway to block the UV radiation [60]. The presence of nano-oxides in sunscreens plays a vital role in the generation of reactive oxygen species (ROS), and this process is based on the photogeneration of electron–hole pairs followed by the interfacial electron transfer (IFET) or energy transfer process [61]. During the energy transfer process, singlet oxygen ($^1O_2$) is produced, and while superoxide ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are formed through IFET process [62, 63]. The produced singlet oxygen and hydroxyl radicals are responsible to show cytotoxicity and mutagenicity [64]. The above phenomenon leads to the oxidation of organic ingredients in cosmetics and ultimately, interacts with the protein, DNA, and lipids and finally, photodamaged the cell [65, 66]. Recently, researchers are trying to minimize the photoactivity of ZnO-NP through surface modification with different antioxidants [63]. Polymer coating with natural antioxidant like chitosan is the most preferable and interesting way surface modification [67].

To check the applicability of ZnO/chitosan nanocomposite as a UV-blocker, the following method can be used. 0.5–4% (by weight) of ZnO/chitosan nanocomposite powder is suspended in water followed by the sonication of 10 min. In this suspension, bleached cotton fabric samples are padded in two dips and nip followed by

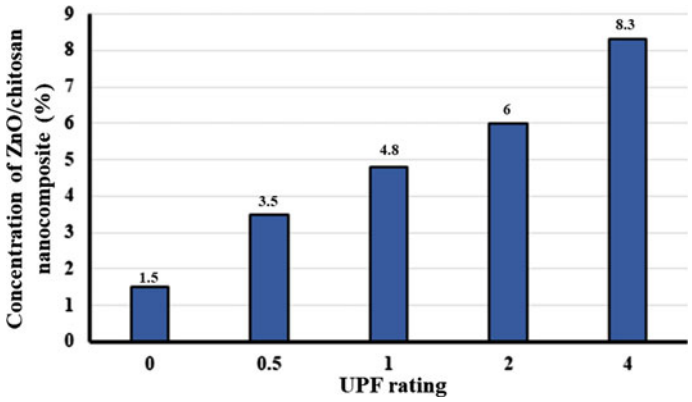

Fig. 6.13 UPF rating of bleached cotton fabric treated with ZnO/chitosan nanocomposite [5]

squeezing to a wet pick-up of 100%. The samples are dried at 100°C1(10 min) and cured at 170 °C (5 min). The treated cotton fabrics are washed with distilled water and finally dry [5]. Figure 6.13 reflects UV protection of bleached cotton fabric treated by ZnO/chitosan nanocomposite. The ultraviolet protection factor (UPF) of the treated samples is higher than that of untreated cotton fabric. UPF rating very much depends on the concentration of ZnO/chitosan nanocomposite which reflects its UV absorption capacity on the surface of the cotton fabric [68].

6.6 Conclusion

In summary, ZnO/chitosan nanocomposite is a good option to the textile industries for a coating over textiles to improve its antibacterial and UV-blocking properties. Various methods are available to synthesize ZnO/chitosan nanocomposite. UV–Vis, FTIR, TEM, SEM, XRD studies can be used to characterize synthesized ZnO/chitosan nanocomposite. The size and stability of ZnO/chitosan nanocomposite depend on its synthesis route. Depending on the application of the ZnO/chitosan nanocomposite, its synthesis route should be selected. The antibacterial properties of ZnO-NPs depend on their size. Coating of textiles with ZnO/chitosan nanocomposite also increases its durability and biocompatibility.

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Chapter 7
Polymeric Nano-Composite Scaffolds for Bone Tissue Engineering: Review

Lokesh Kumar and Dheeraj Ahuja

Abstract Bone tissues have an amazing ability to repair and regenerate. However, complex bone fractures and defects still present a significant challenge to the researchers in biomedical field. Current treatments center on autograft-allograft and metal implant to substitute bone loss. While metal implant and allograft treatments are associated with several complications such as donor site morbidity and limited supply of material. Therefore, scaffolds can provide a new method to resolve such problems by restoring and improving tissue functions. An ideal scaffold should have biocompatible and biodegradable, as well as suitable 3D porous interconnected structure to facilitate cells and tissues in growth with proper circulation of bone mineralization. To date, various biomaterials are available for bone tissue engineering including ceramics, polymers and composites composed by calcium and phosphate bone minerals. Polymeric scaffolds can be modified to improve bioactivity and osseointegration mechanical strength in order to tailoring biological properties. In this chapter, strategies and techniques to engineer new kind of polymer surface to promote osteoconduction with host tissues will be discussed. Also, benefits and applications of polymeric composite scaffolds for orthopedic surgery will be discussed.

Keywords Tissue engineering · Polymer scaffold · Osteoconductive · Biodegradable

Abbreviations

HA Hydroxyapatite

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TE	Tissue engineering
PLA	Polylactic acid
PU	Polyurethane
ECM	Extracellular matrix
PCL	Poly(caprolactone)
PGA	Poly(glycolic acid)
PMMA	Polymethylmethacrylate
PA	Polyamide
PLGA	Poly(L-lactic-*co*-glycolic acid)
TIPS	Thermally induced phase separation
PLA	Polylactide
TCP	Tri-calcium phosphate
TEA	Triethanolamine
DEA	Diethanolamine
PEG	Poly(ethyleneglycol)

7.1 Introduction

7.1.1 Scaffold for Bone Tissue Engineering

An extensive variety of clinical methods have been utilized for replacement or repair of bone or tissue damaged due to any disease or injury. Currently, the most widely utilized healing practice is based on three types of donor graft tissues: allograft, autograft and xenografts. The major limitation of utilizing these healing practices is less availability of donor and donor sites, higher morbidity rate, chances of disease transmission and rejection of grafts [1]. This limitation can be overcome by tissue engineering. Tissue engineering reproduces the damaged tissue by developing biological substitutes rather than restoring them with grafts. This helps in reviving and improving tissue function [2–4]. The first article on tissue replacement was published by Gaparo tagliacozzi in 1597 [5]. Tissue repair and regeneration are natural healing processes that take place after damage on patient's body. For example, liver is one of the organs of human body that can be regenerated after fractional noxiousness [6].

The tissues can be reproduced in two different ways. The first way includes isolation of cells from patient's body and growing them on three-dimensional scaffolds under controlled conditions. The tissues so cultured are then replaced with the defected tissue, and the scaffold is degraded over the time. Another way is directly growing tissue in vivo utilizing scaffold that instigates and targets growth of tissue. The in vivo method, i.e., direct growth of tissue in patient body, is beneficial over in vitro, i.e., growing tissue in culture and then replacing as for in vivo tissue grows in situ and patient's cells are not required. The combination of both in vivo and in vitro is known as tissue engineering triad and is shown in Fig. 7.1. This triad

generally works on three fundamentals, i.e., signaling mechanism, cells and extracellular matrix (ECM). ECM holds the cells and helps in regeneration and development of tissues [7]. The fundamental conception is to utilize inherent biological responses to tissue damage in conjunction with engineering fundamentals [8]. In tissue engineering, regeneration of bone tissue is widely studied area. As per bone tissue engineering fundamentals, bone tissue equivalents are developed by targeting osteogenic differentiation of multipotent mesenchymal stem cell of bone marrow [9]. It is being utilized for implant surgery, where the objective is to harvest the ideal tissue engineered bone construct [10, 11].

Tissue regeneration process is generally achieved by implying three steps that help in attainment of entire process. The first steps involve inoculation or transportation of grown cells to a damaged or injured site followed by transmission of tissue producing biomolecules to a targeted tissue. The final and third step involves growth and differentiation of a required cell type in 3D scaffolds. Among all these three steps or approach, tissue engineering based on scaffold is gaining attention as it has the possibility of assimilating chemical, physical and biological stimuli with scale variation for cell activity.

Therefore, in the last two decades research in the arena of scaffold-based tissue engineering has increased at a rapid rate [12, 13]. Among the varieties of scaffolds, research on biodegradable polymeric scaffolds for tissue engineering is gaining much attention as they cater sensual and structural surroundings for growth of cells and tissue [14–17]. Scaffold is central component that is utilized for delivering drugs, cells

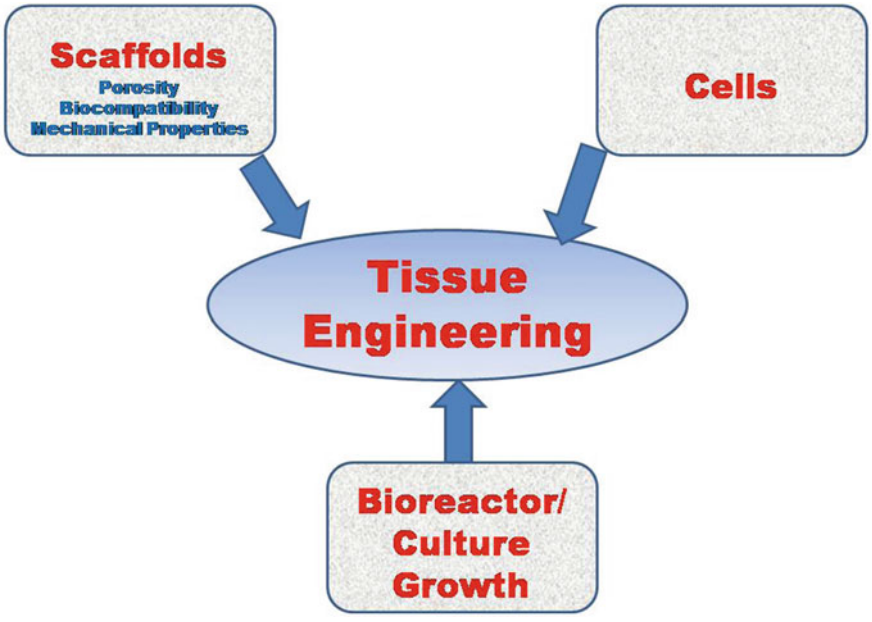

Fig. 7.1 Tissue engineering system (triad)

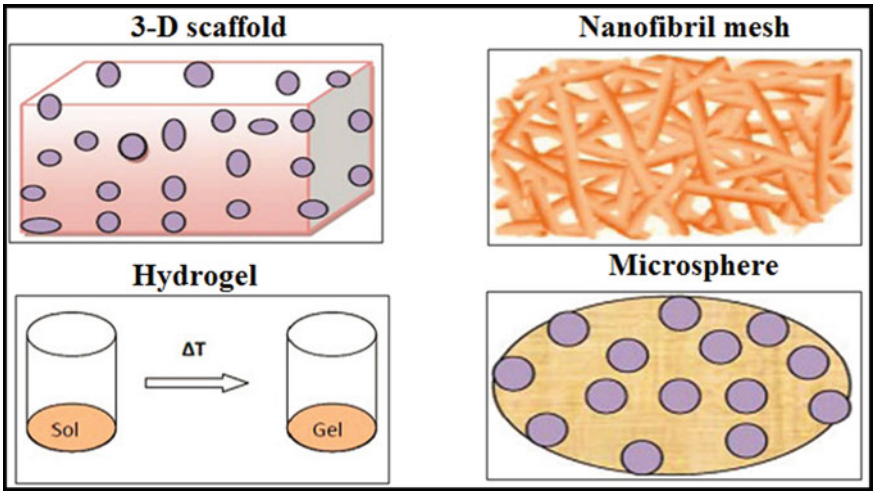

Fig. 7.2 Different types of polymeric scaffolds for cell and drug delivery

and genes into the patient's body. On the basis of this, scaffolds are classified as cell delivery scaffold and drug delivery scaffold. Implantation of cells into fabricated arrangement capable to support 3D tissue formation is referred as "cell delivery scaffolds," while fabricated arrangements capable of high drug loading efficiency and drug release for longer duration are known as "drug delivery scaffolds" [18, 19]. Polymeric scaffolds being utilized for cell or drug delivery application include 3D porous matrix, a nanofibrous matrix, a thermoresponsive sol–gel transition hydrogel and a porous microsphere as shown in Fig. 7.2 [20–23]. These all are being utilized for constant drug discharge formulations and have been practiced in tissue engineering for their possible usage as a cell delivery carrier or supportive matrix [24].

7.2 Properties of Scaffold

Scaffolds are three-dimensional structures formed by the implantation of cells that helps in cell formation. They also help in repositioning of contained structure and generating adequate mechanical settings for the proper healing of the organ by providing mechanical support. Also, they help in growth and attachment of cell, thereby leading to cell formation. Further, it also drops and absorbs cells and biomechanical factors, enables diffusion of vital cell nutrients and expressed products along with exerting specified mechanical and biological influences to change the performance of cell phase. Once the patient's body part or organs is healed, the extraneous part is required to be detached from human body together with clinical and biomechanical point of view. Hence, there are some of the key characteristics that must be considered while fabricating 3D scaffolds for tissue engineering. Generally, the

Table 7.1 Essential properties required for smooth functioning of cell delivery scaffolds and drug delivery scaffolds for tissue engineering

Cell delivery scaffolds	Drug delivery scaffolds
• Tolerable tensile properties to defense cells from tensile forces [32]	• Uniform distribution of drug all over the scaffold [33]
• Desired volume, mechanical strength and shape [34]	• Capability to deliver the drug at fixed interval of time [35]
• Admissible biocompatibility [14]	• Low drug abiding affinity so as to allow stable drug delivery during scaffold injection at a physiological temperature [36]
• Bioadsorption at fixed interval of time [37]	• Dimensionally, structurally and biologically balanced activity for longer duration [36]
• Biocompatible chemical combination with minimum allergic and immune responses [38]	
• An extremely porous and interrelated open pore architecture to concede high cell seeding density and tissue in growth [39, 40]	
• Physical architecture to hold cell adhesiveness and propagation [41]	

scaffold should be biocompatible and possibly biodegradable with desirable surface properties for cell adhesion, mitigation and normal functionality persuaded by the desired mechanical strength and porosity to be able to integrate with the surrounding tissue [17, 25, 26]. In addition, size of scaffold must be identical to the injured surface. Furthermore, biological signals from scaffolds such as small drug molecule, growth factors and cytokines in vitro and in vivo should be delivered in controlled manner as they are important parameters for foundation and enrichment of tissue morphogenesis, viability and functionalities [27–29]. Hence, fabrication should take into account the physico-chemical properties for the release of required biomolecules to direct and regulate biological responses of the cells into particular tissue.

As described in the above section that scaffolds for bone tissue engineering are classified as cell delivery scaffolds and drug delivery scaffolds. Some of the important properties that both types of scaffolds should possess for effective tissue engineering are mentioned in Table 7.1 [30, 31].

Apart from the abovementioned properties for cell delivery and drug delivery scaffolds, some of other important properties that should be considered for scaffold tissue engineering are discussed below:

7.2.1 Biocompatibility

It is referred as the ability of a material to meet the desired application without performing an allergic or harmful immune effect. The scaffold prepared to be seeded

should have admissible biocompatibility and toxicity profile [42]. Also, it must have sufficient surface chemistry for cellular attachment, differentiation and proliferation [43]. Further, it should adhere to the cells with minimal interruption of surrounding tissues. Variety of tissue responses are attained from seeding of scaffolds depending upon their composition [44]. When the scaffold seeded is nontoxic and degradable, new tissue is generated while the nontoxic and biologically effective scaffold assimilates with the neighboring tissues. In case the scaffold is biologically inactive, it may be enclosed with fibrous capsule, whereas it is rejected from the body resulting in the death of neighboring tissue when it is toxic [45–48]. Samandari and Samandari [49] studied the biocompatibility of prepared chitosan-graft-poly(acrylic acid-co-acrylamide)/hydroxyapatite nanocomposite scaffold using multistep model by MTT assays on HUGU cells. It was found that scaffold has good cytocompatibility and cell viability and proliferation enhanced with reinforcement of hydroxyapatite. Kumar and Ahuja [50] synthesized aliphatic polyurethane nanocomposite utilizing modified hydroxyapatite and performed cell culture and in vitro studies in simulated body fluid. It was observed that surface was partially hydrolyzed and prepared nanocomposite was suitable for bone tissue engineering.

7.2.2 Biodegradability

It is referred as the chemical disintegration of a biomaterial by bacteria or other biological molecules inclusive of hormones, acids and body fluids [51]. The developed scaffold shall be degradable. Products resulting from the degradation of scaffold control the response of immune system. Therefore, the degradation products of scaffolds shall be nontoxic and should be easily exterminated from the implanted spot of the body so as to get rid of further surgery to remove it. Further, the rate of degradation of scaffold shall be adjustable so that it can be balanced with the rate of tissue production so that it completely dissipates from the body after the tissue production. Hence, currently the scaffolds are developed from the familiar degradable polymers for scaffold tissue engineering. To impart the above-desired properties, the scaffold shall be able to tune mechanical properties, degradation kinetics and release kinetics for different purposes. The degradable polymers to be utilized for orthopedic injuries must fulfill series requirements like mechanical support during tissue growth, organized degradation to biocompatible breakdown products and controlled release of biomolecules and shall maintain osteoconductive and osteoinductive surroundings.

In the recent past, a great deal of attention is being focused on utilization of biodegradable polymers for the development of scaffolds. The reason for this is their well-acknowledged biocompatibility in vivo in addition to the two major reasons. Firstly, the scaffold prepared using degradable polymers can be tuned for their mechanical properties along with their controlled degradation. Secondly, with the passage of time after complete healing of the injury the architecture of scaffolds completely degrades eliminating the need of second surgery for the rehabilitation of the implant, thereby resulting in the fast recovery of the injured site. Among various

degradable polymeric scaffolds such as polyglycolic acid (PGA) [52], polylactic acid (PLA) [53], polyurethanes (PU) [54] and polycaprolactone (PCL) [55], polyurethane is best delivery scaffolds and offers several benefits in the design of injectable and biodegradable polymer composition [56–59].

7.2.3 Porosity

It is degree of material void space and is a part of void volume by absolute volume and is also referred as "*void fraction*" [60]. Competent porosity, pore size distribution and inter-pore connectivity support vascularization and cell growth [61, 62]. Mondal et al. in 2014 synthesized surface modified and aligned mesoporous anatase titania nanofibers-based mats for esterified cholesterol detection and found that around 61% enzyme molecules were loaded in the mat due to its high porosity of fibers [63].

7.2.4 Targetability

It is the capability of the formulation system to influence their prearranged spot and release their enclosed substances on the injured spot [64]. Formulation systems composed of nanofibers have magnificent capacity to transport their enclosed substances to the injured spot and escape from their side effects. This effective target ability results in the reduction of the dose and frequency of enclosed substances [65, 66]. Gong et al. encapsulated amphiphilic peptide developed by transformation of nanoparticles to nanofibers for growth of immune system after cancer treatment. It was observed that amphiphilic peptide had antitumor properties and low toxicity in mammalian cell indicating good biocompatibility in addition to antibacterial properties, to prevent from bacterial contamination [67].

7.2.5 Binding Affinity

As the name suggests, it is the capacity of the drug to bind the scaffold. It should be low enough to deliver the drug [68]. Varieties of scaffolds have been developed by different researchers utilizing various nanomaterials having binding affinity [69–71]. However, among them scaffold formulation of nanofibers has proved to be having adequate binding efficiency for continuous delivery of the enclosed substance for longer duration or accommodating of cells in their pore structure [72, 73].

7.2.6 Stability

Assessment of physical, chemical and biological activities of the developed scaffold at different environment condition is referred to as stability of the scaffold. The developed scaffolds must have chemical and biological stability along with dimensional stability for longer duration of time. Nanocomposites of scaffold exhibit magnificent stability at physiological temperatures, and their activity is sustained for prolonged period [74–76]. Polyvinylidene fluoride (PVDF)/poly(methylmethacrylate) (PMMA)/hydroxyapatite (HA)/titanium oxide (TiO_2) (PHHT) film scaffold nanocomposites with surface morphology nanowhiskers were developed by Arumugam et al. [77]. The prepared nanocomposites were explored for mechanical stability and in vitro studies for biomedical application. Results showed that nanocomposite scaffolds were mechanically stable and can be used for biomedical applications.

7.2.7 Loading Capability and Deliverance

It is the quantity of drug that can be immersed into the scaffold. The scaffold should possess high drug loading capability in order to deliver the drug for prolonged period after seeding of the scaffold in the body [78]. The drug from the scaffold should be delivered in controlled manner to allow the adequate amount of dose to be delivered to the cells over a given duration [79, 80].

7.2.8 Mechanical Properties

The assessment of developed scaffolds characteristics over different types of forces such as stress, strain, break, dent, stretch or scratch is referred as mechanical properties of the scaffolds [81]. These properties are influenced by the interior structure design of scaffold. Till date, plenty of porous scaffolds have been developed that have strength in the range of 10–30 Mpa. The strength can be altered by varying the porosity of the scaffold [82, 83]. So that during implantation, these properties of the scaffolds are competent with that of tissue at the seeding spot or they are able to protect the cells from ruining tensile and compressive forces and to sustain under physiological conditions [84]. Once the scaffold is implanted, it should impart minimal level of biomechanical function that should continuously recover till normal tissue function has been achieved [85].

7.2.9 *Scaffold Architecture*

Pore size and shape, pore tortuosity, degree of porosity and surface area constitute the architecture of scaffold [86]. Microstructure of scaffold is utilized to examine the movement of nutrients, waste and biological chemicals within scaffold and reciprocal action of cell on scaffold. Movement of cells within the scaffolds is adamant by degree of porosity and interconnectivity of pores [87, 88]. A scaffold with an undefended and interconnected pore arrangement and a high degree of porosity (>90%) is perfect to interconnect and assimilate with the host tissue [33, 89, 90].

7.3 2Dimesional (2D) Versus 3Dimensional (3D) Culture Scaffold

The scaffolds developed are seeded into two types of cultures, i.e., 2D and 3D culture. In the former culture, the cells are grown in a single layer on a glass or plastic over slip. They communicate only in two dimensions, i.e., x and y, while in case of 3D culture, cells are developed on a 3D porous matrix and are capable of connecting in multiple directions. 3D scaffold permits cells to regenerate and retain extracellular matrix (ECM) that is not possible in 2D [91]. In 2D, cells cannot clone the properties of nutrient gradients, signal propagation or the development of bulk mechanical properties [92]. 3D model gives more appropriate understanding of biochemical and biophysical signaling responses of the cells, especially of the outward response appearing in the ECM along with mechanical and chemical responses arising from both adjacent and distant cells. This approach leads to the generation of adequate cell-based assays for manufacturing of suitable biomaterials utilized to examine the cell material communication [93, 94]. The 2D and 3D scaffold with culture is shown in Fig. 7.3.

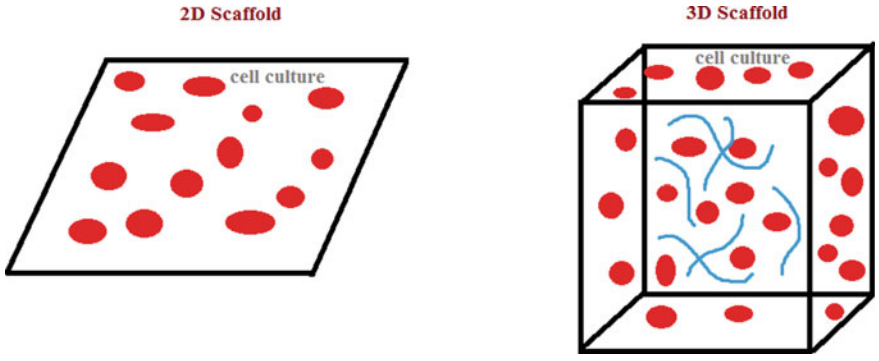

Fig. 7.3 2D versus 3D culture scaffold

7.4 Polymer Scaffold and Processing Techniques

An ideal scaffold for tissue engineering application should possess various important characteristics such as high porosity, surface area, structural strength and specific shapes (3D or 2D) [95, 96]. These characteristics depend on the manufacturing techniques of scaffolds. Till now, numerous manufacturing techniques have been utilized for development of natural and synthetic scaffolds for tissue engineering and regenerative medicines applications. The manufacturing techniques of scaffolds are generally divided into two categories, i.e., conventional manufacturing techniques and rapid prototyping. The former technique is also referred as "non-designed controlled fabrication" method and is used to synthesize scaffolds with irregular microporous structure [97], whereas the latter is also known as "designed controlled scaffold fabrication"; it facilitates fabrication of microporous structure scaffolds with controlled dimensions, location and geometry of pores [98, 99]. In the recent past, a new fabrication technique which is combination of conventional and modern manufacturing method has been used for the generation of porous scaffolds and is referred as combined manufacturing technique [100–103]. The above section summarizes the various fabrication techniques for the development of porous scaffolds (Fig. 7.4).

7.4.1 Conventional Techniques

Conventional techniques inclusive of particulate leaching and solvent extraction [104], emulsion and phase separation [105], gas foaming [106], electrospinning [107], freeze drying [108] or a combination of techniques [109] have been utilized for the fabrication of scaffolds for tissue engineering applications. These techniques

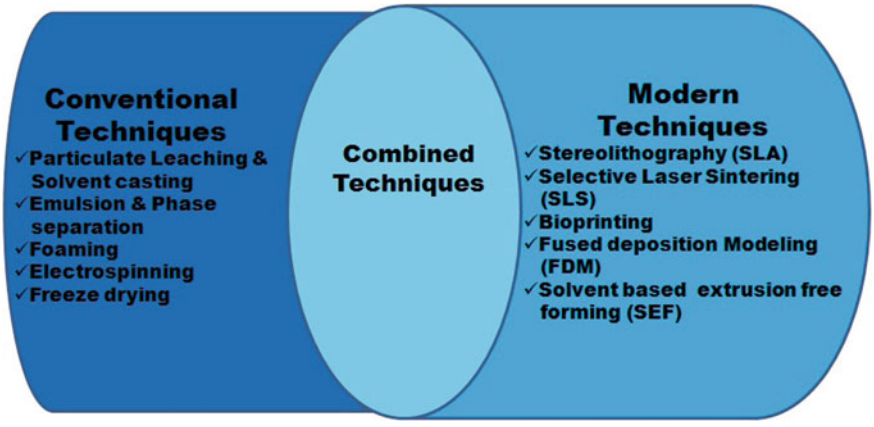

Fig. 7.4 Various manufacturing techniques of scaffold fabrication

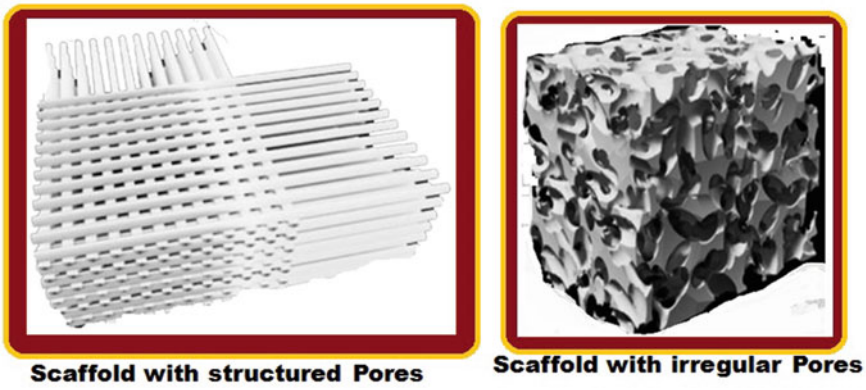

Fig. 7.5 Scaffolds with different pores arrangements

lead to the formation of porous scaffolds with irregular pores and structured pores as shown in Fig. 7.5.

7.4.1.1 Particulate Leaching and Solvent Casting

The technique particulate leaching can be used alone or in combination with solvent casting. Particulate leaching is widely utilized scaffold fabrication technique in bone tissue engineering and regenerative medicines. For this, initially the salt, sugar or wax of specified size is poured into the mold; thereafter, the polymer mixture is poured into the mold followed by hardening and crosslinking of polymer [110]. The scaffolds obtained have the pore size and shape identical to the dimensions of salt, sugar or wax [111, 112]. Gorna and Gogolewski [113] prepared 3D polyurethane scaffolds using salt leaching process for tissue repair and regeneration. In this, new elastomeric biodegradable polyurethanes having an enhanced affinity toward cells and tissues were synthesized using aliphatic diisocyanate, poly(caprolactone) diol and biologically active 1,4:3,6-dianhydro-d-sorbitol (isosorbide diol) as chain extender. The three-dimensional scaffolds showed poor water permeability. By loading the three-dimensional porous polyurethane scaffolds with calcium phosphate salts such as hydroxyapatite or tri-calcium phosphate, their osteoconductive properties can be additionally promoted, thus making them promising candidates for bone graft substitutes.

Solvent casting particulate leaching involves dissolution of polymer solution by homogeneously distributing salt, sugar or wax of specified size in combination with solvent. During the process, solvent evaporates leaving the matrix with salt particles. The matrix so obtained is then immersed in water where salt leaches out to develop a structure with high porosity [114, 115]. This method can be applied for thin membranes of thin wall three-dimensional samples, and under other conditions, the soluble particles are difficult to be separated from the polymer matrix. The major

benefits of this fabrication method are low cost and easy processing in addition to its high porosity with capability of controlling of pore size that make it an ideal technique for the development of 3D scaffolds [116–118]. However, the major limitation of this technique is that the scaffolds synthesized do not have any control on inter-pore connectivity and the pore structure. Moreover, it is time consuming as evaporation of solvent takes days or weeks [119, 120].

7.4.1.2 Emulsion and Phase Separation Method

Thermal-induced phase separation [121, 122] or liquid induced-phase separation [123–125] is other type of manufacturing method for development of scaffolds with interconnected irregular pores. A two-phase uniform mixture of polymer can be unsterilized thermally by changing the temperature leading to liquid/liquid or liquid–solid phase separation. For liquid phase separation, polymer is dissolved in solvent; thereafter, the solvent is separated by decreasing the temperature, resulting in the formation of porous polymer scaffold. This method is known as thermally induced phase separation (TIPS). The scaffolds prepared utilizing these methods have high porosity. Also, their pore size can be adjusted by variation in freezing temperature, type of solvents used, polymeric material and its concentration [126, 127]. Despite of its advantages, the major limitation is their small pore size that can be reproducibly obtained by this process. Furthermore, the technique utilizes organic solvents that may leach some residual after processing, and hence, complete monitoring of the process is required for the complete removal of solvents prior to biological analysis. In emulsion phase separation, polymer is dissolved in solvent and then freeze dried to induce crystallization of the solvent that acts as mold for the pores. These crystals are then removed by freeze drying to yield porous structure. Alteration in processing parameters induces different pore sizes and pore distribution. This technique generates relatively thick scaffolds with porosity greater than 90% and with medium and larger pore sizes [128].

Thermally induced phase separation method was utilized by Guan et al. [126] for preparation of polyurethane scaffolds. Effect of polymer concentration, melting temperature and monomer type effect on porosity and pore architecture were studied. The results showed that polyurethane scaffolds prepared with poly(ether ester urethane) urea monomer have better cell adhesion and growth. Cai et al. [129] developed biodegradable scaffold by blending polylactide (PLA) with natural dextran using phase separation method. The results showed that pore size of the films was around 5–10 mm.

7.4.1.3 Gas Foaming

Gas foaming technique is used to fabricate scaffold without using solvent. In this, gaseous porogens produced by chemical reaction or by release of gases such as

high-pressure carbon dioxide and ammonia are used to foam polymers. This technique results in the formation of scaffolds with sponge-like structure with a pore size of 100–500 μm and a porosity up to 93% that leads to the formation of porous structure. This large pore size and high porosity give expeditious production of fibrocartilaginous tissue and best in growth of mesenchymal tissue along with least inflammatory response [130–132]. Therefore, this method is best suited for the fabrication of polyurethane scaffolds for tissue engineering [58]. One of the major limitations with this method is that scaffolds so obtained may have closed pore structure or a solid polymeric skin [99, 132, 133]. However, combination with articulate leaching can lead to improvement in interconnectivity of pores. Porous nanohydroxyapatite/polyurethane composite scaffold was developed using foaming method by Dong et al. [134]. The prepared scaffolds were studied for biocompatibility and degradation along with morphology, strength and chemical structure. Results revealed that porosity and compressive strength of scaffolds are improved. Manavitehrani et al. [135] synthesized poly(propylene carbonate)-based porous scaffolds using gas foaming technique. Pore size was found to be within 100–500 μm, and biological studies showed biocompatibility and tissue infiltration in the scaffolds.

7.4.1.4 Freeze Drying

This manufacturing technique of scaffold fabrication is based on principle of sublimation. For this, polymers or ceramics are dissolved in water or organic solvents persuaded by emulsification in water phase. The solution containing polymers is dropped in the mold, and the solvent is evaporated by freeze drying to obtain a polymer scaffold with porous structure [136, 137]. Freeze drying is performed by freezing the material and thereafter reducing the surrounding pressure using vacuum and adding sufficient amount of heat to allow the frozen water in the material to sublime directly from solid phase to the gas phase. This technique can be applied to variety of polymers such as silk proteins, PEG, poly(L-lactic) acid (PLLA) and PLGA/poly(propylene fumarate) blends [138, 139].

7.4.1.5 Electrospinning

This method uses electricity for making fibers from a solution and is the most commonly utilized manufacturing method for preparation of nanofiber (NF) polymers and composite [140]. This technique can be used to generate small diameter fibers ranging from 5 μm to 50 nm with large surface area. For fabricating electrospinning fibers, polymer solution is charged using a capillary tip or needle with mechanical pressure through high voltage of around 10–30 kV. The polymer droplets coming out from the needle grow persuaded by evaporation of solvent, resulting in the generation of fine fibers which twin mat into porous scaffolds [141–143].

The diameter of fibers obtained using electrospinning can be varied by changing the different parameters of electrospinning inclusive of electric field voltage, space

among the capillary tip and solution parameters and feeding rates such concentration, solvent, surface tension, molecular weight and viscosity of polymers [144, 145]. In the recent past, this technique has been used to develop nanofiber meshes from a variety of polymers including poly(ethylene-co-vinylacetate) [146], poly(glycolic acid) [147], poly(D,L-lactide-coglycolide) [148], poly(D,L-lactic acid) [149], poly(ethylene oxide) [150], poly(L -lactic acid) [151], poly(ε-caprolactone) [152, 153] and silk [154]. Spider dragline silk protein and collagen-based composite fibers were fabricated by Bofan et al. using this technique [155]. The prepared composite was explored for mechanical properties and biomedical applications. The results showed that tensile strength of fiber improved with increase in silk percentage while a mall reduction was observed in its elasticity. Chitosan and polylactic acid-based blend nanofibers were synthesized to study the combined effect of natural and synthetic polymers [156, 157]. Karchin et al. [158] used melt electrospinning technique to prepare polyurethane scaffold. For this, the biodegradable segmented polyurethanes were synthesized using polycaprolactone diol, 1, 4-butane diisocyanate and 1,4-butanediol which were then melt electrospinned for the preparation of scaffold. The mechanical properties of the resulted scaffolds were similar to in vivo tissue and therefore can be used in bone tissue applications.

The major benefit of using this manufacturing method for scaffold fabrication is that scaffold developed is suitable for cell growth and tissue regeneration. Further, it generates superfine fibers with particular direction, high aspect ratio and surface area that favor the cell growth both in vivo and in vitro. Furthermore, this technique is simple and efficient and can produce both sheet and cylindrical shape [159–161]. Apart from this, there are some of limitations of using this method such as organic solvents used for electrospinning are sometimes toxic that is not good for cells and limited control over pore size [162, 163]. Hence, it is a big challenge to manufacture 3D scaffold with different pore geometry utilizing electrospinning method.

7.4.2 Rapid Prototyping Technique

Although conventional techniques are most widely used for the fabrication of scaffolds, due to their limitations these conventional techniques are being replaced by modern or rapid prototype technique inclusive of stereolithography, selective laser sintering, bioprinting, fused deposition modeling and solvent-based extrusion free forming because these techniques result in the development of 3D scaffolds through layer-by-layer assembly [164–168]. Also, pore size, porosity and shape of the manufactured scaffold can be altered that enhances the cell migration, proliferation and nutrient perfusion as compared to scaffold prepared utilizing conventional techniques [169].

Modern techniques or rapid prototyping methods are also referred as solid freeform fabrication and use computer-aided design (CAD) model to develop a 3D structure with controlled morphology, chemical composition and mechanical properties. The machine used in developing scaffolds using this technique generates the polymer

scaffold in layer-by-layer fashion. For this, the initial layer of the physical scaffold is developed persuaded by thickness of next layer. At last, the fabricated scaffold is detached from the base platform of the machine. CAD program containing scaffold structure design and modeling is used for controlling the layers in the manufacturing machine. For building CAD model of particular tissue regeneration, magnetic resonance imaging (MRI) scans and computed tomography (CT) data are utilized. These techniques are classified on the basis of printing fundamental or on the type of material used for printing [170].

Decreased starting time for producing prototype components, enhanced capability for anticipating part geometry due to its physical existence, prior exposure and contraction of design errors and elaborate calculation of assembling characteristics of components and assemblies are some of the major benefits of these techniques. However, resolution limit that inhibits the designing of scaffold with fine microstructure, use of toxic binders and low quality arrangement are the major drawbacks of these technologies [171, 172].

7.4.3 Combination of Techniques

The abovementioned technologies can be combined to fabricate specific polymer scaffold. For example, phase separation can be combined with particulate leaching [173], electrospinning with freeze drying [174], fused deposition in combination with gas foaming [109], etc. Song et al. [109] developed hierarchical bionanocomposite scaffolds with tunable micro/macroporosity structure utilizing fused deposition modeling in combination with gas foaming to control the pores. The above-prepared scaffold was explored for bone tissue engineering and found that they can be successfully used for bone tissue regeneration. PLA-based scaffolds for tissue engineering application were developed by Salerno et al. [173] utilizing phase separation technology in combination with porogen leaching and $scCO_2$ drying. Scaffolds prepared consisted of large pores and nanoscale pore walls.

Porous scaffolds with nanotopography can be fabricated by combining modern techniques with conventional technologies. In the recent past, progress in the development of tissue engineering scaffolds using combination of modern and conventional techniques was outlined by Giannitelli et al. [175]. The fabricated scaffolds on the basis of achieved level of integration were categorized as assembly, fabrication and technique level.

The various manufacturing technologies for scaffold with their advantages and disadvantages are shown in Fig. 7.6.

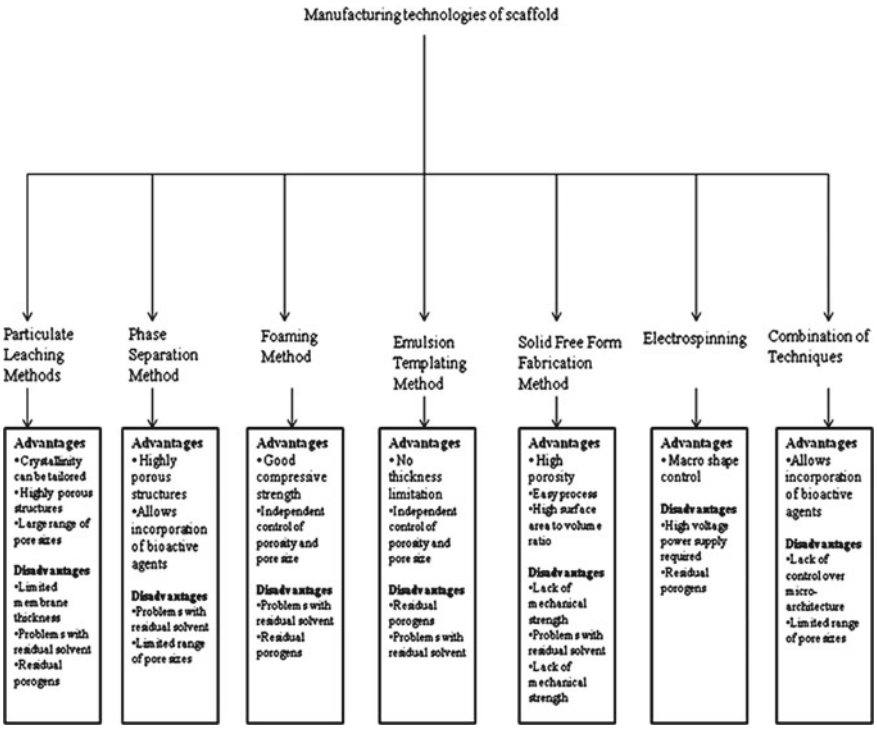

Fig. 7.6 Various Scaffold manufacturing techniques with advantages and disadvantages

7.5 Biomaterials for Tissue Engineering Polymeric Scaffold Manufacturing

A variety of biomaterials inclusive of ceramic, polymers (natural and synthetic) and composite material are used for the preparation of scaffolds for tissue engineering. As the scaffolds are aimed to use for healthcare applications, some of the important characteristics of biomaterials such as biocompatibility, biodegradability, cytotoxicity, adequate mechanical strength and mucoadhesive nature must be taken into consideration for their utilization. A variety of biomaterials like ceramics, natural and synthetic polymer and composites are widely utilized biomaterials for fabricating tissue engineering scaffolds.

7.5.1 Ceramics

Ceramics are inorganic biomaterials that can be categorized as bioinert and bioactive. Alumina and zirconia are bioinert materials while calcium phosphate [176],

bioglass and glass ceramics [177, 178] constitute bioactive materials. Bioceramics may either be osteoinductive (stimulate bone develop) or osteoconductive (support bone develop). All the bioceramics are osteoconductive as all help in bone formation but not osteoinductive. Some of the ceramic biomaterials most commonly used for scaffold fabrication include calcium phosphate-based bioglass and glass ceramics as their composition is alike mineral part of bone [179].

Two most widely used calcium phosphate bioceramics used in tissue engineering scaffolds are tri-calcium phosphate and hydroxyapatite (HA) [180, 181]. Tri-calcium phosphate is very frequently used as degradable scaffold material; on the other hand, HA is non-resorbable and osteoinductive used for coating biomedical implants. Osteoinductivity leads to bone regeneration, thus enabling the implant to assimilate with the surrounding tissue. Further, HA shows improved densification and improved sinterability because to their better surface area, that can expand fracture hardiness as well as other mechanical properties [182].

Henceforth, HA is most widely used bioceramics for scaffold fabrication. It can be prepared by using numerous technologies such as sol–gel processing, emulsion, batch hydrothermal process, mechano-chemical method, chemical vapor deposition, bio-mimetic techniques and ultrasonic spray pyrolysis [183, 184]. Among these techniques, sol–gel is generally used due to its low processing temperature, homogeneous molecular mixing and capability to produce bulk amorphous monolithic solids and nano-crystalline powders [185, 186].

7.5.2 Natural Polymers

Natural polymers represent a convenient alternative to synthetic polymeric material systems as their structure is similar to human bone matrix of tissues. Chitosan and alginate are two most commonly used polysaccharides that have wide application in tissue engineering scaffolds, which do not exist in the human body. But they exhibit good bioactivity and can approach to cell in growth. Alginate is water soluble and has simple gelatin chemistry with calcium ions, thus finding applications to synthesis of scaffolds for bone tissue engineering and liver disease treatment [187]. Chitosan is a derivative of chitin which naturally occurs in the exoskeletons of arthropods. Chitosan with composite scaffolds has been found suitable for skin and bone tissue engineering applications [188].

Fibrin is one of the most attractive natural proteins applied in tissue engineering arena. Fibrin can be used in the treatment of ordinary wound repair and has wide applications as an adhesive in ortho-surgery. It must be produced from human blood vessels, to utilize as an autologous scaffold. Fibrin is not degradable itself unless a protein inhibitor is used to control degradation. Fibrin hydrogels have been used to regenerate soft tissues with chondrocytes [189] Gelatin is the derivative of collagen that is produced by collagen molecules breaking it into single-phase molecule. Further, disadvantages of gelatin are poor mechanical strength and hence

are crosslinked with hyaluronic acid for skin tissue engineering and with alginate for wound healing applications [190, 191].

7.5.3 Synthetic Polymers

Synthetic polymers are preferred over natural properties as their physical, biological and mechanical properties can be adjusted. This can be done by altering the ratio of monomers units or by adding particular groups (e.g., RGD peptide (arginylglycylaspartic acid) that can be successfully recognized by human cells after the implantation in to human body. The products of degradation and degradation kinetics must also be controlled by adequate selection of the segment to form product that can either be released from the body by renal filtration system or can be metabolized into nontoxic products [192]. This can be done by utilizing biodegradable polymers such as polyglycolic acid (PGA), polylactic acid (PLA) and their copolymers like poly(DL-lactic-co-glycolic acid) (PLGA), approved by food and drug administration [193–195]. These synthetic polymers can be degraded hydrolytically and employed regularly due to their by-product degradation that can be easily expelled from the body as water and carbon dioxide. But, decreased pH in the localized area leads to inflammation during degradation. One of the other synthetic polymers, polycaprolactone (PCL), whose structure is analogous to PLA and PGA can also be degraded biologically at physiochemical condition and is generally being used for drug delivery applications as it degrades at a slower rate as compared to PGA and PLA [196–198]. Another most commonly used degradable synthetic polymer that is biocompatible, nontoxic and water-soluble polymer which is liquid at lower temperature and takes the form of elastic gel at body temperature (37 °C) is poly(ethyleneglycol) (PEG) [199]. The polymers based on PEG have been widely used as injectable scaffolds for tissue engineering applications [200]. The hydrophilicity and rate of degradation of PEG and PLA-based scaffolds can be controlled by tailoring the ratio of monomers.

Polyurethane (PU) is another synthetic polymer being utilized as scaffold for tissue engineering applications. The physical, chemical and mechanical properties of PU in addition to their biocompatibility and biodegradability can be altered in a controlled way by changing the composition of hard and soft segment [201]. Biodegradability of PU is generally achieved by integrating hydrolyzable moieties and labile soft segment or by combining with degradable polymers like poly(glycolic acid), poly(lactic-co-glycolic acid), polylactic acid, polycaprolactone, etc., for soft tissue engineering applications [202].

7.5.4 Composites

Scaffolds synthesized from single material show poor properties in terms of biocompatibility, mechanical strength and biodegradability. But composites scaffolds

Table 7.2 Different biomaterials for scaffold fabrication with their advantages and disadvantages

Sr. No	Scaffolds biomaterials	Advantages	Disadvantages
1	Bioceramics (hydroxyapatite)	• Biocompatible • Biodegradable	• Nonresorbable
2	Synthetic polymers (polylactic acid, polyglycolic acid and their copolymers)	• Biocompatible • Hydrophilic	• Degradation products are CO_2 and H_2O creating local acidic conditions
3	Natural polymers (collagen and alginate)	• Biocompatible • Good cell recognition • Simple gelation methods	• Poor mechanical properties
4	Composites (polymer-ceramics, polymer–polymer)	• Capability of altering mechanical and biological properties	• Compromise between "best" qualities of individual components with overall scaffold properties

including two or more bioactive materials enhance the biological properties in addition to mechanical properties of newly developed material. Polymer-hydroxyapatite (HA) (ceramics) composites, PLA/PLGA, PMMA/HA and hydroxyapatite (HA) reinforced with TiO_2 have been developed for application in tissue engineering [203–205]. In this approach, usually the composite matrix is prepared by biocompatible polymer and inclusions of bioceramic (HA, BG, tri-calcium phosphate) particles/fibers. Polymeric composites with ceramics, such as HA, can be used as coating on composites. Scaffolds represent an appropriate alternative of allograft or autograft and they combine the properties of polymers (degradability) and ceramics (bioactive) for tissue engineering application [30, 206, 207]. Advantages and disadvantages of various types of scaffolds are tabulated in Table 7.2.

7.6 Applications

Polymeric scaffold is most commonly used for cell delivery [208], drug delivery [209], genes delivery [179], wound healing and bone tissue engineering applications [210, 211]. For cell delivery application, cells are injected into the scaffolds and administered into the body, whereas for gene delivery application, polymeric scaffolds are used. Polymer scaffolds are architecture in such a way so as to deliver the genetic material as polyplexes, thereby transfecting to seeded cells and expressing the growth of cells to activate morphogenesis of particular cells to create the required tissue [212]. Drugs with low molecular weight that proliferate or differentiate the cells are fused into the scaffolds to activate cellular differentiation and cellular modeling [213]. In the recent past, dexamethasone (DEX) and green tea polyphenols (GTP) were delivered through electrospun polymer ultrafine fibers to attain a adequate balance between effective treatment of keloid and safety to skin [214]. Scaffolds have

shown excellent cell attachment, proliferation and penetration and thus are suitable for tissue engineering applications. The studies show that scaffolds can be used in blood vessels, bones, muscles, skins, neural tissue and other stem cells such as heart, cartilage, ligament and urinary tract [212, 215, 216]. Polymeric fibrous scaffolds due to high porosity, porous architecture, well interconnectivity and high surface area can be utilized for wound dressing. They not only heal the wound but also expel out the extra fluid from the wound area. Further, they also support rinsing of exogenous microorganism, thereby speeding the healing process [217–219]. Thus, all these make porous polymeric scaffolds an ideal biomaterial to be as tissue engineering and regenerative medicine biomaterial.

7.7 Conclusion and Future Prospective

In reviewing the published literature on polymeric composite scaffolds with bioactive properties, it was revealed that, in the recent past, new polymeric scaffold nanocomposite has been fabricated utilizing conventional, modern and combination of techniques. New materials and combination of composite scaffolds designs based on new fabrication method are being proposed continuously to advance bioactive and biocompatibility of composites. Combination of techniques methods is being widely used as it gives scaffold with required pore size and high porosity. Further, this review article highlights required properties of the scaffolds for bone tissue engineering and the numerous biomaterials being utilized for scaffold and its composite preparation. It is found that bioceramics, hydroxyapatite (HA), can be used as filler in polymer matrices to develop nanocomposite of scaffold. This has been found that HA is significantly associated to produce bionanocomposite scaffolds with similar structure and composition to human bones. It is well known that homogeneous dispersion of filler in polymer matrix plays a key role and mainly enhances osteoconductivity and mechanical properties. Moreover, nanoscale organized composites provide a better microenvironment for cell in growth in terms of cell adherence and proliferation. Hence, ceramics/polymer composites can be developed to enhance the mechanical and biological properties for biomedical applications. Overall, it was concluded that polymer scaffold composites can be used as tissue engineering scaffolds.

Despite of the well-known utilization of scaffolds, if we look into practicality and convenience, still there is a need to develop new degradable polymer composites that can meet all the needs of surgical implants, drug and cell delivery.

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Chapter 8
Biodegradable Polyvinyl Alcohol/Starch/Halloysite Nanotube Bionanocomposite: Preparation and Characterization

P. Manju and P. Santhana Gopala Krishnan

8.1 Introduction

The researchers' attention has been drawn to the need to replace petroleum-based polymers with appropriate alternatives derived from biomass due to growing environmental and sustainability concerns [1]. As a result, biopolymers have gained widespread acceptance and have experienced significant growth in a variety of industries during the previous few decades [2]. Biopolymers are an important component of bionanocomposites that have piqued the interest of researchers. They are an excellent alternative for the matrix of bionanocomposites and provide numerous benefits that have helped bionanocomposites gain widespread acceptance [3]. Biopolymers are an excellent alternative to petroleum-based polymers, because they are renewable in nature and reduce carbon footprint and solve degradability difficulties because they are mostly biodegradable in nature. Important biopolymers widely being used are poly(lactic acid) (PLA), starch (ST), poly(butylene succinate) (PBS), poly(vinyl alcohol) (PVOH), chitosan, etc. [4–10].

Researchers from different areas of science are investigating the use of nanotechnology for different material property improvements [11]. For this, nanostructures such as nanoclay, nanowhiskers, nanocrystals and nanofibres are being used. Halloysite nanotubes (HNTs) are now the most widely used nanostructure in the development of bionanocomposites [4]. This is due to the benefits provided by this nanomaterial, which include its tubular structure, nano-scale lumen, high aspect ratio, hydrophilic nature, low cost, abundant availability, ease of dispersion in polymer matrix by applying shear force, environmental friendliness and cytocompatibility

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properties [12]. As a result, it improves the thermal, mechanical, flame and barrier properties of bionanocomposites, culminating in the creation of a hybrid class of bionanocomposites. The use of these materials has spread to a variety of fields, including biomedical, tissue engineering scaffolds, drug delivery, cancer cell isolation, bone implant, cosmetics, etc. [1]. Bionanocomposites are being studied in order to find out a solution to the disadvantages of biopolymers such as weak mechanical strength, limited thermal stability and poor barrier qualities, among others [13]. As a result of the synergy between nanotechnology and renewable resources, a large range of applications in various industries with better features emerged. Since polymers made from renewable resources are more environmentally friendly, their use is being increased nowadays. Preparation and characterization of bionanocomposites using halloysite nanotubes (HNT), starch (ST) and poly(vinyl alcohol) (PVOH) are discussed in this chapter.

8.2 Poly(Vinyl Alcohol)

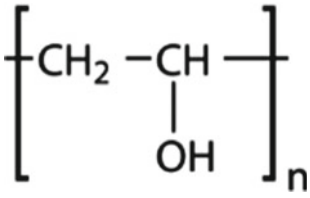
PVOH is a biopolymer made by hydrolysis of polyvinyl acetate, which degrades through microbial degradation and hydrolysis [8, 14]. The chemical and physical properties of PVOH depend upon the initial length of vinyl acetate polymer and the degree of hydrolysis. Properties of PVOH are given in Table 8.1. The repeating unit of PVOH is given in Fig. 8.1. PVOH has good mechanical properties, oxygen barrier properties and film forming ability. Limited biodegradability and high water permeability are the disadvantages of PVOH [15].

PVOH is mainly classified into two: partially hydrolysed and fully hydrolysed PVOH. Partially hydrolysed PVOH is mainly used in food applications. It is also used in the synthesis of poly(vinyl butyral) and vinylon fibres [16]. Vinylon or Vinalon is heat and chemical resistant fibre fabricated from PVOH and is used in textiles, ropes and shoes. PVOH is also widely used in membranes, packaging, textile industry, paper industry, adhesives, coatings and biomedical applications [17]. PVOH

Table 8.1 Properties of PVOH and ST

Property	PVOH	ST
Appearance	Creamy to whitish powder	White powder
Density (g/cc)	1.19–1.31	1.5
Molecular weight (g/mol)	20,000–400,000	$3.12–5.52 \times 10^6$
Glass transition temperature (°C)	75–85	Varies according to moisture content and source in the range of 20–150
Melting temperature (°C)	180–190 for partially hydrolysed and 230 for fully hydrolysed	160–210

Fig. 8.1 Structure of PVOH



is biocompatible with human tissue and can absorb protein molecules and undergo cell adhesion without any toxicity. The global producers of PVOH are DuPont, Nippon synthetic chemical industry, Eastman chemical company, Sekisui chemical company and Anhui Wanwei Group [17].

ST can be considered as the best material candidate to be blended with PVOH because it is a completely biodegradable, cheap and widely abundant biopolymer [7, 15]. PVOH/ST blends have been investigated for decades in terms of blend ratio and plasticizer content to achieve good material compatibility and properties [15].

8.3 Starch

ST is an important food resource, which belongs to the polysaccharide family, widely present in leaves, stems, roots, tubers and fruits in the form of water-insoluble granules [18]. ST is the end product of photosynthesis which is the chemical storage of energy. ST can be extracted from food products such as potato, wheat, rice, barley, maize, banana and mango [19]. The dimensions of the ST granules vary according to the source ranging from 0.5 to 175 μm.

ST is a semi-crystalline polymer consisting of 20–30% amylose and 70–80% amylopectin (Fig. 8.2). The ratio of amylose and amylopectin varies according to the difference in the plant resource. Amylose is a linear polymer, in which α-D-glucose units linked by α-(1,4) glycosidic bond linkages, while the amylopectin is the branched counterpart with α-(1–4) linked backbone and α-(1–6) linked branches [20]. The properties of ST are given in Table 8.1. ST completely biodegrades in soil and compost, is non-toxic and has a low cost. The processability of ST is difficult because of its inherent brittleness and less flexibility. Besides, the melting and degradation temperatures of ST are very nearby which is very difficult during the processing of ST. Therefore, gelatinized, plasticized and thermoplastic starch (TPS) is generally used [7].

The important applications of ST include textiles, adhesives and paper binders, textiles, chemical productions and fermentation. The major global producers of ST are Cargill, Ingredion, Tate and Lyle, Archer Daniels Midland Company, Riddhi Siddhi Gluco Biols and Gulshan Polyols [21].

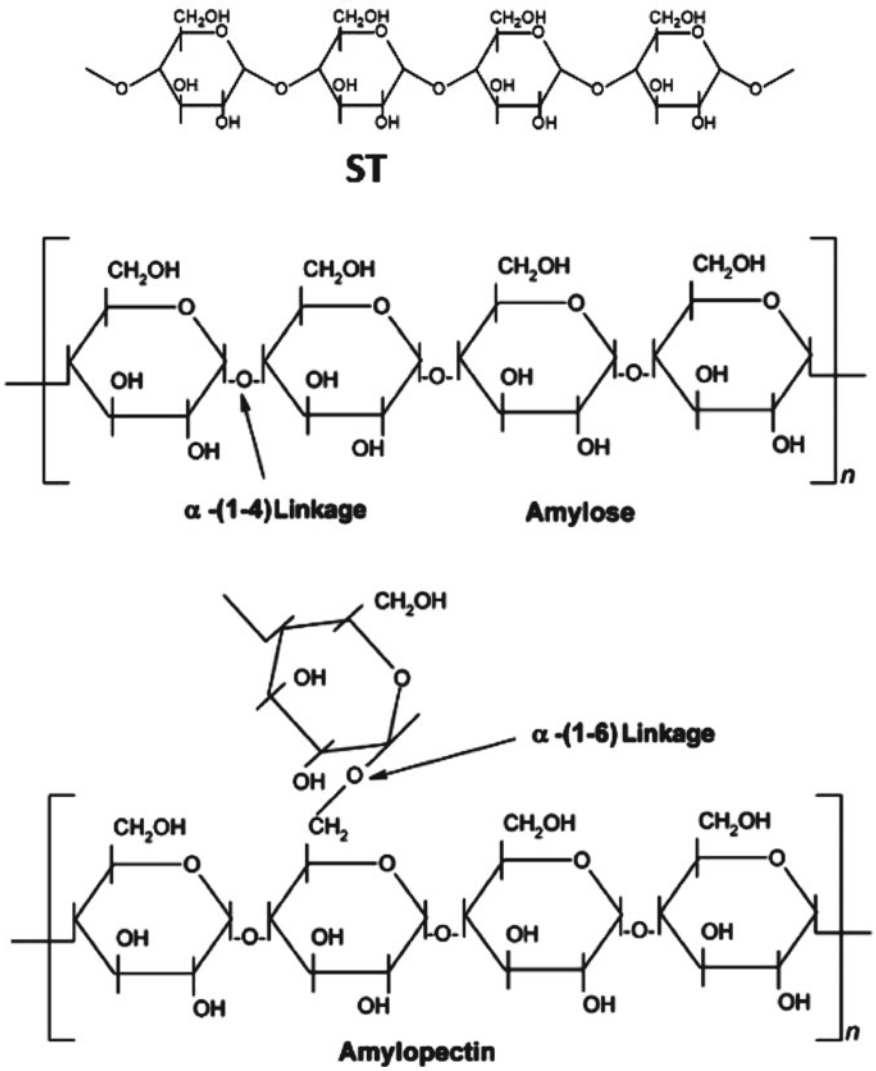


Fig. 8.2 Chemical structure of ST, amylose and amylopectin

8.4 Halloysite

HNT is a clay mineral belonging to the kaolin group that was called after Baron Omalius d'Halloy (1707–1789), a Belgian geologist who originally discovered it [4, 5, 22]. HNT is a two-layered aluminosilicate with the chemical formula $Al_2Si_2O_5(OH)_4.nH_2O$, and it shares chemical similarities with other clays such as

kaolinite, dickite and nacrite [23]. However, the shape of crystals of unit layers separated by a monolayer of water molecule differs primarily. It can be found in a variety of worn rocks and soils. HNT is extracted from natural sources and is typically white in colour, making it easy to grind into powder. The specific gravity of HNT is in the range of 2–2.65, and the cation exchange capacity varies in the range between 2–60 meq/100 g. The surface area of HNT is about 184.9 m^2/g. HNT is mined in China, New Zealand, America, South Africa, Brazil and France, among other places [12].

HNT comes in a variety of shapes and sizes, including spheroidal, short tubular and platy particles. The hollow tubular structure, with a diameter of less than 100 nm, is the most prevalent, in which the aluminosilicate sheets are rolled up like a scroll (Fig. 8.3). The length of HNTs ranges from 0.2 to 1.2 μm, with inner and outer diameters of 10–30 and 40–70 nm, respectively. The structural unit of HNT is made up of two sheets: Si-tetrahedra and Al-octahedra arranged in a hexagonal pattern. Inner aluminol (Al–OH) and outer siloxane (Si–O-Si) hydroxyl groups are located between layers and on the surface of the nanotubes, respectively. These hydroxyl groups can be used to attach various functional groups by surface modification techniques. The interlayer space of the HNT mineral contains a layer of water, resulting in a layer thickness of up to 1.1 nm. Since these water molecules are bound together by weak interactions, they can be easily removed by drying HNT. This results in the production of HNT in a range of 0.7–1.1 nm interlayer spacing. As a result, it is assumed that hydrated HNT has a diameter of 1.1 nm and dehydrated HNT has a diameter of 0.7 nm. [12].

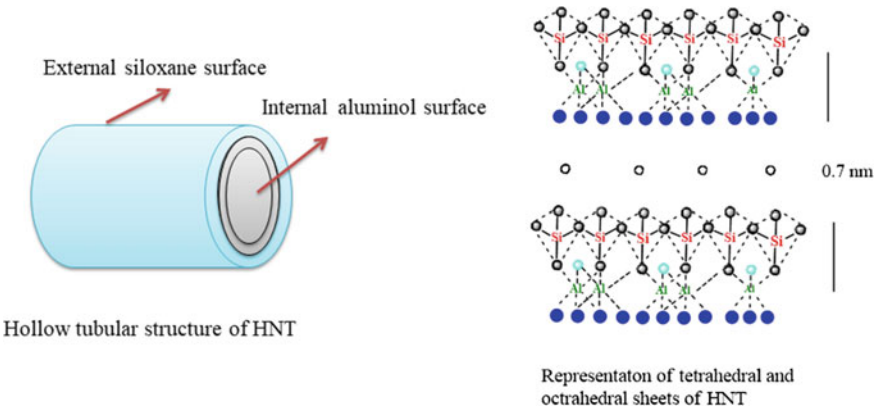


Fig. 8.3 Structure of HNT

8.5 PVOH/ST/HNT Bionanocomposite: Preparation

The most common methods employed for the preparation of PVOH/HNTs are solution casting technique, melt processing and electrospinning. As literatures discussing the PVOH/ST/HNT are limited, the methods for preparing PVOH/HNT and ST/HNT will also be discussed in this section.

8.5.1 Solution Casting

Solution casting is a suitable method to be performed on a laboratory scale. The thermal degradation of polymers used for solution casting will be minimum because of the absence of higher temperatures and shear in this method [15]. The polymer is dissolved in a suitable organic solvent, and the solution is casted on mould plates in different thicknesses to obtain the films. In the case of PVOH/ST/HNT films, PVOH and ST shall be mixed in powder form at room temperature. This mixture was dissolved in 200 mL deionized water at 35 °C which was then vigorously mixed using a magnetic stirrer at 500 rpm and 85 °C for 3 h. Then, equal amounts of these clear solutions were poured into casting moulds and dried at 50 °C for 24 h [15]. In addition, the PVOH/ST films shall be plasticized with 30 wt% glycerol (GLY) to fabricate PVOH/ST/GLY films. The amount of ST and GLY was determined through trial-and-error methods. For the preparation of PVOH/ST/GLY/HNT films, the HNT suspension was prepared by mixing weighted amounts of HNT with 100 mL of deionized water using a mechanical mixer at 500 rpm for 2 h at 50 °C, followed by sonication for 1 h in degas mode for preparing a dispersed and bubble-free HNT suspension. This suspension was added drop by drop to 100 mL of PVOH/ST/GL solution at 50 °C by continuous mixing for 30 min using a mechanical stirrer. This solution was further homogenized using a magnetic stirrer for 30 min proceeded by a final sonication procedure to remove any bubbles. Then, the films were dried at 50 °C for 24 h and stored in a desiccator [15]. A modified solution casting technique was performed for developing a non-woven membrane comprising of chitosan/PVOH/HNT for air filtration purposes. To begin, the chitosan/PVOH blend casting solution was prepared by dissolving chitosan and PVOH in a 200 m mol adipic acid solution. Then, polymer solutions were cast onto a glass plate to obtain films with several thickness. Finally, 100 mL of the HNT dispersion was vacuum filtered onto the polymer side of the composite membranes to develop the hybrid non-woven membranes [24].

8.5.2 Electrospinning

Electrospinning is a technique to synthesize fibres, in which a high electric voltage is applied to a continuous flux of polymer solution. The fibres thus formed will be in

micro/nanosize. A schematic representation of electrospinning is given in Fig. 8.4. PVOH/HNT bionanocomposites are spun into fibres using this technique. Cheng et al. prepared PVOH/HNT nanofibres by electrospinning technique with 10 wt% PVOH solutions with a concentration of HNT being varied from 5 to 25 wt%. The electrospinning device was maintained with a voltage of 15 kV and an injection rate of 0.2 mL/min. The collector was a paper-wrapped rotating metal drum, and the distance from the needle was maintained at 15–20 cm [14]. PVOH/HNT/chitosan nanofibres were produced by electrospinning technique, in which the chitosan and PVOH were mixed in a ratio of 30/70 with 3 and 5% of HNT [25].

Electrospinning of ST is difficult because of achieving ST in fibre form because of its lower strength, water resistibility, thermal instability and poor processing behaviour. Also, it has to be noted that electrospinning of ST is only possible from ST having high amylose content [26]. But with the advent of electrospinning and modifying the conventional electrospinning technique, researches for electrospinning of ST have also commenced recently [27]. In addition, electrospinning of ST with other biopolymers such as PVOH, PLA and polycaprolactone was widely accepted so that the disadvantages of both the biopolymers can be overcome to develop a hybrid system [28–30]. Solution of 7 wt% PVOH in water was formed, and ST ranging from 1 to 5 wt% was added to this. This solution was electrospun in an electrospinning device maintained in a specific voltage [31]. Bubble electrospinning is a recent technique developed to produce fibres from aqueous solutions. Liu and He produced nanofibres from PVOH/ST blends using this method [29]. If a polymer

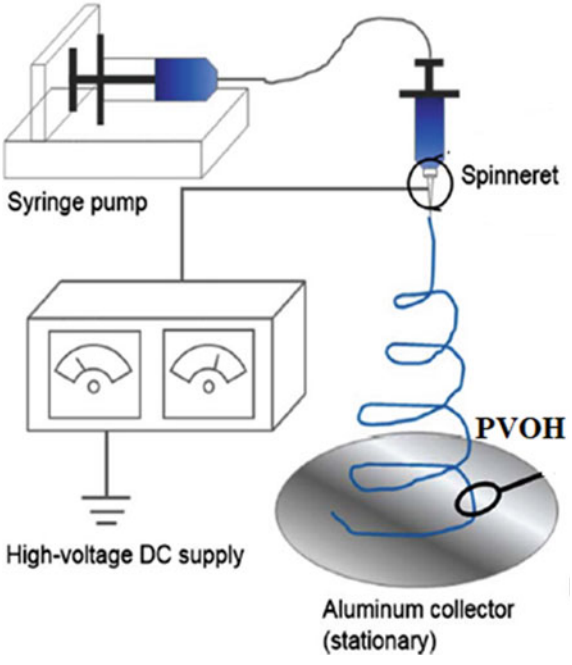


Fig. 8.4 Representation of electrospinning

bubble ruptures under an electrostatic force, the surface tension leads to surface minimization of film fragments along with the formation of some daughter bubbles. If the electrostatic force ejects some fragments upwards, the process can be called as bubble electrospinning. If a stream of blowing air is used instead of electrostatic force to pull up the polymer bubble, the process is termed as blown bubble spinning [29].

8.5.3 Melt Processing

Melt processing is a technique that specializes in large-scale manufacturing with more productivity and efficiency. Even though solution casting seems to be the easy processing technique for ST/HNT bionanocomposites, this is not practical in polymer processing industries. Studies about melt processing of plasticized ST/HNT bionanocomposites have commenced recently only. Generally, ST/HNT bionanocomposites are being fabricated by following two steps. ST and plasticizer such as GLY and HNT were blended in mechanical stirrer for 3 h at room temperature, followed by processing in a twin-screw extruder at a screw speed of 60–150 rpm with temperature from hopper to die which was set as 110, 115 and 120 °C [32–34]. Melt processing of ST/PVOH was also reported by different researchers. ST, PVOH and plasticizers were mixed in a high-speed mixer at room temperature for 3 min. After conditioning for 12 h, the mixture was charged into a torque rheometer and mixed further at 105 °C, for 10 min and 80 rpm. These mixtures were then compression moulded to obtain the ST/PVOH films [35]. Further, they extended their research to synthesize ST/PVOH/MMT films by a similar processing method [36].

8.6 PVOH/ST/HNT Bionanocomposite: Characterization

8.6.1 Chemical Interaction Analysis

The chemical interactions present between HNT and ST or PVOH matrix are identified using Fourier transform infrared (FT-IR) analysis. FT-IR analysis also helps in identifying the characteristic peaks of ST, PVOH and HNT. Thus, any changes in characteristic peak values of ST or PVOH or HNT can be concluded as the interactions between them. This might be evident by the red shift or blue shift phenomenon of the peaks [33]. As reported by Schmitt et al., the peak corresponding to the internal and external hydroxyl groups of HNT was shifted to lower wavenumbers in ST/HNT bionanocomposites which was due to the formation of interactions between OH groups of HNT and C-O-C groups of ST [33]. Similar findings were reported by Ren et al. for potato ST/HNT bionanocomposites [34].

8.6.2 XRD Analysis

From the XRD pattern of the bionanocomposites, the incorporation of HNT can be confirmed by the presence of characteristic peaks of HNT. The intensity of the peaks was found to increase with HNT concentration. The presence of the characteristic peaks of both ST/PVOH and HNT confirms its dispersion of HNT in the polymer matrix. The appearance of any new peaks may pave light for the evidence of interaction that occurred between ST/PVOH with HNT [34]. XRD analysis also helps in studying the changes in the microstructure of ST/PVOH by the addition of HNT [33]. The addition of HNT has been reported to change the crystalline structure of HNT as reported by Schmitt et al. Incorporation of 2 wt% HNT was found to induce the formation of B-type structure of ST by reducing the formation of V_H-type structures. In the B-type structure, the double helices of amylopectin are packed in the hexagonal unit cell with 36 water molecules per unit cell, whereas in V_H type, the single helices of amylopectin are packed in the orthorhombic unit cell with 16 water molecules per unit cell. Moreover, the V_H-type crystals are formed by the addition of any complexing agent to amylose [33]. Upon addition of 0.25 and 0.5 wt% of HNT into PVOH, shifts in the characteristic peaks of PVOH were observed in the XRD pattern of PVOH/HNT bionanocomposites. These shifts can be considered as slight intercalation of PVOH into HNT as reported by Abdullah et al. [15]. In contrast, Cheng et al. reported that there were no changes for the characteristic peaks of PVOH by the addition of higher concentrations of HNT in the range 5–25 wt% [14].

8.6.3 Morphological Analysis

For the morphological analysis, the specimens will be mounted on the stub using double side tape and coated with a thin layer of gold/platinum. The images will be collected at the specified operating voltage and magnification. In the processing of ST, a plasticizer is an inevitable component, and hence, the percentage of plasticizer that has to be added shall be optimized during the fabrication of ST/HNT bionancomposites. This is because plasticizer in a higher percentage might affect the dispersion of HNT and hence the properties also. Similarly, the dispersion of HNT in ST/PVOH matrix is an inevitable factor in determining the mechanical and thermal properties of the bionanocomposites. As a result, characterization of bionanocomposites through microscopic techniques such as scanning and transmission electron microscopy (SEM and TEM) and atomic force microscopy (AFM) has importance in modifying the properties by examining the dispersion of HNT [33]. A figure showing the agglomeration of HNT as obtained from TEM analysis is given (Fig. 8.5). A typical AFM image for PVOH/ST/GLY/HNT is comprised of three major areas, in which the black area corresponds to the amorphous phase of the bionanocomposite,

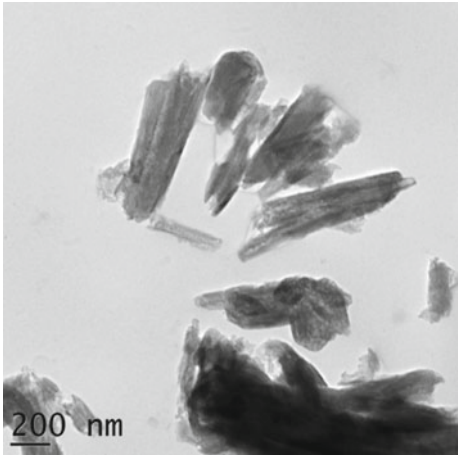


Fig. 8.5 Agglomeration of HNT as evident from TEM analysis (micrograph from authors collection)

light and dark brown area corresponds to the crystalline phase, and light and yellow areas correspond to the fully and partially embedded HNT in the matrix [37].

8.6.4 Mechanical Properties

The mechanical reinforcement performance of nanoparticles on bionanocomposites depends on the effective load transfer from the matrix to the nanoparticles. This will be more effective when there are strong interactions at the nanofiller matrix interface and if the nanofillers are dispersed uniformly in the matrix. The three main interactions between matrix and fillers are micromechanical interlocking, chemical bonding and van der Waals force [14]. The HNTs are held together in bundles with van der Waals force. Hence, it is vital to disperse nanotubes well in the polymer matrix to achieve improved mechanical properties for the composites. Thus, the applied force can be shifted to HNTs, bringing the improvement of mechanical properties [14].

Tensile strength, Young's modulus and elongation-at-break shall be determined employing Universal Testing Machine (UTM) [34]. The incorporation of 7 wt% HNT in ST matrix has increased the tensile strength of bionanocomposites by 47% as reported by Ren et al. A figure representing the mechanical properties of ST/HNT bionanocomposites as observed by Ren et al. is given in Fig. 8.6 [34]. They also reported that, besides the presence of HNT, the selection of plasticizers can affect the mechanical properties of the bionanocomposites. The use of a mixture of plasticizers was found to be a promising way to optimize the mechanical properties of these ST-based bionanocomposites. Bionanocomposites which used a mixture of plasticizer such as GLY and sorbitol have better mechanical properties such as tensile strength, Young's modulus and elongation-at-break than all the other bionanocomposites [34]. HNT can enhance storage modulus and Young's modulus by maintaining ductility

also. Generally, for all ST bionanocomposites, a reinforcement effect is observed by the addition of HNT along with an increase in modulus. This is because of the effect of HNT which has a high elastic modulus of about 140 GPa [38, 39]. The segmented motion of the ST chains helps in enhancing the storage modulus of the bionanocomposite with an increase in HNT. Young's modulus was increased by 84% by the addition of 4 wt% HNT [33]. By the addition of modified HNT, the tensile strength of ST/HNT bionanocomposites was increased by up to 144% and Young's modulus was improved up to 29% without compromising ductility [33]. The tensile strength of PVOH/ST/HNT was found to be improved by 20 and 3.4%, respectively, with the incorporation of 0.25 and 0.5 wt% HNTs. Young's modulus was enhanced by 148% with the addition of 1 wt% of HNT. However, strength was decreased beyond the HNT loading of 0.5 wt% due to agglomeration of HNT as evidenced by SEM analysis [15].

Zhou et al. reported that PVOH-HNT films have higher tensile strength and elongation-at-break values than PVOH films. Tensile strength and elongation-at-break values for films containing 5% HNT were 56 MPa and 320%, respectively,

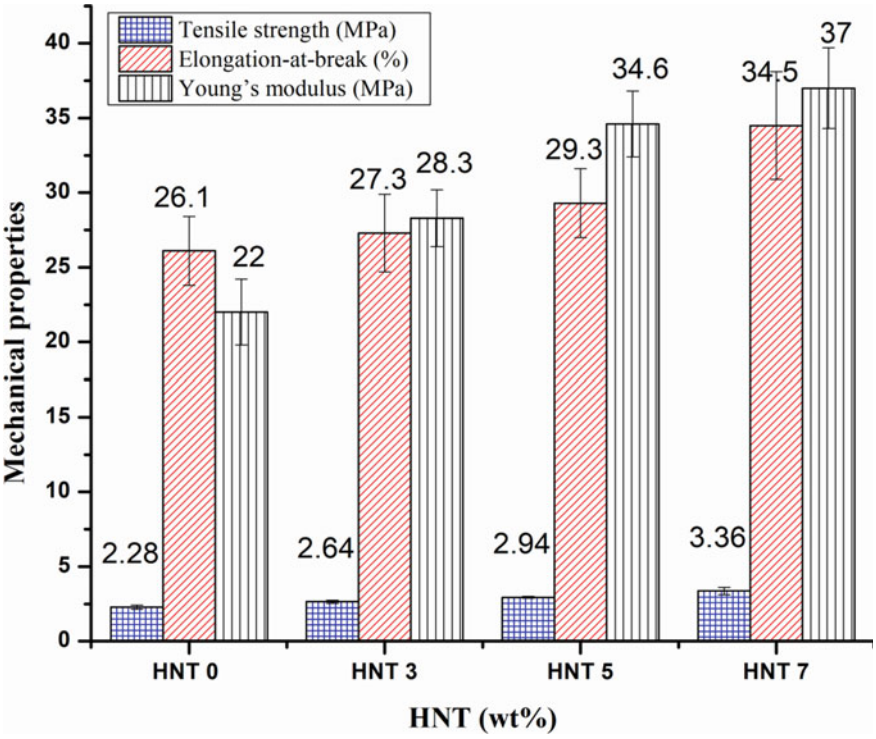


Fig. 8.6 Mechanical properties of ST/HNT bionanocomposites

which were higher than those for neat PVOH films. However, as the HNT concentration is increased beyond 7%, the mechanical characteristics are shown to decline, which could be due to HNT particle agglomeration [40].

8.6.5 Thermal Analysis

The incorporation of nanofillers in the polymer matrix induces two main effects on the thermal stability of the bionanocomposites.

(i) a barrier effect that improves the thermal stability.
(ii) a promoter effect which increases the thermal degradation process [34].

The barrier effect of nanofiller increases the thermal stability of polymer matrix by the fine dispersion of nanofiller in the polymer matrix and through the strong interactions developed between nanofiller and polymer matrix. The better dispersion of nanofiller increases the pathways for the escape of combustion gases developed and the formation of char residue at the surface of the matrix [41]. The presence of many hydroxyl groups on the edges of nanofillers catalyses the degradation of the polymer through which the nanofiller acts as a promoter for polymer degradation [42, 43]. But the barrier effect will be predominant when the nanofillers are well dispersed in the matrix [43]. Thus, the thermal stability will be increased [15].

The primary stage of degradation of PVOH and PVOH/HNT bionanocomposites was reported to be in the range of 280–300 °C, due to the elimination of water molecules. During this stage, the acetate groups remaining in the PVOH, due to incomplete hydrolysis of PVOH, will form polyenes. During this stage, degradation of bionanocomposites will be higher than that of neat PVOH, because of the plasticization effect of HNT on PVOH chains, and the degradation will be accelerated. During the second stage of degradation in the range, 350–460 °C, chain scission occurs. But during this stage, the thermal stability of bionanocomposites will be higher than that of PVOH because of the barrier effect of HNT in the PVOH matrix. The residue yield, which is formed by the degradation of polyenes formed during the primary stage, will also be higher for bionanocomposites, and these residues will provide enhanced thermal stability [44]. For ST/HNT bionanocomposites, the degradation occurs in three stages [7]. The primary stage below 100 °C corresponds to the evaporation of water and the plasticizer used. The second and third stage (100–400 °C) corresponds to the degradation of amylose and amylopectin chains. By the addition of HNT, the degradation temperatures were shifted to higher values for the second stage. The values were higher for bionanocomposites having a higher concentration of HNT [33, 34].

The addition of HNT improves the thermal stability of ST and PVOH [33, 34]. This is because of the high thermal stability of HNT and the interactions between HNT and ST and PVOH. The incorporation of 7 wt% HNT has led to the enhancement of maximum degradation temperature for ST/HNT bionanocomposites which was due to the good dispersion of HNT [33]. As reported by Abdullah et al., the addition

of HNTs increased the thermal stability of PVOH/ST/GLY/HNT bionanocomposites which was evident from the increase in decomposition temperature and reduced weight loss. The decomposition temperature at 5% weight loss was increased to 468 °C from 460 °C by the addition of 1 wt% of HNT. A similar trend was observed for T_{50} and T_{90}, where the HNT loading was increased from 0.25 to 1 wt% [15]. Because of the hydrogen bonding in between hydroxyl groups of PVOH, interchain and intrachain interactions occur between polymer chains of PVOH. Thus, the addition of HNT will interrupt the intermolecular and intramolecular interactions between the polymer chains and changes the physical structure and crystallization behaviour of PVOH. With the increase in the concentration of HNT, T_c of PVOH also shifts to higher temperatures [45]. The T_c of poly(lactic acid)-HNT bionanocomposites was also found to increase with respect to increase in concentration of HNT [5]. The T_g of PVOH was found to increase by 5.72 °C from 74.83 to 80.55 °C by incorporation of 25 wt% of HNT as reported by Cheng et al. Similarly, T_m was increased from 221 to 226 °C by the incorporation of 25 wt% of HNT [14].

8.6.6 Water Absorption Capacity

PVOH and ST have higher sensitivity towards water always. Studies have reported that the incorporation of HNT can reduce the water absorption of PVOH and ST. This was explained based on the structure of HNT which inhibits the diffusion of water molecules in PVOH/ST and thereby decreases the water absorption capacity [15]. The water vapour transmission rate of PVOH/ST/GLY/HNT films was reduced by 52.34% with increasing the HNT content from 0 to 5 wt%. The decrease in value was less pronounced when the HNT content increased from 1 to 5 wt% resulting from the agglomeration of HNT at the high concentrations [37]. The moisture absorption capacity of ST was increased with the addition of HNT, and with further increase in the HNT, the moisture absorption capacity was found to decrease which might be due to the agglomeration of HNT [46].

8.7 Conclusions

ST is an important promising biopolymer of the polysaccharide family. Similarly, PVOH also is a significant biopolymer with many advantages. Naturally, occurring HNT shall be incorporated into ST or PVOH or ST/PVOH. The different preparation methods and characterization techniques of the nanocomposites were analysed in detail. The effect of HNT on the mechanical and thermal properties of different systems was also investigated. Moreover, investigations on ST/PVOH/HNT bionanocomposites are yet to be explored to widen its applications in different fields.

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Chapter 9
Environmentally Friendly Bionanocomposites in Food Industry

Subajiny Sivakanthan and Podduwala Hewage Sathiska Kaumadi

9.1 Introduction

Petroleum-based (fossil fuel-based) materials found versatile applications in human life. However, there are rising concerns over the use of petroleum-based products such as their adverse effects on the environment and human and animal health. In recent years, there is a mounting interest in developing bio-based materials as alternative sources for petroleum-based (fossil fuel-based) chemicals to protect the environment as well as the well-being of humans and animals. However, biopolymers in industrial applications have some drawbacks primarily due to their hydrophilicity and poor barrier properties [1]. The use of composite materials, engineered materials made from two or more constituent materials with significantly different physical or chemical properties from their constituent molecules [2], is widely practiced in various applications.

Nowadays, bionanocomposites are gaining popularity in various industrial applications. Bionanocomposites are materials consisting of natural or synthetic biodegradable polymers of biological origin and nanomaterials (1–100 nm). In these materials, the biopolymer matrix makes up the continuous phase, and the nanomaterials (organic and/or inorganic materials) are the dispersed phase [1, 3].

Composite materials exhibit robust physical, chemical, and mechanical properties compared to their constituent materials. However, conventional composite materials, which do not have nanomaterials, differ from nanocomposites in their

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mechanical properties due to the high surface to volume. Consequently, many varieties of nanocomposites have been developed using different polymer matrices and nanofillers. Bionanocomposites are found to be promising candidates in many applications, including the food industry, biomedicine, tissue engineering, paint, packaging, coating, solar energy, etc. [2–4]. This chapter aims to give an insight into the applications of bionanocomposites in food industries.

9.2 Environmentally Friendly Bionanocomposites

Growing concern about environmental pollution due to the high impact of plastic waste has led industries and academic researchers to develop sustainable and green polymers extracted from natural resources. Due to the deleterious consequences of plastic pollution, biodegradable polymers started to receive an enormous level of attention concerning non-degradable polymers based on petroleum sources [5].

Synthetic polymers are used widely by humans in their day-to-day lives in sectors such as agriculture, construction, food industry, and biomedicine. Factors such as low cost, convenience, good performance, durability, superior mechanical, and other properties make these petrochemical-based plastics popular in commercial and industrial markets [6]. Due to the inability of these plastics to degrade naturally, issues have arisen with the accumulation of plastic as waste [7]. These fossil-based sources are finite in quantity and will reach an endpoint due to the higher consumption. Because of this, these polymers cannot be regenerated fast enough when compared to the rate of consumption [8].

Nowadays, the development of environmentally sound "green" materials has become a growing trend globally to reduce plastic waste accumulation as a consequence of the issues caused by non-degradable plastics. Biodegradable polymers such as cellulose, starch, poly(lactic acid) (PLA), and polycaprolactone have gained great attention as raw materials for making environmentally friendly plastics. Factors including abundance, low cost, biodegradability, and renewability of these biopolymers make them suitable to replace non-biodegradable plastics [9-10]. Biodegradable polymers have the ability to undergo at least one step of the degradation process through the metabolism of naturally occurring organisms, moisture, oxygen availability, and temperature without leaving any harmful residues to the environment. Categories of biopolymers consist of (a) natural polymers; plant carbohydrates including starch, cellulose, chitosan, agar, etc., and animal or plant proteins such as soy protein, corn zein, gluten, collagen, whey protein, casein, (b) biodegradable synthetic polymers; poly(lactic acid) (PLA), poly(glycolic) acid (PGA), poly(caprolactone) (PCL), etc., (c) microbial polymers derived via microbial fermentation such as microbial polyesters including poly (hydroxy alkanoates) (PHA) and microbial polysaccharides such as pullulan and curdlan. Though biopolymers are posing the ability to replace non-degradable plastics, their poor mechanical and barrier properties such as low heat distortion, brittleness, high gas and vapor permeability, and poor resistance to protracted processing have caused limitations in

the applications [11]. To overcome these shortcomings, biocomposites are considered promising candidates.

Materials that consist of two or more constituents with different physical and chemical properties are recognized as composites. If materials with various properties are combined creating a matrix consisting of enhanced traits than the separated component individually, it falls into the frame of a composite. Composites are a combination of two constituents: matrix and reinforcement. The continuous phase is called a matrix which generally includes a polymer, metal, or ceramic, etc. The matrix plays a role in maintaining fibers in the proper direction/arrangement in space and protects them from abrasion or environment. Enhancement of physical or mechanical properties of the matrix gets done by using reinforcement materials. Composites are better in strength, electrical, biological, thermal, and environmental applications [12]. There are three main categories of composite: (1) laminated composites, (2) fibrous composites, and (3) particulate composites. Fillers used in polymer matrix help in the reduction of costs, decrease shrinkage of the matrix, lower the coefficient of linear expansion, increase thermal conductivity, and lower resistivity. Filler's shape, size, and orientation in the polymer matrix play a role in improving the mechanical properties of the matrix.

Composites with at least one phase in the dimensions of nanometers (about 1–100 nm in length) get categorized as nanocomposites. Nanotechnology used in these composites influences the characterization, preparation, and structure of the materials [13]. Based on matrix materials, nanocomposites can be classified into three classes: (1) nanocomposites of a polymer matrix, (2) nanocomposites with a metal matrix, and (3) nanocomposites with a ceramic matrix. Nanocomposites get occupied for improving the mechanical, optical, and barrier properties of the composites. Large surface-to-volume ratio and surface activity of nano-sized materials are some of the factors that contribute to the enhancement of properties of the polymer nanocomposites [13]. These nanocomposites are inexpensive, light in weight, exhibit sound processability, transparent with enhanced barrier attributes to water/gasses, etc. The demerits of these matrices include increasing viscosity, difficulties in dispersion, increasing sedimentation rates, converting into black if different carbon-containing nanoparticles get employed, etc. Fabrication of nanocomposites is a challenge with both pre-polymerization and post-polymerization methods. Direct release of nanoparticles into the aquatic resources during industrial discharge and indirectly through surface runoff to the soil is common. Via many chemical–physical processors, the transformation of nanoparticles takes place in the environment. Many organisms (both aquatic and terrestrial organisms) ingest these nanoparticles. Mentioned downsides of the nanocomposites triggered the formation of bionanocomposites [12].

Materials comprise particles with at least one dimension that falls between 1 and 100 nm, and a constituent of the biological origin or biopolymers gets defined as bionanocomposites [1]. Generally, composites consist of two or more components that exhibit different properties both physically and chemically after combining that when they were individual components. In the case of bionanocomposites, they are made up of biopolymers and inorganic solids such as metal oxides that lie in the nanometer range with novel and multifunctional properties including

biodegradability, biocompatibility, and antimicrobial activity [14]. Nanofillers such as nanoclay, zinc oxide, and titanium dioxide have a large surface-to-volume ratio that improves the polymer matrix–filler interaction to enhance the overall performance of the material including thermal and barrier properties [1]. When compared with neat polymer matrixes and early composites, bionanocomposites exhibit enhanced mechanical and barrier properties, where they withstand thermal, storage, and transportation stresses [15].

Based on the matrix type, the origin, shape, and size of the reinforcement bionanocomposites can be classified. Based on the shape of the particle reinforcement, these can be classified into particulate, elongate, and layered.

Elongated particle bionanocomposites

Elongated particles such as carbon nanotubes and cellulose nanofibrils are utilized to reinforce this type of bionanocomposites and exhibit superior mechanical properties than other composites [12]. A higher aspect ratio of the reinforcement is caused by the enhancements of the properties of elongated particle bionanocomposites [15].

Particulate bionanocomposites

Isodimensional particles are commonly used in reinforcement. Due to the low aspect ratio and the reinforcing effect being moderate, it aids in enhancing the resistance to flammability and reduces the permeability and the cost of the composites.

Bionanocomposites reinforced with layered particles

Layer polymer nanocomposite can be classified into three subclasses based on the dispersion of particles in the matrix. When there is no partition between the layers of the matrix due to the particle–particle interaction, flocculated/phase-separated nanocomposites are generated. Since individual laminas are not separated, these composites are called microcomposites [14]. Intercalated nanocomposites are obtained when polymer chains are intercalated between sheets of the layered nanoparticles. When there are partitions in between individual layers, exfoliated nanocomposites are produced [12].

9.2.1 Properties and Applications of Bionanocomposites

Properties of bionanocomposites such as mechanical properties, biocompatibility, biodegradability, and antimicrobial activities are highly dependent on their high surface area, morphology, and shape. Parameters such as the process used in bionanocomposite fabrication, type of fillers and their orientation, type of adhesion at the matrix interface, degree of mixing of two phases, nanoparticle characteristics, the volume fraction of nanoparticles, size and shape of nanofillers, name of the interphase developed at the matrix interface, and the morphology of the system affect the nature of the properties of the bionanocomposites [14]. Bionanocomposites exhibit improvements in modulus, solvent or gas resistance, and

dimensional stability than the neat polymers. These composites also offer benefits such as transparency, low density, better surface properties, and recyclability. Nanoparticles use in bionanocomposites help in achieving uniform dispersion of the particles in the biopolymer matrix leading to an ultra-large interfacial area between the constituents. A large organic or inorganic interface alters the relaxation behavior, molecular mobility, and consequent thermal and mechanical properties of bionanocomposite materials [16]. Effective dispersion of nanoparticles in the bionanocomposites enhances the performance of the material. Various factors such as choice of solvent, size of nanoparticles, and type of mixing govern the uniformity of distribution of nanoparticles in the biopolymer matrix. Techniques including plasma modification, chemical modification, grafting, or coasting with bio-moieties are used in surface modification of the nanofillers to enhance their dispersion in the matrix. Biocompatibility and hydrophobicity/hydrophilicity of the bionanocomposites can be also improved by using surface modification techniques. Criteria including controlled crystallinity of nanoparticles and fixing their size and shape are also important when it comes to the development of high-performance bionanocomposites [3].

Bionanocomposites exhibit various applications in sectors such as automotive, electrics, pharmacy, paper/packaging, and biomedicals [17]. Bionanocomposites developed employing biopolymers such as PLA, poly-(butylene succinate) (PBS), and polyhydroxybutyrate (PHB) are optically transparent; therefore, they are commonly utilized in the food industry. Due to the factors such as less expense, less pollution, and antimicrobial activities, bionanocomposite films are used in the production of food packaging [14]. For the development of durable load bearings, automotive parts, and car building blocks, cellulose nanofibers are utilized in automobile industries [18]. Nanocellulose-based biocomposites also are used in water treatment industries to remove contaminants such as fluorides and chlorophenols [19]. Bionanocomposites are used in the biomedical industry such as drug release systems, wound dressings, vaccination, and bioengineering due to their biocompatibility and non-toxic properties. Electrical, magnetic, and optical properties of bionanocomposites make them include in the diodes, displays, solar cells, sensing and medical devices, etc. [14]. When considering the various properties of bionanocomposites, the present chapter focuses on the application of these materials in the food industry.

9.3 Bionanocomposites in the Food Industry

In the industry of food, for the applications such as food packaging, chemically synthesized polymeric materials are employed widely [20]. Simplicity, lower production cost, higher durability, and the flexibility of these synthetic plastic materials manage to gather special attention toward becoming the most utilized packaging material. However, non-biodegradability has become the major drawback of these synthetic polymers.

Environmental concerns about non-degradable plastic have raised interest toward biodegradable alternatives in the food industry [21]. In food industries, to extend the shelf life and to enhance the quality of the food, the packaging is occupied [11]. More than 40% of plastics get employed as packaging, including food packaging such as films, sheets, bottles, cups, tubs, and trays. The environmental impact of long-lasting plastic packaging waste is becoming an alarming concern among the public due to the limitations of their waste disposal methods. After plastic packages complete their usage life, it is desirable if they possess the ability to get degraded under natural conditions [22].

Exploration of bio-based and or biodegradable materials from renewable sources would be a sound solution in the substation of synthetic plastic materials to reduce the growing waste problems. Biodegradable packaging materials developed from renewable biological resources exhibit excellent qualities including biodegradability at the end of their life. Bioplastic materials consider being one of the most potential eco-friendly substitutes for non-degradable, non-renewable plastic packaging materials [23].

Nevertheless, as conventional packaging, biopolymer-based packaging must provide various functions such as containment and protection of food to maintain its sensory quality and safety and provide information about the food to the consumer [24]. So far, the utilization of biodegradable plastics in the food packaging sector is minimum due to several factors, such as poor barrier properties and mechanical properties. Before the commercial use of biodegradable primary packaging materials, factors including degradation rate under various conditions, changes that occur during the storage, the potential for microbial growth, and the release of harmful compounds to the food products must be concerned. In real applications, high hydrophilic properties with poor processability of biopolymer-based packaging materials generate severe limitations for their usage at the industrial level. Biopolymers frequently get blended with synthetic or less frequently get chemically modified to eliminate the mentioned problems.

Recently, hybrid of organic/inorganic systems, particularly material with layered silicate dispersed at a nanometric level in a polymeric matrix, has gathered greater attention. Nanocomposite incorporated with biopolymers exhibits enhanced properties such as good mechanical properties, oxidation stability, decreased solvent uptake, and biodegradability compared to the neat polymers. The application of bionanocomposites in the packaging sector expands the use of biodegradable polymers which helps in the reduction of waste products associated with food industries [16]. Compared to the base polymer class, bionanocomposites are exhibiting much improved properties due to the high aspect ratio and high surface area of nanoparticles incorporated. Thus, these advances provide a base for the development of bionanocomposites as packaging material posing improved properties [25].

The utilization of biopolymers in food packaging applications poses both positive and negative outcomes. The ability to get degraded under natural conditions, degradation without the release of toxic substances, minimum alteration of food components, ability to incorporate active components, and ability to develop as edible packages are some of the benefits of biopolymers in food packaging. The limitations of these

biopolymers in food packaging are poor barrier properties, low tensile strength, brittleness, low thermal properties, higher gas/water permeability, etc.

The incorporation of nanomaterials into polymer matrixes is a novel technique in food packaging. Bionanocomposites are used to extend the shelf life of fresh products such as fruits and vegetables by controlling the respiration of the products. The quality of meat, poultry, and seafood products in fresh or frozen states can be enhanced due to the retardation of moisture loss, reduced lipid oxidation, etc., using the packages developed with bionanocomposites. Several benefits of bionanocomposites in food packaging are included below [26].

- Biodegrade under natural conditions.
- Can be developed as edible packages.
- Help in enhancing the shelf life of the food products.
- Improve quality and the properties of food.
- Enhance properties of barrier including oxygen and moisture.
- Protection against lipid rancidity.
- Facilitate incorporation of an active agent such as antioxidant and antimicrobials.
- Facilitate controlled release of active agents.
- Can be used in multilayer food packages together with nonedible films.
- Facilitate the use of biosensors and nanochips for food quality checking.
- Low in cost and effective in waste utilization.

9.4 Properties of a Bionanocomposite that Make It Suitable as a Food Packaging Material

9.4.1 Mechanical Properties

Packages are prone to various types of stresses during their usage. Hence, characterization of mechanical properties of bionanocomposites which get occupied in the development of food packaging is necessary [27]. Tensile strength (TS), elastic modulus (E), and elongation at break (ε) are the main mechanical properties in the usage of food packaging applications [28]. The capability of the material to resist stress before its ruptures is called TS. The TS of these materials affects the physical integrity and barrier properties. Fracture strain or ε is the ability of a material to stretch before breaking. This helps to determine the extensibility or the flexibility of the packaging materials [29].

Starch and PLA are the widely used materials in food packaging due to their desired properties and availability and production at an industrial scale. PLA exhibits an acceptable level of mechanical properties for the incorporation in the production of packaging materials. Biodegradable polymers including starch, whey protein, soy protein, and chitosan do not exhibit improved mechanical properties up to an acceptable level for packaging material development. As a result, the incorporation

of different compositions of nanoparticles into biodegradable films has been initiated. The mechanical properties of bionanocomposites are highly dependent on the biopolymer type, nanoparticle type, and nanoparticle content.

Different reinforcement effects of nanoparticles in bionanocomposites can be achieved based on the nanoparticle dispersion state, surface area, polydispersity, and organo-modification, etc., which would lead to their grafting to the matrix of polymer to expand the mechanical properties [30]. Characteristics of bionanocomposites such as Young's (E) and shear (G) moduli, thermal expansion coefficient (α), and electrical conductivity (σ) can be influenced by the effects of the above-mentioned factors. The affinity between biopolymers and nanoparticles is the key factor for the homogeneous distribution of nanofillers in the polymer matrix. TS of bionanocomposites gets increased at lower nanofiller contents, where biopolymer length gets increased with increasing nanofiller content. This is highly dependent on the type and the content of the nanofiller used. Some studies state that aggregated nanofiller facilitates the movement of polymer chains as ball bearings [31]. Soft polymer matrices adjacent to stiffer filler become mechanically more constrained. Furthermore, effects of greater reinforcement can be achieved using fillers with higher specific surface area resulting in enhanced mechanical properties of the materials. The molecular weight of polymer matrices also bears an impact on the reinforcement and the mechanical properties such as TS of the bionanocomposites [32]. Changes in mechanical properties of various bionanocomposites of various studies are discussed below.

According to the study of Arrieta et al. [33], PLA reinforcement with nano-TiO_2 (nano-titanium dioxide) (1–5%) showed improved TS and Ɛ by 80%. PLA which is reinforced with cellulose nanofibers (5%) exhibited no observable difference in TS, decreased Ɛ by 36%, and E increased by 40% [33]. As stated in the study of Hassannia-Kolaee et al., biopolymer of whey protein–pullulan reinforced with nano-SiO_2 (nano-silicon dioxide) (1–5%) improved the TS of the material by 70% and Ɛ decreased by 32%. MMT (Montmorillonite) (1–5%) reinforced whey protein–pullulan biopolymer matrix exhibited increased TS up to threefold and decreased Ɛ up to 4.5-fold [34]. Chitosan reinforced with cellulose (5–40%) exhibited reduced Ɛ of high molecular weight chitosan up to threefold, Ɛ of low molecular weight chitosan reduced up to 12-fold, in high molecular weight chitosan, TS increased up to 75%, in low molecular weight chitosan, TS increased up to 60%, and E of high molecular weight chitosan and low molecular weight chitosan increased up to 40 and 75%, respectively [35]. Kefiran reinforced with nano-ZnO (nano-zinc oxide) (1–3%) exhibited increased TS up to 100% and increased elongation at break up to 12-fold. Kefiran which is reinforced with nano-TiO_2 (1–5%) improved elongation at break up to fourfold, reduced TS by around 45%, and reduced E by 50% [36-37]. Starch–PVA biopolymer matrix reinforced with nano-SiO_2 (0.05–0.5%) caused an improvement of TS of the matrix up to 70% and reduced the Ɛ up to 60%. Starch–PVA matrix with MMT (1–5%) improved the TS and E by 80% and fourfold, respectively [38].

9.4.2 Barrier Properties

In general, food is kept in a package after production before consumption to serve a number of functions. Packaging provides protection to the product to eliminate the harm that might occur due to the factors dirt, light, oxygen, microorganisms, moisture, etc. The packaging materials must be safe, inert, cheap, light, and easy to dispose of. Good packaging material must have the ability to withstand stress during the process and be resistant to stress during storage. The presence of oxygen inside packaged food is one of the major causes of food deterioration. The reactions coupled with oxygen, including nutrient loss, rancidity, color change, aerobic microbial growth, effects on respiration rates, and production of ethylene, are some of the many causes of the deterioration of packaged food. Therefore, the permeability of packaging materials to gases and other small molecules is one of the major factors that must be considered in the case of barrier properties [34].

Determination of the permeability of polymeric materials, including bionanocomposites, can be done by using the adsorption rate of gas molecules hooked on the matrix at the atmosphere-polymer boundary and the diffusion rate of adsorbed gas molecules through the matrix. The rate of formation of free volume holes in the polymer by the random or thermal motion of polymer chains is the dependent factor of the absorption rate. Diffusion occurs due to the hurdles of gas molecules to adjacent voids (empty holes). Therefore, the penetrability of polymeric materials is reliant on allowed hole size, grade of polymer motion, exact polymer–polymer, and polymer–gas interaction. These mentioned factors are highly reliant on the intrinsic polymer chemistry and external properties such as structural features, the polarization of the polymeric chains, hydrogen bonding structures, additional intermolecular connections, polydispersity, molecular weight, grade of cross-linking, crystallinity, temperature, and pressure. The overall gas diffusion is also directly dependent on the film's thickness [31].

No polymer displays the necessary barrier and mechanical properties to all packaging submissions. Therefore, polymer mixtures or compound multilayer schemes are broadly occupied. As an instance, material such as ethylene vinyl alcohol (EVOH), which is highly subtle toward water yet blockade to oxygen gets sandwiched with bilayers of the hydrophobic polymer as polyethylene (PE) to provide a higher barricade to oxygen in a very moist situation. Polymer direct blending can also be used in enhancing blockade properties that do not get gained through single layers of polymers. Nonetheless, polymer blends provide packages with improved barricade properties, and these arrangements pose higher manufacture costs, need the use of superior adhesives which complicate their instruction, and are problematic in recycling. Hence, the polymer industry tries to improve these properties of monolayer polymer materials using technologies such as nanocomposites [37].

In nanocomposites and or bionanocomposites, there are nanofiller dispersed into a homogeneous polymer matrix. This diffusion of nanofillers to the polymer matrix

distresses the blockade properties of similar materials in two ways, first by the formation of a "tortuous path" for the dissemination of gas. Since nanofillers are impermeable in nature, the molecules of gas have to diffuse around them instead of taking a straight path perpendicular to the surface of the film. Due to this reason, the mean pathway for the dissemination of the gas via the film is elongated. Out of all dissimilar forms of nanomaterials, including spheres, fibers, rods, tubes, and plates, layered nanomaterials are the greatest suitable to recover the blockade properties. Other than the nanofiller content and the aspect ratio, the state of exfoliation also moves the blockade properties of nanocomposites.

In the second way of dispersion of nanofillers, nanomaterials can impact the barricade properties by producing changes in the polymeric matrix. The polymer chains in the vicinity of the nanomaterials get partially stopped if the interactions of nanomaterial–polymer are favorable. Thus, the gas molecules which pass via these interfacial zones will have weakened movement. Interfacial region effects are important on these polymeric materials that exhibit high permeability to gases. When related to micrometric fillers, the nanofillers consist of an advanced aspect ratio than a higher surface area-to-volume ratio which in terms help to enhance the properties of the composites/biocomposites. Barrier properties of various bionanocomposites are shown in Table 9.1.

9.4.3 Thermal Properties

The processing of polymers and biopolymers is highly dependent on thermal properties such as glass transition temperature, and melting point. Molecules of the amorphous region of the polymers stay frozen under lower temperatures called "glassy state". This is the state where polymers are hard, rigid, and brittle. Once the polymers are heated under higher temperatures, their chains wiggle making those polymers more flexible giving the name "rubbery state". The temperature where the shift occurs from the glassy state to the rubbery state of the polymer is recognized as glass transition temperature. The amorphous region is the place where glass transition occurs. The crystalline state stays without getting affected. The melting point of the polymer is combined with the region of crystalline. At the temperature above the melting point of a certain polymer, the viscosity of the polymer reduces fast and it improved the ability to process the polymer. Various studies have reported the thermal properties of different bionanocomposites, and some of them are summarized below.

- Bionanocomposites of starch reinforced nano-ZnO (2–4%) exhibited glass transition temperatures in uppers polymer around 39.1 °C, in lower polymer around 36.6 °C, and neat polymer around 34.7 °C [44].
- Starch reinforced with MMT at ratios of 1–7% exhibited melting points around 243.0, 221.5 °C, and 191.7, respectively, in upper, lower, and neat polymers [49].

Table 9.1 Effects of nano-reinforcing agents on barrier properties of various bionanocomposites

Biopolymer	Nano-reinforcing agent	Improvements/changes in biopolymer properties (on water vapor permeability (WVP), water vapor transmission rate (WVTR), and oxygen transmission rate (OTR)	References
PLA	Nano–ZnO_2	Nano-ZnO_2 resulted in about 37% decrease of WVP with increased ZnO_2 content from 1–3%	[31]
PLA	Cellulose nanocrystals	Reductions of 34% in WVP were obtained for the cast films containing 1 wt.% of surfactant-modified cellulose nanocrystals while OTR for nano-biocomposites with both 5 wt.% of surfactant-modified cellulose nanocrystals decreased by 48%	[40]
Whey protein–pullulan	Nano-SiO_2	WVP of the film decreased around 33% with the increase of nano-SiO_2 content up to 5%	[41]
Chitosan	Cellulose nanofibers	WVP reduced by 27%. For the film with glycerol at the concentration of 18% and and cellulose nanofibers at 15%	[42]
Chitosan	Nano-TiO_2	WVP decreased significantly TiO_2 concentration of 1% and 2%	[43]
Starch	Nano-ZnO_2	WVP reduced by 50% with increased ZnO content up to 3%	[44]
Starch–PVA	Nano-SiO_2	WVP decreased by 30% at the nano-SiO_2 concentration up to 2%	[45]
Chitosan	Cellulose nanocrystals	By the addition of 3% of cellulose nanocrystals, WVTR reduced by 45% and OTR decreased by 38%	[46]
Alginate	Cellulose nanocrystals	By the addition of 5% of cellulose nanocrystals, WVTR reduced by 15% and OTR decreased by 45%	[46]

(continued)

Table 9.1 (continued)

Biopolymer	Nano-reinforcing agent	Improvements/changes in biopolymer properties (on water vapor permeability (WVP), water vapor transmission rate (WVTR), and oxygen transmission rate (OTR)	References
Whey protein isolate	MMT	Addition of 15% (w/w protein) MMT into 10% (w/w dispersion) whey protein isolate-based cast films or coatings resulted in reduction of OTR by 91% for glycerol-plasticized and 84% for sorbitol-plasticized coatings and reduction of WVTR by 58% for sorbitol-plasticized cast films	[47]
Whey protein isolate	Nanoclays	At the filler ratio of 9%, WVTR and OTR were reduced by approximately 50%	[48]
Kefiran	Nano-ZnO	WVP decreased about 17% with increasing content of nano-ZnO up to 2%	[36]

- Chitosan developed incorporating cellulose nanofibers provided glass transition temperatures around 125.3, 138.1 and 130.1 °C in upper, lower, and neat polymers [42].
- Whey protein consisting bionanocomposite reinforced with nano-SiO_2 exhibited glass transition temperatures around 34.06, 27.09, 29.08 °C, and melting points of 117.34, 115.64 and 117.68 °C in upper, lower, and neat polymers [41].
- PLA reinforced with nanofibers exhibited glass transition temperatures and melting points in upper, lower, and neat polymers 57.2, 56.3, 56.9, 168.0, 167.9 and 168.1 °C, respectively [50].

9.5 Application of Bionanocomposites in the Packaging of Food

9.5.1 Dairy Products

The application of bionanocomposites as a packaging material is limited in the dairy industry due to the shortcoming of the high sensitivity of biopolymers toward moisture. Packaging of high moisture-containing dairy products such as yogurt, milk, and ice cream employing biopolymer packaging is not possible due to the mentioned cause. Therefore, the utilization of biopolymers as a packaging material in the dairy industry is limited to low moisture-containing products only including cheese. Few researchers have reported the application of bionanocomposites in the packaging of different dairy products.

Gammariello et al. (2011) evaluated the effects of bio-based coating that consisted of silver (Ag)/MMT nanoparticles together with modified atmosphere packaging on sensory and microbial decay of the Fior Di cheese. Various concentrations (0.25, 0.50, 1.00 mg/mL) of Ag nanoparticles were incorporated in a sodium alginic acid solution (8 wt./vol %) before the coating of the cheese. Up to 30% CO_2, 5% O_2, and 65% nitrogen were included in the modified atmospheric packaging. According to the study, modified atmospheric packaging combined with Ag-based nanocomposite induced the shelf life of the Fior Di cheese. Cheese stored in conventional packaging exhibited a shelf life of around three days. Coated cheese stored in modified atmospheric packaging achieved a shelf life of more than five days regardless of the Ag nanoparticle concentration. This study stated that the strategy for shelf life prolongation using this method could get practiced by the industries due to its simplified application. Somehow, it is necessary to remove the developed coating containing nanoparticles before eating the Fior Di latte. By some means, further studies must be conducted to evaluate the safety of silver that migrates to the food [51].

Meira et al. (2016) have developed starch/halloysite/nisin nanocomposite films as active antimicrobial packaging. Scanning electron microscopic image exhibited that the films were homogeneous and halloysite nanotubes dispersed in a starch matrix. The surface of films was aggregated as higher levels of nisin were added. According to the X-ray diffraction (XRD) spectra, a decrease in polymer crystallization occurred due to the alteration of starch peaks after halloysite nanotube and nisin incorporation. The incorporation of HNT caused increased mechanical properties of the developed films. By some means, addition of nisin causes a decrease in E and TS values. The thermal stability of biofilms consisting of nisin was decreased. They showed a Tmax value around 20–26 °C lower than the films without nisin. The antimicrobial activity of the films was tested, against the microbes including *Clostridium perfringens, Staphylococcus aureus,* and *Listeria monocytogenes* present in skimmed milk agar. Nisin consisted films were capable of inhibiting all mentioned microorganisms. Minas Frescal cheese surface was applied with inoculated *Listeria monocytogenes,* and, after four days, 2 g/100 g nisin-containing bionanocomposite caused a reduction in the initial microbial count of the bacterium. About 6 g/100 g nisin-containing

films were able to inhibit *Listeria monocytogenes* completely. Results of this study exhibited that nisin in starch/halloysite films could be a barrier control for the dairy products such as cheese [52].

In a study by Incoronato et al. (2011), Ag/MMT embedded agar, antimicrobial packaging material was developed and texted the effectiveness of the packaging material toward the quality and the deterioration of Fior Di latte cheese. The presence of spoilage-causing and beneficial microbes was observed during the study. The sensory quality of the cheese was evaluated via a panel test. The result of this study exhibited improved shelf life of the tested cheese. Ag cations hinder the proliferation of microorganisms without affecting the beneficial microbiota and the quality of the sensory traits of Fior Di latte. According to this study, the active packaging system is suitable for the quality and the shelf life enhancement of the tested cheese product [53].

Youssef and El-sayed (2018) prepared novel bionanocomposites using chitosan/polyvinyl alcohol (PVA)/titanium nanoparticles. These bionanocomposites were tested as packaging materials for soft white cheese. Characterization of developed biofilms was done using a scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), and XRD. Chitosan/PVA/titanium nanoparticles containing films posed excellent mechanical properties. Improved antimicrobial activities especially against gram-positive bacteria such as *Staphylococcus aureus,* gram-negative bacteria such as *Pseudomonas aeruginosa*/*Escherichia coli,* and fungi such as *Candida albicans* were also recorded. During the experiment, soft white cheese was packed in developed bionanocomposite material and stored at 7 °C for about 30 days. The results of microbial analysis of bionanocomposite with chitosan/PVA/titanium nanoparticles exhibited decreased total bacterial count, mold, yeast, and coliform activities. This study concluded that these chitosan/PVA/titanium nanoparticles consisting of bionanocomposites can be used in food packaging applications including, cheese [13].

9.5.2 Fruit and Vegetable

Increasing demand for healthy and nutritious food has promoted the urge to circulate fresh fruits and vegetables for consumption. Fresh products are highly vulnerable to spoilage and physical damage, moisture loss, chemical alterations, and microbial deterioration. The post-harvest life of the fruits and vegetables can be improved using packaging including plastic films and coating. Though plastic is used as packaging for many fruits and vegetable, they have become a global concern due to its non-degradable nature. Due to the aforementioned issue, bioplastic films/coatings are used as eco-friendly alternatives to conventional packaging. Generally, bioplastics alone do not pose superior properties; therefore, they can be reinforced with nanoparticles to improve this shortcoming. The nanoparticles employed in this process can also exhibit antimicrobial, antioxidant activities. Many studies have been reported

on reviewing the effects of biopolymer-based nanocomposite films and coatings in packaging whole or cut fruit and vegetables to extend their shelf life [54].

The effects of bionanocomposites on the preservation of fruits and vegetables were experimented with by employing chitosan-based nanocomposites. As the study concluded, chitosan is one of the excellent bioplastic materials which contributes to improving the shelf life of fresh products, including fruits, vegetables, and meat. The addition of nanoparticles of ZnO, Ag, and TiO_2b together with phytochemicals such as essential oils and fruit extracts in the matrix of chitosan increases the properties of antimicrobial, mechanical, and barrier. The utilization of these bionanocomposites in food packaging can improve the shelf life of various food products while contributing to a reduction of non-degradable plastics and additive usage [55].

In a study by Maneerat and Hayata (2006), the antifungal activity of TiO_2 photocatalytic reaction on plastic films upon *Penicillium expansum* was studied in vitro as well as on fruits. The TiO_2 photocatalytic reaction caused a reduction in the conidial germination of fungi. The amount of TiO_2 added was correlated to the actions of the pathogen fungi, *Penicillium expansum.* The presence of the number of viable colonies of *Penicillium expansum was reduced* as the amount of TiO_2 increased. The development of *Penicillium* rot on apples was reduced by the application of bionanocomposite films with TiO_2 photocatalytic reaction. Tomato fruits applied with the same bionanocomposite exhibited no rot after a week of storage. Brown lesions rot on lemons has decreased since the application of bionanocomposites with TiO_2. Findings from this experiment suggest that the TiO_2 photocatalytic reaction is effective in providing antifungal activity against *Penicillium expansum* that can be utilized for disease control post-harvest [56].

Negatively charged polysaccharide, alginate matrices reinforced with sepiolite and palygorskite fibrous clay were developed. Fibrous clay surface created strong bonds between hydroxyl and carboxyl groups of the polysaccharide. The primary interaction mechanism to obtain the stability of the bionanocomposite was the hydrogen bonding between the –OH groups of biopolymers and the silanol groups of the fibrous silicate. The improvement of mechanical and barrier properties, stability, and water absorption reduction was achieved via the compatibility of the biopolymer and the clay. Tested fillers of zein together with fibrous clay were also tested as fillers for these films. Zein employment decreased the hydrophilic nature of pristine clay, resulting in improved properties in biohybrid fillers. These biohybrid fillers incorporated films that exhibited excellent barrier properties to vapor and gas, reducing the water uptake at higher humidity. As concluded by this study, these modified bionanocomposites can be employed in packaging fruits and vegetables such as apples. They will be a sustainable eco-friendly alternative in the food industry [57].

9.5.3 Meat and Poultry

Microbial contaminations, oxidation of lipid, and protein make products such as meat poultry highly vulnerable to spoilage. Growing demand for healthy, safe, and

nutritious food has increased the need to pack these products as a processing step. The utilization of packaging technologies assists in keeping the quality and safety of food products. Fresh food packaging helps to reduce microbial spoilage, lipid/protein oxidation, and enzymatic deterioration of the food. By this, the tenderness, color, aroma, etc., can be preserved. Currently, most packaging materials used in food production industries are not biodegradable, which causes environmental concerns [58].

The development of biopolymer-based packaging materials can help in reducing fossil fuel-based materials in the industry. Bionanocomposites open a new road for high-performance, lightweight green composite to substitute the non-degradable packaging materials. When it comes to food packaging, special considerations must be provided to obtain higher barrier properties. For that, nanostructures can be employed to improve the active or intelligent properties of the food packages. According to many studies, use of bionanocomposites can be effective in the extension of the shelf life of meat products. Below it has discussed the effects of bionanocomposites on the quality of meat and poultry products [11].

In a study by Nagarajan et al. (2015), Tilapia and squid skin, gelatin biofilm together with cloisite Na+ nano clay, and ethanolic extract of coconut husk was developed. These films were utilized as a packaging material for mackerel meat powder and stored for 30 days at 28–30 °C. Meat powder packaged in bionanocomposites exhibited lower moisture content, total volatile base, peroxide value, and pH than meat powder packed in polyethylene films [59].

In a study by Morsy et al. (2014), turkey breast and beef were stored in pullulan film consisting ZnO and Ag nanoparticles and oregano/rosemary extract for three weeks at 4 °C. An inhibitory effect of pathogens such as *S. aureus, L. monocytogenes, and E. coli* was observed. Ag nanoparticles exhibited a better effect on microbes when compared to ZnO and extracts [60].

Bionanocomposite developed with chitosan and nanocellulose was employed in storing ground meat for 6 days at 3 and 25 °C. The meat exhibited reduced color change and lipid oxidation. A reduction in the growth of mesophilic aerobes and psychrotrophic microbes was observed. Added carbon nanotubes contents assisted in the improvement of the antimicrobial activities of these biofilms [61].

9.5.4 Application of Bionanocomposites in Novel Food Packaging Systems

Active packaging

The urge for the utilization of food products up to a maximum level became essential with the growing population and the land resource reduction. This is where packaging came into play as an important material that assists in improving the shelf life of food products throughout the food supply chain. Further, several improvements in packaging systems enable various food products around the world with maximum shelf

life and safety [62]. According to significant demand changes of today's consumers and market trends, novel packaging systems such as active packaging became important; when compared to the conventional packaging materials, this packaging system is developed to interact with both internal and external environments of the packaging and the food product [63]. Active packaging is defined as a package that alters the conditions of the packaged food to improve the shelf life or to increase the food safety and sensory properties, together with maintaining the quality of the packaged food. Here the conditions inside the package get controlled to improve the quality of the food product that has been packed [64]. In this section, it has been focused on the employment of bionanocomposites as active packaging systems.

Antioxidant packaging

Lipid oxidation of food products with higher lipid content especially, with a higher degree of unsaturated fatty acids, is prone to deterioration. Lipid oxidation of food items, such as nuts, oils, fish, and meat products, results in giving off flavor due to rancidity and makes the products less suitable for human consumption. Degradation of polyunsaturated fatty acids as a part of lipid oxidation causes the formation of toxic aldehydes and loss of nutrients in the foods.

Increasing demands for safer and healthier food products have taken the attention toward developing novel technologies such as the addition of antioxidants to foods or packaging material. Novel food packaging technologies such as vacuum packaging and modified atmosphere packaging get used to eliminate the presence of oxygen inside the packaging system to minimize the food spoilages such as oxidation. Nonetheless in scenarios where food products such as meat which cannot be stored without oxygen, the application of antioxidants on the surfaces of the food or incorporation into the packaging material can be an alternative [65].

Antioxidant packaging is a type of active packaging method in which antioxidant properties get incorporated or coated with the packaging material to minimize the oxidation of the food components. According to many studies, the release of incorporated antioxidants from packaging materials such as butylated hydroxytoluene (BHT) and tocopherol from polythene films to foods with higher lipid contents delays the oxidation of fats and the denaturation of the proteins. The utilization of natural antioxidants instead of synthetic additives in antioxidant packaging became a new trend. Tocopherols, plant extracts, and essential oils present in herbs including rosemary, tea, etc., are used as natural antioxidants in these kinds of packaging [66].

Bionanocomposites consisting of antioxidants are developed together with nanoparticles and the bioactive molecules in a matrix of natural polymer. In bionanocomposites, natural or synthetic antioxidants can be employed. Nanoparticles get used to reinforce the polymer matrix and, in some cases, to release antioxidants into the food system. Bionanocomposite films containing antioxidant components are produced via casting or extrusion methods. These developed films get occupied as packaging for fatty foods [31].

Many studies have developed numbers of antioxidants consisting of bionanocomposites; in the study of Infurna et al. (2020), bionanocomposite films were developed based on natural chitosan, pectin, halloysite nanotubes, and antioxidants via solvent

casting method. Halloysite nanotubes and antioxidants consisting of halloysite nanotubes (quercetin and vanillic acid) effects on optical properties, mechanical properties, photo-oxidation, and wettability were determined. The optical properties and morphology of the films were not negatively affected by the halloysite nanotubes and antioxidant containing halloysite nanotubes presented. The halloysite nanotubes created a decreased water contact angle, and quercetin and vanillic presented in halloysite nanotubes did not change the wettability of the films. The rigidity of the films was decreased due to halloysite nanotubes in the chitin: pectin blend. With the addition of halloysite nanotubes: quercetin and halloysite nanotubes: vanillic, the rigidity of the system improved. Halloysite nanotubes played in improving the compatibility of the bionanocomposite and provided antioxidant properties to the matrix. The presented halloysite nanotubes cause a higher resistance toward photo-oxidation [67].

Hybrid chitosan, nano-silver bionanocomposite films (CSSNC) were developed using synthesized nanoparticles together with chitosan through the green ex situ method out of the extracts of fruit waste. Around six films of different compositions of CSSNCs were made using techniques including FTIR spectroscopy, thermogravimetric analysis, XRD, SEM, and UV–visible spectroscopy. Out of all developed CSSNCs, the film of CS9AGEI was optimized for further biological assays where the antimicrobial activity exhibited a high zone of inhibition toward *E. coli* and *S. aureus.* According to the antioxidant activities of the CSSNCs, radical scavenging against nitric oxide, 2,2-diphenyl-l-picrylhydrazyl (DPPH), and total reduction capacity was determined [68].

Bionanocomposite of polylactic acid nanofibers together with antibacterial property containing silver nanoparticles and antioxidant activity bearing Vitamin E has developed through electrospinning technology. In the study of Munteanu et al., the characteristics including structure and morphology of the bionanocomposies were carried out in the study using SEM, XRD, and transmission electron microscope (TEM). Nanofibers employed assisted in inhibiting the growth of *E. coli, Salmonella typhymurium*, and *Listeria monocytogenes.* According to the results obtained from the test of DPPH (2,2-diphenyl-l-picrylhydrazyl), the antioxidant activity was around 94%. Test on fresh apple and apple juice exhibited PLA/Ag/Vitamin E bionanocomposites' ability to reduce the polyphenol oxidase activity. Silver nanoparticles incorporated multifunctional electro-spun PLA nanofibers together with antioxidant Vitamin E can be used as food packaging since they pose a higher surface area of nanofibers together with antimicrobial and antioxidant activities. As concluded, this developed type of bionanocomposites can be utilized as food packaging for fruits and juices [69].

Polymethylmethacrylate (PMMA) and nano-hydroxyapatite (nHA) incorporated polymer-ceramic nanocomposite/composites were developed in the study of Doğan et al. Nanocomposite showed different enzyme activities when compared to the composites. Samples synthesized in acetone exhibited increased enzyme activities on glutathione reductase and glucose-6 phosphate dehydrogenase enzymes. These samples also inhibited glutathione peroxidase and catalase enzyme activities.

Samples synthesized tetrahydrofurans were given inhibitory actions for glucose-6 phosphate dehydrogenase enzymes and catalase [70].

Binary/ternary polylactic acid-based bionanocomposites consisting of nano-lignin and metal oxide particles (Ag_2O, TiO_2,) were developed in the experiment of Lizundia et al. (2020) through solvent casting technique. Antioxidant activity of ternary-based bionanocomposites was performed and obtained a synergic effect of lignin and metal oxides. According to the study, these developed bionanocomposites pose striking renewable additives for food packaging and the biomedical industry with higher antioxidant and antimicrobial properties [71].

Antimicrobial active packaging

Food products undergo different deterioration mechanisms such as microbial, physical, chemical, biochemical, and textural deterioration, based on the ingredients, production methods, packaging systems, etc. Microbial deterioration is more dominant when compared to other deteriorations and causes more damage to the food products. While techniques such as canning, heat treatment, and dehydration are used in improving the shelf life of the food, special attention must be given to the packaging since it also plays an important role in protecting the products from environmental hazards (e.g., moisture gain, dust, insects, etc.). Based on the factors such as reduction of demand for chemical usage in food products, growing demand for fresh-like, nutrient-rich foods, increasing food demand due to the world population growth, and new packaging techniques such as active packaging, intelligent packaging came to the play to secure the food safety. Since microbial spoilage is dominant among many food products, the incorporation of antimicrobial agents into the packaging became a promising technique in the active packaging system [72].

According to the intended use, the design of the antimicrobial can differ. When developing antimicrobial packaging, the following factors should be considered.

- Residue antimicrobial actions, the processing method of the packaging, and the chemical nature of the packaging material—the selection of an antimicrobial agent is dependent on the processing method of the package including extrusion, printing, etc., and its compatibility with the material. As an example, since higher processing temperature may affect the antimicrobial agents, it is important to carry out the processing methods such as extrusion at a low temperature.
- Traits of the antimicrobial agent and the food product—physicochemical characteristics such as pH and the water activity of food have a great effect on the antimicrobial agent. The pH of food ionizes the active site of the antimicrobial agent, causing an alteration of its biological activities. Unique microflora of the foods must also be considered when selecting the antimicrobial agent.
- Temperate during storage—this has a direct effect on the activity of the antimicrobial agents. Increased storage temperature increases the migration of antimicrobial agents. Temperature is also related to the activity of residual antimicrobial activity.
- Physical properties of the packaging material.

The product, the container, and the space between them create the packaging system. In antimicrobial packaging systems, active agents are put into packaging

material: films, edible coating, bilayers, sachets, etc., there are several forms of antimicrobial packaging as mentioned below [73].

As mentioned in Macromolecular Symposia, a chitosan-based bionanocomposite was developed incorporating carvacrol as the antimicrobial agent [74]. In the study of Youssef et al. a chitosan/PV-based bionanocomposite was developed incorporating TiO_2 as the antimicrobial agent [75]. A PLA/NC bionanocomposite consisting *Mentha piperita* and *Buniun percicum* was developed in the work of Talebi et al. [76]. Thymol incorporated PLA bionanocomposite was developed by Zhu et al. [77]. TiO_2 nanoparticles incorporated chitosan film was developed in the study of Kaewklin et al. [78]. Bionanocomposites-consisting antimicrobial agent ZnO was employed in controlling *Staphylococcus aureus* and *Candida albicans* [79]. Halloysite, Ag NP, Titania, layered silicate, and cellulose nanofibers incorporated PLA bionanocomposites were able to control *Listeria monocytogenes*, *E. coli,* and *Bacillus subtilis* [80]. *S. aureus, Bacillus cereus, E. coli 0157:H7 P. aeruginosa, and S. typhimurium* were controlled by the bionanocomposite developed of soybean polysaccharide with zataria multiflora boiss and mentha pulegium essential oil [81–82].

Since the urge of replacing plastics with alternative materials becoming more popular, scientists have put special focus on biopolymers. Cellulose, chitosan, lignin, starch, polylactic acids, etc., are used as biopolymers due to their availability and abundance to get used as food packages; these biopolymers must pose non-toxic and must have the preferable level of properties. In each biopolymer, polymeric chain configuration, substituents, and hydrogen bonds play an important role in improving the properties. Most of the biopolymers exhibit antimicrobial activity including chitosan. Most of the biodegradable non-toxic biopolymers including cellulose, starch, chitosan, alginate, etc., answer the ecological problems. However, biopolymers such as polysaccharides pose several shortcomings such as inferior properties (mechanical, barrier). This is where nanomaterials come to the act to overcome these issues. The incorporation of nanomaterials into the biopolymer matrixes assists in improving mechanical properties and barrier properties. The most common nanomaterials used in the field are montmorillonites, kaolinite, ZnO, TiO_2, etc.

Intelligent packaging

The major role of packaging is to provide safety to a product against spoilage that occurs due to exposure to different external environments. Packages can be seen in different sizes and shapes to provide ease and convenience to the customer or consumer. Some of the functions of packaging systems include providing defense, convenience, containment, and information. In the current food industry, conventional packaging no longer holds sufficient play due to upraising consumer demands and product complexity. Within two decades, packaging systems including active packaging and intelligent/smart packaging came into play to provide enhanced protection, convenience, etc., to the food product as well as the customer [83].

According to the European Commission, intelligent packaging systems are "materials that monitor the conditions of packaged food or the environment that surrounds the food". Intelligent packaging is a technology that employs communication roles to make decisions to enhance the shelf life, safety, and quality of the food product.

These packaging systems provide information and indicate issues that occur due to the changes in the internal and external environment of the package. Intelligent packaging systems monitor product quality and track the product for traceability. These packages have become capable of providing information regarding both food products and the integrity of the packaging. Indicators, sensors, and data carriers are the main technologies utilized in these packages. Information supply is done by sensors as well as indicators, while the data carriers offer management of logistics in the supply chain. Intelligent packaging systems can be adopted by various packaging types, including primary, secondary, and tertiary [84].

Bionanocomposite is considered a reliable candidate for the production of smart packaging due to the properties they offer such as better electrical properties, easy to handle, lightweight, and biocompatibility. Nanomaterials can be employed in the detection of gases, contaminants, and microorganisms or to obtain responses that occur due to environmental changes. A range of polymeric composites reinforced with magnetic, electric, optical, and mechanical sensitive nanofillers get exploited as sensors [31].

Microbial and gas detection

The quality of packaged food can be determined using indicators via the reactions between metabolites generated during the multiplication of the microbes in the product and the indicator. In many cases, the utilization of natural dyes in biopackaging for the determination of colorimetry is done. In a biofilm of chitosan, a time–temperature indicator consisting of an anthocyanin pH indicator was employed to determine deviations in the temperature during the storage time of the products. Gas indicators including oxygen and carbon dioxide can also be used as intelligent packages. These packaging systems indicate the presence of these gases based on the color changes due to the chemical or enzymatic reactions and the amounts of gases present. Essential oxygen detectors are recognized as methylene blue indicators, which change the color due to oxidation or reduction. Silver and titanium dioxide are nanostructures indicators used to a greater extent due to their higher sensitivity and stability. Cytotoxicity in metal particles makes them unsuitable in the packages of food. UV-activated oxygen detector consisting of redox dyes, an electron donor glycerol, and UV absorbing nanoparticles including TiO_2 in the film of carrageenan is one of the developed studies for the gas detecting intelligent packaging [85].

Intelligent packaging also gives signals if the food system inside the package is contaminated with microorganisms ensuring food protection and stability sometimes by releasing antimicrobial agents from the packaging. κ-Carrageenan/locust bean gum-based films consisting of thermosensitive poly (*N*-isopropyl acrylamide) nanohydrogels were developed in the study of Fuciños et al. (2015), which releases natamycin in the packaging system as a response to environmental triggers. Yeast and the *Penicillium commune* were employed as indicator microbes. Natamycin consisting of biofilms exhibited more antimicrobial activity than the films without it. As the study stated, this difference might be occurred due to the natamycin's protection from degradation in the biofilm enabling its release when the temperature increases [86].

9.6 Safety Concerns

Currently, consideration of the safety of food packaging systems consisting of nanomaterials gets a concern as these nanomaterials get leached into food products with increased time and storage temperature. In three ways, nanoparticles in these food packaging systems can affect humans: contact, ingestion, and inhalation. The degree of migration of nanomaterials, rate of toxicity of nanomaterials utilized, and particle size may determine the health effects of the packaging material with nanomaterials. The release of nanomaterials from degrading bionanocomposites might also cause harm to the environment [87].

The evolution of toxicological effects of nanoparticles in food packaging is done in vitro and in vivo. Smaller particles of food packages get absorbed easily and distributed into the organ to cause damage to the cells such as active oxygen species development in the cells. Studies included mice have exhibited carbon nanotubes generated asbestos, length-dependent toxicity when injected into the animals [88].

According to risk assessment regulations of Europe Union No. 10/2011 on plastic materials, different toxicological properties of nanomaterials must be assessed on a case-by-case basis. GRAS (generally recognized as safe) is important when nanoparticles are incorporated in the packaging materials. Prior to confirming the safety of nanomaterials, as suggested by the Institute of Food Science and Technology, the nanoparticles should be treated as "potentially dangerous materials" to minimize their toxic effects on humans [89].

9.7 Conclusion

Today, a number of studies have been carried out with the intention of development and improvement of bionanocomposites for utilization in food packaging applications. These studies upon bionanocomposites aim to improve and develop eco-friendly, greener plastic packaging materials that would help to improve the shelf life of the food product by preserving them while reducing the waste generation coupled around the food industry. Though there are various types of experiments performed around biopolymers at the laboratory and industrial levels, still, there is a need for a better understanding of the compositions, structures, and processing properties of bionanocomposites for the utilization in various sectors. Though there are topics discussed regarding the incorporation of nanomaterials into polymer complexes, there are still enough space for the variations and the improvement in the development of bionanocomposites. In this section, it is discussed that bionanocomposites bear great potential as a greener solution in replacing conventional non-degradable plastics materials. However, it is still necessary to further analyze the functional properties of these bionanocomposites before they get used in the food industry to give better competition against conventional plastics.

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