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REVIEWS

Composites Based on Chitin Nanoparticles and Biodegradable Polymers for Medical Use: Preparation and Properties

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Abstract—Chitin nanocrystals and nanofibrils possess attractive mechanical and biological properties, which makes them promising for application in different fields. This review discusses the application of chitin nanoparticles as a filler in composites based on biodegradable polymers. Different polymer matrices are considered: synthetic, semisynthetic, and natural (proteins and polysaccharides). Since chitin nanoparticles and nanofibrils possess a high aspect ratio and high Young's modulus, the main attention is given to the mechanical properties of the composites. Due to the high bioactivity of chitin, composite materials based on it feature interesting biological properties.

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INTRODUCTION

Polymer and polymer composites play a significant role in the technological development of our society [1]. The number of research papers dealing with polymers and composites for medical use is continuously increasing (Fig. 1); this reflects the interest in and demand for new polymer and composite products, drug delivery systems, and many other materials in practice.

The human body possesses certain tissue regeneration potential [2, 3] and, with different injuries, if spontaneous healing can occur, the task of physicians is to provide optimum conditions for functional tissue to form, rather than scar tissue, etc. [4]. Also, modern regenerative medicine, which uses cells of different human tissues, opens new possibilities for the treatment of traumas and diseases when the regenerative potential is not sufficient [2, 5, 6]. In all these cases, materials from biodegradable polymers capable of decomposing into safe products after fulfillment of the required function and release from the body to eliminate the necessity for surgical reintervention are in demand [7]. Such materials must be biocompatible; i.e., they and their metabolic products should not cause a pronounced immune response. Medical materials also should meet certain other requirements: appropriate mechanical characteristics; an open porous three-dimensional structure similar to the extracellular matrix; a matrix surface suitable for cell fixation, proliferation, and differentiation; and the presence of bioactive molecules, such as cytokines and growth factors [6]. The priority of the above-mentioned characteristics primairly depends on the field of application of a certain item and material.

In the field of medical goods, composites are being studied extensively, since the use of several components can offer a material new properties or eliminate

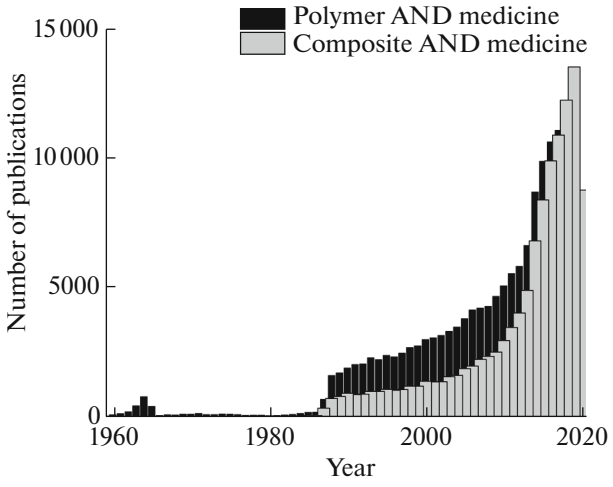

Fig. 1. Number of studies published at different times upon search queries polymer AND medicine and composite AND medicine, according to the PubMed database.

disadvantages of a polymer matrix. For example, synthetic polymer materials and natural polymers after required purification procedures are inferior to human tissues in mechanical characteristics, such as rigidity, strength, and impact strength, especially upon comparison of specific characteristics [8–10]. The use of a reinforcing filler is one of possibilities to correct this disadvantage. Compared to larger size particles, nanosized fillers show a high efficiency of reinforcing effect on the polymer matrix. This phenomenon is attributed to both reaching of the percolation threshold at a low content of filler and the formation of a considerable volume fraction of interfacial polymer layer [11–13]. The properties of the interfacial layer differ from the in-bulk polymer matrix in glass transition temperature, viscoelastic and dielectric characteristics due to the limited mobility of chains near a developed specific surface area of the filler [13, 14].

In medical materials, cellulose nanocrystals and nanofibrils, layered silicates, carbon nanotubes, graphene, hydroxyapatite (HAP) particles, and polyhedral oligomeric silsesquioxanes are used [15–18]. Along with cellulose nanocrystals and nanofibrils, chitin nanoparticles (CNP) can act as a reinforcing filler. Both fillers have anisometric morphology with an aspect ratio from ten to several hundred, as well as possess impressive modulus of elasticity (41–220 GPa) [19]. Also, these structural polysaccharides occur in a wide range of natural sources [20, 21]. However, a slight difference, namely, the presence of acetylated or free amino group in the C2 position instead of hydroxyl group in cellulose [22], governs differences in a number of properties, including a pronounced bioactivity of chitin. This polysaccharide has an anti-inflammatory effect, accelerates wound healing, and restricts generation of bacteria [23–26], which can be an additional advantage when CNPs are used as a filler for medical materials. This review considers medical composites based on biodegradable polymers filled with nanocrystals or nanofibrils of chitin; special attention is given to methods for the preparation of materials and their mechanical and biological properties.

1. COMPOSITES BASED ON SYNTHETIC BIODEGRADABLE POLYMERS

1.1. Polyesters

Polyesters are hydrophobic aliphatic polymers capable of decomposing to monomers upon hydrolysis of the ester group and thereby naturally released from a body [27]. Since quite short aliphatic chains are between the ester groups, polyester can decompose within the time that is suited for biomedical field. The unique character of polymers of this class consists in their vast variety and synthetic versatility. Polyesters can be obtained from many monomers by ring-opening and condensation polymerizations [28]. However, upon preparation of composites with polyesters as the polymer matrix filled with hydrophilic-surface particles, such as nanofibrils and nanocrystals of chitin and cellulose, an urgent question arises whether the particle distribution in composite is uniform [29–31]. This problem can be solved by the surface premodification of particles [32, 33]; however, the surface modification can prevent the formation of the percolation network of a filler through hydrogen bonding between particles [34].

1.1.1. Polylactide (PLA). Polylactide is one of the most common polyesters obtained from renewable sources, which decomposes upon hydrolysis to form lactic acid. This polymer has found application in different fields (package, textile fibers). Medical goods based on PLA are known: retention sutures, fasteners for surgery and orthopedics [35].

PLA exists in two optical forms, D-lacide and L-lactide; their physical properties can be controlled by varying the relative content of D- and L-forms. Crystalline poly-L-lactide (L-PLA) is a hard transparent polymer with a tensile strength of 45–70 MPa and an elastic modulus of 4.8 GPa. Poly-DL-lactide (DL-PLA) is an amorphous polymer with a considerably lower tensile strength of about 20 MPa and a modulus of 1.9 GPa [36, 37]. The rate of PLA degradation depends on the crystallinity and porosity of a material.

Among potential applications of PLA is the use as a material to substitute bone defects due to its good biocompatibility, biodegradability, and nontoxic decomposition products [38]; however, insufficient mechanical characteristics of the material restricts the application of this polymer in this field. Reinforcing of the PLA matrix by different fillers, such as HAP and cellulose particles, is one of the ways to eliminate this disadvantage [36]. Chitin, which is used for reinforce-

ment in different matrices, also attracts attention of researchers, especially in terms of biodegradable package [39–42].

The most of works in the fields of biomedical application are aimed at the design of PLA- and chitin-based materials for bone tissue repair. In [43], PLA-based composites filled with α-chitin nanocrystals obtained by acid hydrolysis, which were surface acetylated by acetic anhydride in the presence of pyridine, were prepared. The course of acetylation was monitored by changes in the IR and ^{13}C NMR spectra. PLA and modified chitin were dispersed in dicholoromethane and composite films were obtained by casting. The content of the filler in the composites varied from 1 to 10 wt %. The glass transition temperature and also melting point of composites (55 and 149.3°C, respectively, at 4% of chitin) were slightly lower than those of pure polymer (57.5 and 149.6°C). The strength and Young modulus of the nanocomposites progressively increased with an increase in the loading of the filler until its content reached 4 wt %. At this concentration, the strength and Young modulus reached the maximum values exceeding those for pure PLA films by 45 and 37%, respectively (Table 1).

In [30], α-chitin nanocrystals (l = 150–400 nm, d = 5–55 nm) obtained by acid hydrolysis were modified by grafting of oligomeric L-PLA chains onto the particle surface by the ring-opening polymerization of L-lactide (Fig. 2). Then, L-PLA-based composites, reinforced by the modified polysaccharide in one case and the nonmodified polysaccharide in another case, were synthesized; the content of the filler varied from 1.25 to 10 wt %. The amount of grafted chains according to the ^{13}C NMR spectral data was 35.33–42.45 wt %. The study of mechanical properties of materials showed that the tensile strength and elastic modulus of the resulting nanocomposites depend on the filler content. The highest strength and elastic modulus were observed at 5% filling. Note that the films with modified chitin showed better properties compared to the materials with unmodified chitin, which is due to a more homogeneous distribution of the grafted chitin in the matrix and a better adhesion at the interface. At polysaccharide concentrations above 5%, the polysaccharide undergoes aggregation in the matrix and, as a consequence, the mechanical properties of the nanocomposites deteriorate. To study biological properties, the proliferation and viability of MC3T3-E1 murine preosteoblasts on the obtained materials were assessed; the control sample was a pure PLA film. The assessment of the cytocompatibility of the materials, which is based on counting of living cells of this cell culture for 7 days, showed that the addition of CNPs increased the rate of proliferation of MC3T3-E1 cells. In [44], the same material was used to obtain a matrix with a fiber diameter of 400–800 nm by electrospinning. The 5% content of the polysaccharide was found to be optimum for reaching the maximum elastic modulus and strength of the material. Matrices filled with the modified chitin demonstrate an increased cytocompatibility compared to pure PLA for MC3T3-E1 preosteoblasts. It was also shown that the inclusion of the modified chitin also improves the adhesion and proliferation of MC3T3-E1 cells at the initial growth step.

In another study, DL-PLA films were synthesized and, after their keeping in a solution of dopamine in Tris-HCl buffer a layer of an aqueous suspension of α-chitin was deposited on these films by vertical coating. Since dopamine can undergo spontaneous oxidative polymerization [45]; its preliminary treatment with a solution results in the surface modification of DL-PLA film by polydopamine, a hydrophilic polymer. The concentration of nanoparticles in a suspension of chitin was sufficient to form a chiral nematic phase, which upon vertical deposition resulted in orientation of polysaccharide particles on the PLA film surface [46]. The scanning electron microscopy (SEM) study of the film containing 5 wt % of the filler showed that more than 90% of nanoparticles were oriented along the direction of suspension flow. Keeping the composites in phosphate-buffered saline for two weeks caused neither changes in the morphology of films nor a decrease in the weight of the samples. Despite the fact that the elastic modulus (2.5 GPa) and tensile strength (30 MPa) of the material with 5% of chitin exceed those for the pure PLA film and the composite films obtained by mixing PLA with α-chitin, they do not reach values typical of tubular bones. For example, the values of the elastic modulus for the human femoral bone are 17–27 and 6–13 GPa and the tensile strengths are 80–150 and 50–60 MPa in the longitudinal and transverse directions, respectively [47]. At the same time, these materials can serve for the repair of trabecular bones, for which the elastic modulus and ultimate tensile strength are in a range of 0.05–0.5 GPa and 1–20 MPa, respectively [47]. Besides improvement in the cytocompatibility, the developed multilayered materials exhibited higher osteogenic activity compared to the nonfilled film.

The addition of plasticizers is one of the methods to facilitate dispersion of chitin in the PLA matrix. In [48], the plasticizer was triethyl citrate (TEC). Suspensions of α-chitin nanocrystals obtained by acid hydrolysis were mixed with TEC and ethanol (a solvent for TEC). The resulting dispersions were loaded together with PLA into an extruder to obtain composites with 3 wt % of the polysaccharide, from which films were obtained using a hot press. The amount of plasticizer in the composite was 2.5, 5, and 7.5 wt %. Also, pure PLA, a plasticizer-containing PLA, a plasticizer-free composite, and a composite containing 3% of chitin were obtained by extrusion. According to the SEM data, the plasticizer-free composite contains considerable aggregations, the number and size of which considerably decrease upon addition of the plasticizer. IR spectroscopy shows that the band of

Table 1. Mechanical characteristics of composites based on synthetic biodegradable polymers and chitin nanoparticles

Matrix	Filler	*d*, nm	*l*, nm	Type of item	Preparation method	Optimum concentration, wt %	Young modulus, composite/matrix, GPa[a]	Tensile strength, composite/matrix, MPa[a]	Tensile elongation, composite/matrix, %	Ref.
PLA	NC, Ac-α-chitin, H(HCl)	8–17	114–320	Film	Solution casting	4	1.6/1.1	36.3/25.1	6/31	[43]
L-PLA	NC, α-chitin-g-L-PLA, H(HCl)	5–55	150–400	Film	Solution casting	5	1.4/0.6	30.5/18.7	11/6	[30]
L-PLA	NC, α-chitin-g-L-PLA, H(HCl)	5–55	150–400	Nonwoven material	Electrospinning	5	2.0/0.8	4.3/2.6	48/62	[44]
DL-PLA	NC, α-chitin, H(HCl)	40	220	Film	Solution casting, vertical deposition of a chitin suspension onto a film	5	2.552 ± 0.097 0.754 ± 0.053	30.02 ± 1.05 7.18 ± 0.82	12.5/130	[46]
PLA	NC, α-chitin, H(HCl)	11 ± 5	300 ± 150	Film	Extrusion, hot pressing	3, chitin 5, TEC	1.94 ± 0.02 1.86 ± 0.10	54.4 ± 1.7 60.6 ± 0.6	4.6 ± 0.1 7.9 ± 0.4	[48]
PLA	NF, α-chitin	90	20000 (20 μm)	Blade	Extrusion, pressure casting	2, chitin 1, PEG	3.2 ± 0.8 3.5 ± 0.1	52 ± 6 60.4 ± 0.3	10 ± 2 4.1 ± 0.5	[49]
PCL	NC, β-chitin, H(HCl)	18	2200 (2.2 μm)	Film	Solution casting	2.5	590/350 MPa			[69]
				Film	Hot pressing	10	930/320 MPa			
PCL	NC, α-chitin, H(HCl)	20	300	Film	Solution casting	20	0.5/0.2	24/22	550/700	[71]
				Nonwoven material	Electrospinning	25	18/12 MPa	17/4	250/670	
PDO	NC, α-chitin, H(HCl)	20	300	Nonwoven material	Electrospinning	10–15	61/18 MPa	13/5	100/250	[74]
PU (PCL/MDI)	NC, α-chitin, H(HCl)	20 ± 10	250 ± 50	Film	Solution casting	3	6.5/3 MPa	20.8/11.3	575.4/800	[80]
PU (Castor oil/TDI)	NC, Ac-α-chitin, H(HCl)	12.2 ± 3.4	211.6 ± 67.4	Film	Solution casting	6	1.8/0.98 MPa	5.7/2.7	290/200	[82]
STPU (HMDI/PBSD)	NC, α-chitin, H(HCl)	11.1 ± 1.4	176 ± 34	Film	Solution casting	1	288.8 ± 7.6 238.8 ± 6.7 MPa	14.2 ± 1.3 26.5 ± 2.3	420/762	[84]
PU (LDI/PBSD)	NC, α-chitin, H(HCl)	10 ± 1	222 ± 31	Film	Solution casting	3	256 ± 26 135 ± 5 MPa	7.2 ± 0.5 8.1 ± 0.3	249/1018	[85]

NF, nanofibrils; NC, nanocrystals; Ac-α-chitin, acetylated α-chitin particles; H(HCl), acid hydrolysis with hydrochloric acid.

[a] If no other measurement unit is given.

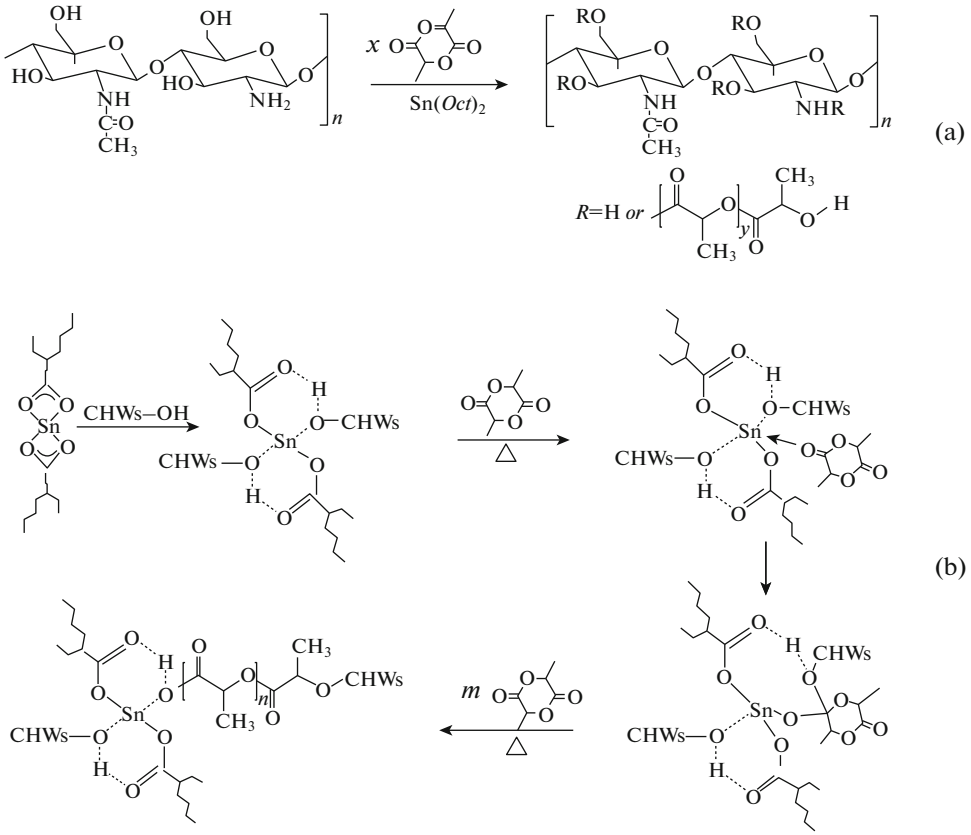

Fig. 2. Scheme for chitin modification (a); mechanism of the ring-opening polymerization of L-lactide in the presence of $Sn(Oct)_2$ catalyst and hydroxyl groups of chitin (b) [30].

O–H stretching vibrations at 3510 cm^{-1} for pure PLA upon addition of chitin and TEC becomes broader and shifts to 3494 cm^{-1}, which suggests hydrogen bonding between the matrix and the filler. The glass transition and melting points decrease upon addition of the plasticizer. The Young modulus of the composites increase compared to pure PLA upon addition of chitin particles both with plasticizer and without it (Table 1). However, the strength with addition of the filler decreases, which can be due to the hydrolysis of PLA upon extrusion and the presence of a slight amount of agglomerations. Comparison with the PLA synthesized with the plasticizer suggests that the addition of chitin increases the strength at the same concentration of TEC.

In [49], polyethyleneglycol (PEG) was used as the plasticizer upon extrusion of composites based on PLA and chitin nanofibrils. First, chitin–PEG mixtures were prepared; they demonstrated the absence of agglomerations upon drying in contrast to aqueous suspensions of the polysaccharide. Also, no agglomerations were observed in extruded samples. After extrusion by pressure casting, plates were obtained. The addition of PEG and polysaccharide has a considerable effect on the color and transparence of the studied materials. It was found that the most transparent and colorless composite was obtained at the lowest content of chitin (2 wt %) and the highest content of PEG (10 wt %). Both a strong decrease in the Young modulus and tensile stress and an increase in the tensile elongation were observed upon plasticization of PLA (up to 180%). The addition of chitin nanofibrils to the already plasticized PLA has no reinforcing effect, despite an improvement in the dispersion of chitin in the matrix. This can be due to the fact that PEG having a high affinity to polysaccharide covers the surface of CNPs and prevents its interaction with PLA. This is confirmed by the fact that the Young modulus (2.9 GPa) of the plasticizer-free composite with 2% of chitin is higher than that of the composites with plasticizer and only the composite with 1% of PEG and 2% of chitin possesses higher modulus (3.2 GPA), i.e., the low amount of the plasticizer is sufficient to provide distribution of the filler, but, at the same time, is not sufficient to prevent the interaction of nanofibrils with the matrix. Note that the composite with higher-molecular-weight PEG demonstrated higher values of the Young modulus and tensile stress.

Also note that PLA in the form of micro- and nanoparticles have been being studied extensively as a drug delivery system [50–52]. To obtain stable particles of PLA suspensions, it is often necessary to use

amphiphilic stabilizers: low-molecular-weight surfactants, polymers, such as PEG, polyvinyl acetate (PVA), and dextrans [52, 53]. In addition, diblock and triblock copolymers of PLA with PEG [54, 55] and dextran [56] are extensively used in the preparation of PLA-based particles. Yet one method for the preparation of an aqueous dispersion of hydrophobic particles using solid particles as a stabilizer is known; such dispersion are referred to as Pickering emulsions. In [57], PLA microparticles (the diameter of the main fraction was 100–200 μm) stabilized with chitin nanocrystals were obtained. More than 50% yield of microparticles was observed upon application of a 1% suspension of polysaccharide particles, while a 2.5% solution of PVA was used to achieve such yield in using this polymer as a stabilizer.

Thus, bone tissue repair is the main application of PLA–chitin composites. Although composite materials based on PLA with chitin can be of great interest as surgical suture materials, research works in this field are relatively few [58]. Among the most common methods for the preparation of composites are electrospinning, extrusion, and preparation of films from a solution.

1.1.2. Polycaprolactone (PCL). Polycaprolactone is in-demand crystalline polyester, which is easy to process, since it is soluble in a wide range of organic solvents, has a low melting point (55–60°C), and a low glass transition point (–60°C). This polymer possesses, on the one hand, a relatively low tensile strength (23 MPa) and, on the other hand, an extremely high tensile elongation (>700%) [59]. PCL degrades quite slowly (2–3 years); therefore, it is used for the development of long-living implants [60]. However, matrices made of pure PCL demonstrate a low adhesion and proliferation of cells [61, 62]. To eliminate these disadvantages, both inorganic fillers, such as bioceramics [63], HAP [64], forsterite [65], and carbon nanotubes [66] and organic fillers, such as surface-modified cellulose nanocrystals [67] and chitosan [68], are added.

In [69], nanocomposite materials based on PCL and β-chitin nanofibrils (l_{av} = 2.2 μm, d_{av} = 18 nm) were described for the first time. Composite films were prepared either by mixing with an aqueous suspension of PCL stabilized by poly(ethelene oxide)–poly(propylene oxide) copolymer and a suspension of chitin followed by water evaporation at 75°C or by hot pressing the mixture of pre-lyophilized suspensions of the polymer and the polysaccharide. The content of the filler varied from 0.5 to 2.5 wt % in the former case and from 0.5 to 10 wt % in the latter case. The mechanical properties of the prepared composites were studied by dynamic mechanical analysis. At a chitin content of 5% and less, both methods for the material preparation are characterized by a decrease in the modulus and destruction of the sample above 50°C due to melting of PCL regardless of the chitin content. However, the modulus of the 10% composite obtained by hot pressing come to plateau near 42 MPa starting from 50 up to 190°C. The transition of the matrix to a viscous-flow state at these temperatures can be explained by the formation of a stable network of the filler. For the films obtained by evaporation, the elastic modulus at room temperature increases from 0.35 GPa for the nonfilled matrix to 0.59 GPa for a 2.5% composite. The elastic modulus of the hot-pressed materials was 0.32 GPa for pure matrix and 0.93 GPa upon 10% filling at room temperature. At identical concentration of chitin (2.5 wt %), the elastic modulus is higher in films obtained by casting. It is likely that the difference in the elasticity moduli emerges initially due to a higher crystallinity of the matrix for the samples obtained by casting.

In [70], lyophilized α-chitin nanocrystals were surface modified through grafting of PCL chains by the ring-opening polymerization of the polymer. After modification, the particle length of the polysaccharide became much shorter (about 100 nm) compared to particles after hydrolysis. Composite plates were obtained by pressure casting. The content of grafted PCL chains was 89.60, 91.20, and 94.78 wt %. With an increase in the portion of the grafted PCL chains, the tensile strength and elongation increase, since intertangling of PCL chains facilitates stress transfer onto the polysaccharide. For example, the tensile strength was 19 MPa for the sample with 89.60% grafting and 29.7 MPa for the sample with 94.78% grafting. Conversely, the Young modulus decreases with an increase in the weight fraction of grafted PCL chains from 400 (89.60 wt %) to 300 MPa (94.78 wt %). Also, the hydrophobicity of the material increases with an increase in the content of PCL.

In [71], 2,2,2-trifluoroethanol (TFE) was used as a solvent for the preparation of PCL-based composite materials. Nanocrystals of α-chitin (l_{av} = 300 nm, d_{av} = 20 nm) obtained by acid hydrolysis and lyophilized were dispersed in TFE using ultrasound (US). Polycaprolactone dissolved in TFE was added to this system, stirred, and subjected to ultrasonic treatment. Films were obtained by casting and matrices with a fiber diameter of 200–400 nm were obtained by electrospinning. The content of the filler in both cases varied from 5 to 30 wt %. According to the SEM data, the filler was distributed homogeneously in the film; however, the IR spectra of the composites display no considerable changes in the characteristic band of the ester group at 1724 cm^{-1} compared to pure PCL films, which suggests a weak adhesion of the polysaccharide and PCL. This can be the reason for the fact that the strength of composite films upon addition of the filler changes insignificantly. For example, the strength is 22 MPa for pure PCL, 18 MPa for the 5% composite, and 25 MPa for the 30% compsite. At the same time, the elastic modulus of the films increases with an increase in the

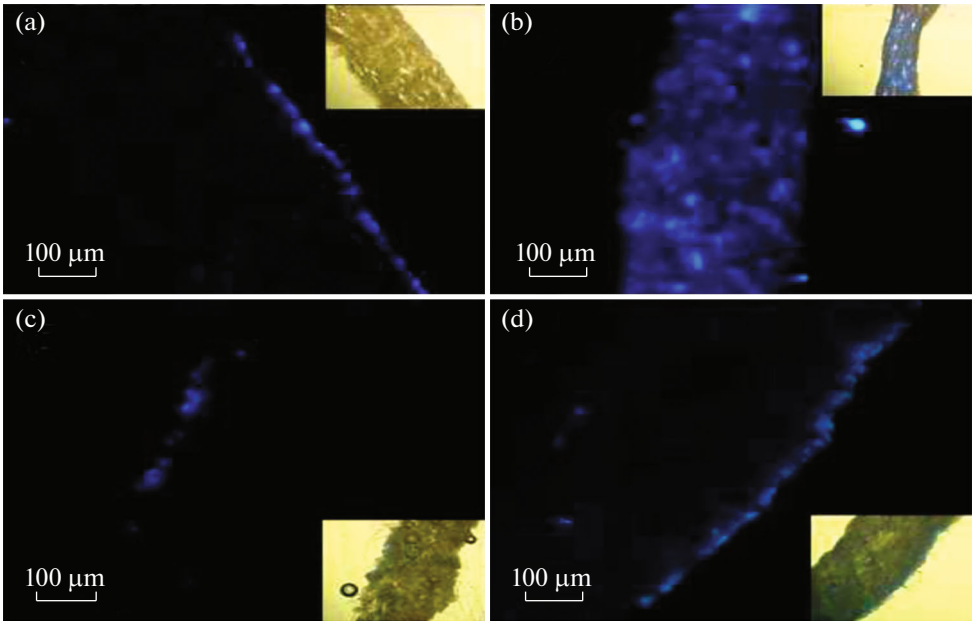

Fig. 3. (Color online) Fluorescence spectroscopy images for cross section of 30% chitin–PCL matrix at first (a) and 14th day (b) of inoculation of dermal fibroblasts; fluorescence spectroscopy images for the cross section of pure PCL matrix at the first (c) and 14th day (d) of inoculation of dermal fibroblasts. Inset: images under natural illumination [64].

amount of chitin compared to pure film (0.2 GPa); the highest elastic modulus was observed for the 20% film (0.5 GPa). The presence of the filler considerably decreases the tensile elongation: 700% for pure film and 400–550% for chitin-filled films. Matrices demonstrate a slightly different behavior. They are characterized by an increase in the strength with an increase in the concenrtration of chitin: 4 MPa for the nonfilled matrix and 18 MPa at 30% of chitin. The addition of the polysaccharide leads to an increase in the elastic modulus and decreases two-fold the tensile elongation. In [72], it was noted in the study of these matrices that the addition of chitin strongly decreases the diameters of matrix fibers. For example, the fiber diameter is ~1 μm at the zero content of chitin and 200–400 nm upon filling. The effect of chitin on the cell proliferation was assessed by the example of cultivation of human dermal fibroblasts on the obtained materials for 14 days. The fluorescence microscopy images of the cross section of the matrices show that cells propagate not only on the surface, but also migrate inwards the matrices at the 14th day. No such infiltration of cells was observed for the pure PCL sample (Fig. 3). Thus, filling of the PCL-based matrices with chitin considerably improves cell infiltration and migration.

1.1.3. Polydioxanone (PDO). Polydioxanone is a colorless crystalline polymer (the crystallinity reaches 55%), which is used since 1980s as a monofibrous suture material. The modulus of pure PDO is 1.5 GPa [60]. Besides suture materials, PDO is applied in orthopedics as a material for fixing screws for small bone and osteochondral fragments [73].

The analysis of literature data showed that, at the present time, the possibilities of preparation of PDO–chitin composites have almost not been studied. For example, we know only one work [74] where nanocomposite PDO–chitin matrices were obtained. For this purpose, α-chitin nanocrystals (l_{av} = 300 nm, d_{av} = 20 nm) obtained by acid hydrolysis and lyophilized after purification were dispersed by ultrasonic treatment in TFE and mixed with polydioxanone dissolved in TFE with the aid of ultrasound. Matrices where the filler content varied from 5 to 20 wt % were obtained by electrospinning. All matrices are characterized by a homogeneous distribution of the filler; with an increase in the polysaccharide loading the resulting fiber became thinner: the filler-free fiber diameter was 500 nm and that at a chitin content of 5–20% was 100–300 nm (Fig. 4).The inclusion of the polysaccharide considerably improved the mechanical properties compared to pure PDO. The modulus of the composite with 15% of chitin is 3.4-fold higher than that of the pure polymer: 61 and 18 MPa, respectively. The highest strength of 13 MPa was achieved at 10% filling and exceeded 2.6-fold the strength of the PDO matrix (Table 1). The tensile elongation decreased to 80–100% compared to the nonfilled matrix (250%). The addition of chitin also favored an increased in the rate of proliferation of RSC96 Schwann cells compared to pure PDO. Preliminary results showed that these matrices can act as materials for neurotization.

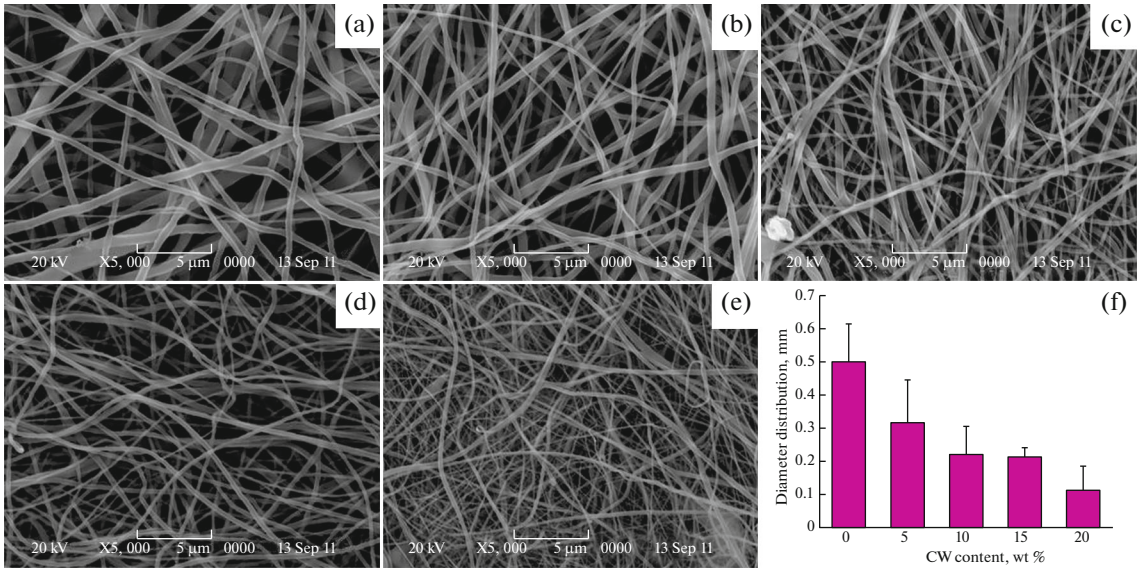

Fig. 4. (Color online) SEM images for PDO–chitin matrices with different chitin contenta: (a) 0, (b) 5, (c) 10, (d) 15, and (e) 20%; mean fiber diameter distribution at different loadings of chitin (f) [74].

1.2. Polyurethanes (PUs)

Polyurethanes constitute a broad class of polymers being promising for medical purposes, in particular, for the development of medical implants, such as heart pacemakers and blood vessel grafts. Polyurethanes are usually obtained by the polycondensation of diisocyanates with bi- and polyfunctional hydroxyl-containing derivatives. Biodegradable PUs are designed using biocompatible aliphatic diisocyanates, including lysine diisocyanate (LDI) and 1,4-diisocyanatobutane (DIB) [60]. The mechanical properties of PUs vary in a wide range, since they depend on such factors as the nature and length of chain segments between urethane groups, crystallinity, and structure (linearity or network). Degradable polyester urethanes were developed by the reaction of LDI with polyester diols or triols based on D,L-lactide, caprolactone, and other copolymers [75]. Aliphatic polyesters in PU form soft segments. The biodegradable elastic polyesterurethane DegraPol is used for the development of high-porosity frameworks for tissue engineering [76].

The application of PU-based biodegradable materials in such fields as orthopedics requires high mechanical properties, which necessitate the use of fillers. To reinforce PUs, nanocrystals or nanofibrils of cellulose [77] and HAP [78, 79] are often used. In recent decade, the number of studies dedicated to the reinforcing properties of CNPs with respect to PUs increased significantly.

In [80], α-chitin nanocrystals (l_{av} = 250 nm, d_{av} = 20 nm) obtained by acid hydrolysis were used to fill PUs based on PCL and methylenediphenyldiisocyanate (MDI). The content of the polysaccharide in casted films was from 1 to 5 wt %. The filled materials demonstrate improved mechanical properties compared to the starting PU film. The addition of 3% of chitin provides the maximum strength of 28.8 MPa and the highest elastic modulus of 9.6 MPa is achieved at 5% filling, which is 1.8- and 3.2-fold higher than that of the PU film, respectively (Table 1). With an increase in the concentration of chitin, the tensile elongation decreases and its values for all composites are lower than that for the pure film. It was shown by IR spectroscopy that the interaction between the matrix and the filler occurs through hydrogen bonding mainly in the amorphous region. Despite the fact that the authors did not propose potential application of the obtained materials, this work is promising in terms of understanding the mechanism of reinforcement of PU matrices using CNPs and can be useful in the design of biomedical materials.

In recent times, there is an increased interest in vegetable oils as renewable natural sources of polyols for the synthesis of PUs [81]. In [82], biocomposite materials based on highly elastic PU, obtained from castor oil and toluylenediisocyanate (TDI), and α-chitin were synthesized. Polysaccharide nanocrystals isolated by acid hydrolysis were surface acetylated using acetic anhydride in the presence of pyridine (Fig. 5a). The average length and diameter of CNPs after modification were 212 and 12 nm, respectively. The preliminary prepared prepolymer from castor oil and TDI (Fig. 5b) was mixed with chitin nanocrystals and 1,4-butanediol in tetrahydrofuran (THF) to obtain composites (Fig. 5c). Hydrophobic acetyl groups at the surface of modified chitin particles favored their homogeneous distribution in THF and, consequently, in the PU films after evaporation of the solvent. The content of the filler in the films varied from 2 to 10 wt %.

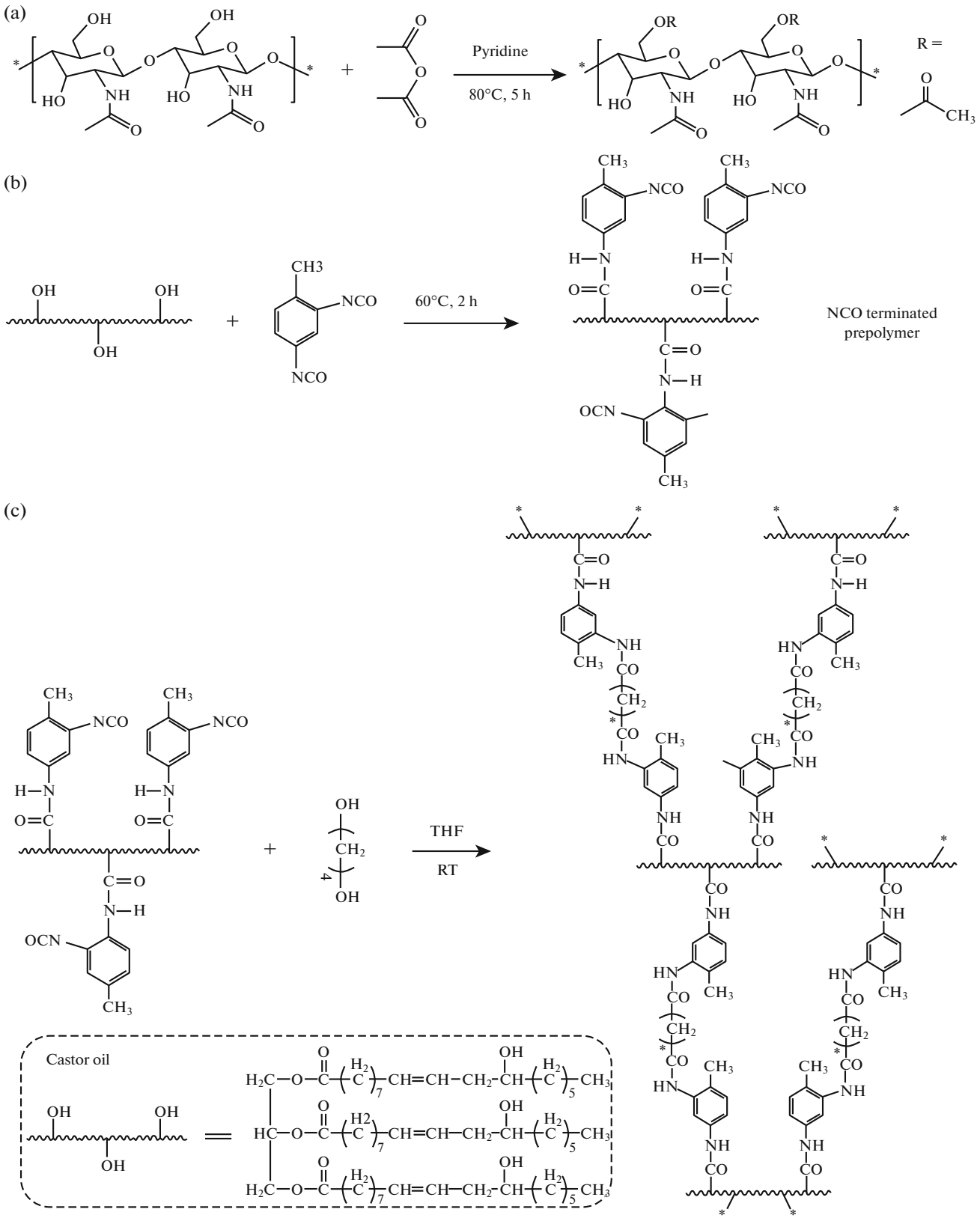

Fig. 5. Schemes for surface acetylation of chitin nanocrystals (a), synthesis of polyurethane prepolymer (b), and synthesis of polyurethane (c); inset: chemical structure of castor oil [82].

The Young modulus of the nanocomposites progressively increases with an increase in the concentration of nanocrystals, in particular, from 0.98 for pure PU to 4.01 MPa for the composite with 10% chitin, the highest strength of 5.6 MPa being achieved at 6% filling (Table 1). The relative tensile elongation with an increase in the content of the filler behaves nonuniformly: for composites with 2 and 4% of chitin its value increases compared to the nonfilled film, but this index decreases at a loading of 6% and more. The increase in mechanical properties is caused first of all by the formation of rigid three-dimensional network between acetylated chitin nanocrystals after reaching the percolation threshold (5.7%) due to residual hydroxyl groups. However, at a higher concentration of the filler chitin particles can undergo aggregation to result in a decrease in the strength, which is seen by the example of 10% composite. In this case, the modulus continues to grow due to the formation of a rigid network.

In [83], it was studied how ultrasonic treatment influences the preparation of composite films based on PU, synthesized from poly(ethylene glycol) adipate and TDI, and α-chitin nanocrystals (l = 100–650 nm, d = 10–70 nm) obtained by acid hydrolysis. During the work, two series of films were obtained by evaporation: the mixture of chitin and PU dispersions was exposed to ultrasound in one case and not exposed in another case. The content of the polysaccharide in the solid phase was 5, 10, 20, and 30 wt %. The SEM images show that a more homogeneous distribution of the filler in the matrix can be achieved using ultrasonic treatment. The strength of composites of both series exceeded that of the nonfilled film; a higher concentration of chitin provided a higher strength. However, the strength of the ultrasound-treated composites was higher than that of the nontreated ones: at 30% filling it was 12.2 and 6.83 MPa, respectively. Upon addition of the filler, the tensile elongation successively decreases. For example, it is 1200% for the pure film and about 200 at 30% loading of the polysaccharide in both series.

Segmented thermoplastic polyurethanes (STPUs) relates to shape-memory polymers. These cross-linked crystalline polymers consist of soft (usually formed by polyester or macrodiol) and rigid segments resulted from the reaction of diisocyanate with low-molecular-weight diol. Due to the thermodynamic incompatibility between soft and rigid segments, these PUs segregate into phases and are good candidates for shape memory materials. The soft segment acts as a switching segment (responsible for the fixed form), which provide glass transition and melting points to be in appropriate temperature ranges. The rigid segments act as network nodes and provide elasticity required for the shape recovery. Shape memory polyurethanes usually exhibit high compatibility, which causes a considerable interest in them due to biomedical applicability [81].

In [84], α-chitin nanocrystals (l_{av} = 176 nm, d_{av} = 11 nm) obtained by acid hydrolysis were used to reinforce STPU with a rigid segment based on 1,6-hexamethylenediisocyanate (HMDI) and a soft segment of polybutylenesebacatediol (PBSD) from castor oil. The content of the filler in the obtained films varied from 0.25 to 2 wt %. Chitin nanocrystals, acting as a nucleating agent for the solid phase, increased its crystallinity and, consequently, the rigidity of the material thereby increasing the Young modulus. On the other hand, the nucleation effect restricted orientation of the soft amorphous segment and crystallization under strain thereby decreasing the tensile strength. Upon addition of chitin, the tensile elongation decreases from 762% typical of the nonfilled matrix to 400–460% at a filler content of 1% and less; however, further increase in the concentration of chitin to 2% results in a dramatic decrease in the elongation to 68% (Table 1). The shape of pure PU film after thermomechanical deformation recovered by 52%. Upon addition of chitin, which favored an increase in the physical cross links between rigid domains, 1% composites restored their shape up to 70% in the first thermomechanical cycle and up to 90% in the second cycle. The filler has no any effect on the shape stability, which is due to the fact that polysaccharide has almost no effect on the soft segment of the polymer. Incubation of L-929 murine fibroblasts on the film samples of pure PU and 1% composite at 37°C demonstrated the cytocompatibility of both materials, the cell count on the composite film after the first day being higher than that on the nonfilled polymer.

In [85], STPU bionanocomposites filled with α-chitin nanocrystals (l_{av} = 220 nm, d_{av} = 10 nm), isolated by acid hydrolysis, were synthesized. PU obtained based on LDI and castor oil PBSD was dissolved in THF. Chitin predispersed in THF was added to a solution and the final mixture was subjected to ultrasonic treatment. The content of the filler in the films after solvent evaporation was 1, 3, and 5 wt %. As in the previous case, the chitin particles acted as nucleating agents, due to which the crystallinity and, correspondingly, rigidity of the samples increased with an increase in the concentration of the filler. The tensile strength and elongation of the composite materials decreased compared to the nonfilled material. The pure PU film restored its shape by 82.3%. This value increased to 83.6% upon addition of 1% of chitin and to 85.5% upon addition of 3% of the polysaccharide in the first termomechanical cycle. This is due to the fact that, as noted above, the initial shape restores through a crystalline structure. Note that the composites restored their shape in both dry and wet states within approximately identical time. In the first cycle, the shape stability of the 3% composite (96.6%) is higher than that of the 1% material (94.3%) and pure PU (94.7%); however, in further cycles the effect of the filler on the shape stability was not observed. The composite material with a filler content of 3% is cyto-

compatible, which was shown by the example of L-929 murine fibroblasts, and differs in the hemocompatibility.

The literature analysis showed that the possibilities of preparation of biomedical composites based on PU and chitin are studied quite poorly; in particular, we could not find any work on the design of matrices from these materials by electrospinning. At the same time, PU–chitin composite films are promising for the design of wound coatings and STPU composites can be applied for the fabrication of blood vessel stents and cava filters.

Summarizing the chapter on composites based on CNPs and synthetic polymer matrices, we can remark the following. α-Chitin nanocrystals obtained by acid hydrolysis in hydrochloric acid are the most common filler for composites (Table 1). α-Chitin nanofibrils were used only in one work and β-chitin nanocrystals were used in another work. Some researchers apply surface modification of the polysaccharide particles or use low- or high-molecular-weight plasticizers in order to improve the filler distribution in the hydrophobic polymer matrix. Using chemical modification of the nanocrystal surface, a higher reinforcing effect of the filler on the matrix could be achieved; however, the effect of plasticizer is not so unambiguous, since the reinforcing effect and improvement in the filler distribution are neutralized by the effect of plasticizer itself on the mechanical properties of the composite. Nevertheless, an increase in the elastic modulus was also observed in the most of works where chitin nanocrystals without preliminary modification were used. Note that the optimum concentration of the filler in the most of work is less than 10 wt %, which is due to both difficulties in the preparation of higher-filled materials and a negative effect of aggregation of CNPs. All researchers that studied the biological properties of composites based on chitin and synthetic polymers note that the addition of polysaccharide particles into the composition improves the adhesion and propagation, and increases the rate of proliferation of different cells of mammal tissues.

Note that, in many works, upon preparation of materials based on chitin and biodegradable synthetic polymers chitin was dissolved in cosolvents for both components (ionic liquids, hexafluoroisopropanol) or solvents for this polysaccharide (methanol/$CaCl_2$). Thus, materials based on chitin and PLA [86–89], PCL [90], polyglycolide [91], PLA-co-glycolide [92–94], and polybutylene succinate [95] were obtained.

2. COMPOSITES BASED ON SEMISYNTHETIC BIODEGRADABLE POLYMERS

Semisynthetic biodegradable polymers may include polyhydroxyalkanoates (polyesters), since despite the bacterial biosynthesis of polymers their chemical structure strongly depends on the composition of nutrient medium. Supplementing the medium with metabolic precursors of required monomers, one can control the structure and, consequently, properties of the resulting polyhydroxyalkanoate. The variety of monomer units is great and includes residues of 3-hydroxy acids: saturated and nonsaturated, linear and branched ones, as well as acids with different functional groups. The number of carbon atoms in the monomer unit varies from 3 to 18 [96]. Nevertheless, poly(3-hydroxybutyrate) (P3HB) and its copolymer with poly(3-hydroxyvalerate) (P3HB-P3HV) [97] are the most common polyalkanoates. However, P3HB due to its high melting point (173–180°C) possesses a narrow temperature processing window; in addition, it is fragile due to the formation of large-size spheroliths upon crystallization [98]. These disadvantages can be overcome using P3HB copolymers, such as P3HB-P3HV [98].

Among weak points of P3HB-P3HB are insufficient tensile strength and Young modulus, which is mainly due to a slower rate of nucleation and crystallization of the polymer compared to pure P3HB and, consequently, a lower crystallinity [97]. The addition of finely divided filler as additional sites of crystallization is one of the ways to eliminate this drawback and, at the same time, to offer new properties. However, the addition of nonmodified α-chitin nanocrystals and modified grafted oligomeric chains of P3HB-P3HV conversely caused difficulty in crystallization, which was manifested in a decrease in the crystallization temperature of the material and emergence of a low-temperature melting peak of polymer [99]. This feature is likely due to hydrogen bonds emerging between the free hydroxyl groups of α-chitin and the carbonyl groups of P3HB-P3HV restricting migration of polyester chains required for crystallization [99]. Nevertheless, crystallization was accelerated by the addition of α-chitin nanocrystals where the hydroxyl groups of the polysaccharide have been acetylated, this effect being more evident in using the filler with a high degree of substitution [100]. The addition of both unmodified and acetylated α-chitin nanocrystals improved the mechanical properties of the material (tensile strength and Young modules) compared to pure P3HB-P3HV (Table 2). The reinforcing effect of the modified nanocrystals is more pronounced: e.g., at a content of 5 wt % the Young modulus is 2000 MPa for composite films with modified particles, 1850 MPa for composite films with unmodified particles, and 1200 MPa for pure P3HB-P3HV; the tensile strength is 26, 22, and 18 MPa, respectively [100]. Acetylated α-chitin nanocrystals were used to prepare a 3D matrix of P3HB-P3HV/α-chitin by combination of thermally-induced phase separation and salting-out (washout of salt particles after stabilization of the matrix) [101]. This combination of methods made it possible to obtain a material with micro- (~10 μm) and macropores (100–300 μm) (Figs. 6a, 6b), respectively. The addition of polysaccharide nanocrystals

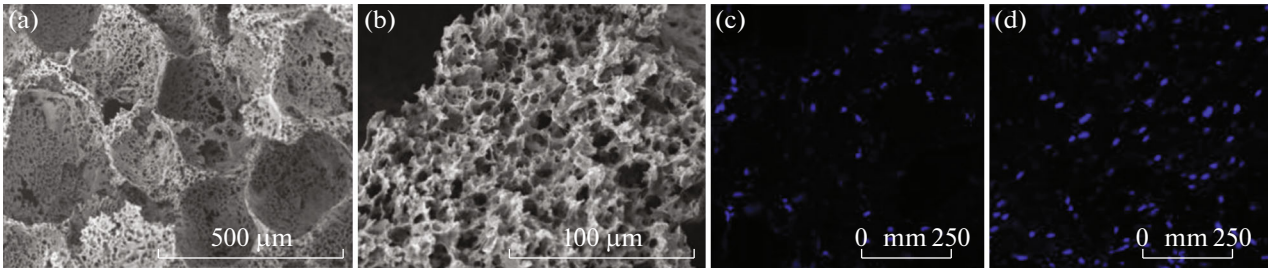

Fig. 6. (Color online) SEM images (a, b) for 3D P3HB-P3HV/α-chitin matrix with polysaccharide content of 10%; confocal microscopy of human adispose tissue stem cells after 7 days of culturing on the pure P3HB-P3HV matrix (c) and P3HB-P3HV/α-chitin composite matrix (d) [101].

improved the mechanical characteristics of the material under compression (the compression modulus was 7 and 5.5 MPa for the composite and pure P3HB-P3HV, respectively), as well as increased the adhesion of stem cells of human fat tissue compared to pure P3HB-P3HV (Figs. 6c, 6d) [101].

In [102], α-chitin nanocrysatls ($l = 210 \pm 25$ nm, $d = 18 \pm 4$ nm) were shown to have a reinforcing effect on the mixture of PLA/P3HB-P3HV polymers (the ratio was 85/15). The composite was obtained by melt mixing; polysaccharide nanoparticles were added to the melt as a 20% suspension in *N*,*N*-dimethylacetamide and, to improve the compatibility of the polymer matrix and the filler, the former was modified by maleic anhydride. The polymer mixture of PLA/P3HB-P3HV was chosen as the composite matrix, since so high addition of P3HB-P3HV makes it possible to obtain a homogeneous porous structure in the material on exposure to CO_2 under increased pressure (4.14 MPa). In such a way, composite materials with a filler content of 0.5–5 wt % were obtained. The addition of α-chitin nanocrystals in an amount of ≤2% decreased the density of the material from 0.75 g/cm^3 typical of the filler-free polymer mixture to 0.4–0.5 g/cm^3 for the composite. Together with almost two-fold decrease in the density, the specific tensile strength of the composite increased compared to the nonfilled PLA/P3HB-P3HV (from 20 to 37 MPa g^{-1} cm^{-3}). Note that the pore size of this material is on the average 1.5 μm, which is not sufficient for the growth of mammal cells [61].

3. COMPOSITES BASED ON NATURAL POLYMERS

Natural polymers possess a number of advantages, which make them attractive for the preparation of medical materials on their basis. For example, all natural polymers are biodegradable, many of them are widespread and biocompatible after appropriate purification, and, in addition, they possess a great number and variety of functional groups [103, 104]. The drawbacks of these polymers pose certain restrictions on the methods for preparation of materials on their basis and their subsequent application. For example, natural polymers are not intended for operation at high temperatures and, therefore, often can be processed only in a narrow temperature range [103]. In addition, the most of crystalline natural polysaccharides decompose at temperatures below the melting point [105]. Due to these limitations, methods for the preparation of composites based on natural polymers and CNPs, where solutions and suspensions of polymers are used as the basis, come to the fore. Melt techniques for the preparation of composites from natural polysaccharides can be applied only in the cases where efficient methods of plasticization are developed, e.g., in using as the polymer matrix of thermally plasticized starch [106].

3.1. Natural Protein Polymers

3.1.1. Collagen. Collagen is one of the most commonly used materials in tissue engineering, regenerative medicine, cosmetology, dentistry, and other medical fields [9, 107–110]. It is a protein which forms hierarchically structured triple helices and a key component of the extracellular matrix of different vertebral tissues [111]. Medical-use collagen is usually applied as xenografts (sections of animal decellularized tissues) extracted from cattle and pigs; they are nonotoxic and biocompatible and rapidly decompose in a human body [9]. However, despite good biological properties of pure collagen, its mechanical properties and structural stability are not sufficient for the adequate substitution of human tissue fragment. Physical and chemical treatment, and intermolecular cross linking lead to a change in the properties of matrix [9]. To improve the mechanical characteristics of collagen-based matrices, synthetic and natural polymers, such as PLA, poly-ε-caprolactone, chitosan, and cellulose, are added to their composition [112–114]. Chitin also can act as a reinforcing filler in collagen-based matrices.

For example, a composite material with a fiber diameter of 35–100 μm was obtained by wet spinning of the suspension containing collagen and α-chitin fibrils; the content of the polysaccharide varied from

Table 2. Mechanical characteristics of composites based on semisynthetic and natural biodegradable polymers and chitin nanoparticles

Matrix	Filler	*d*, nm	*l*, nm	Item type	Preparation method	Optimum concentration, wt %	Young modulus, composite/matrix, GPa[a]	Tensile strength, composite/matrix, MPa[a]	Tensile elonga-tion, composite/matrix, %	Ref.
P3HB-P3HV	NC, Ac-α-chitin, H(HCl)+Ac	20–40	150–400	Film, porous matrix	Solution casting; thermally induced phase separation and salting out	5	2.0/1.2 (film)	26/18		[100, 101]
PLA/P3HB-P3HV (85/15)	NC, α-chitin, H(HCl)	18 ± 4	210 ± 25	Porous matrix	Extrusion with addition of a suspension, CO_2 at 4.14 MPa	0.5–2	650/550 MPa	37/20 MPa/(g cm^3)	15/60	[102]
Collagen	NF, α-chitin, DA	10–20	200–1000	Fibers	Wet spinning	15–20	12.1/5.8 (dry) 1.0/0.1 (wet)	500/310 (dry) 47.3/1.9 (wet)	13/17(dry) 9/9 (wet)	[115]
Fibroin	NC, α-chitin, H(HCl)	8–74	180–820	Porous matrix	Freeze drying	50	3335/443.5 kPa cm^3 g^{-1}			[127]
Chitosan	NC, α-chitin, H(HCl)	6–8	100–200	Hydrogel	Covalent cross linking of a suspension	13.3	169/2.5 kPa	0.135/0.0032		[140]
HBC	NC, α-chitin, H(H_2SO_4), CMC nanoparticles	17 ± 3	130	Hydrogel	Physical cross linking of a suspension	0.5				[141]
CMC/DDA	NC, α-chitin, H(H_2SO_4)	15	150–250	Hydrogel	Covalent cross linking of matrix components	0.5	67/43 kPa			[142]
Chitosan/PEO (1/1)	NC, α-chitin, H(HCl)	10–30	400	Nonwovern material	Electrospinning	50	4.3/0.4	34.9/4.6	0.9/1.3	[144]
Chitosan	NF, α-chitin	20	600–800	Fibers	Wet spinning	0.1–0.3	1.2/0.75	290/220		[139]
Chitosan/PEO (9:1)	NF, α-chitin	20	600–800	Nonwoven material	Electrospinning	20				[146]
Chitosan/PVA/HAP	NC, α-chitin, H(HCl)	35	500	Nonwoven material	Electrospinning	17	6/2 МПа (without GA) (wet) 9/2 MPa (with GA) (wet)	1.3/0.8 (without GA) (wet) 1.5/1.7 (with GA) (wet)		[147]
Chitosan	NC, α-chitin, H(HCl)	5–70	150–800	Film	Solution casting	2.96		83.8 ± 2.9 64.9 ± 0.7	8/12	[148]
Chitosan	NF, α-chitin, DA	6.2 ± 1.1	250 ± 140	Film	Solution casting	90	9/2.5	150/40		[150]
Chitosan	NC, α-chitin, H(HCl)	20	300	Film	Solution casting	3	1.3/1.0	110/40	12/8	[151]
Chitosan	NF, α-chitin, M	10	1000	Film	Solution casting	70	5.4 ± 0.7/2.2 ± 0.2	141 ± 3/52 ± 5	8/42	[152]
Chitosan	NC, NF, α-chitin, H(HCl), M	30 (NC) 85 (NF)	200 (NC) 5000 (NF)	Film	Solution casting	67	4/2 (NC) 4/2 (NF)	40/40 (NC) 100/40 (NF)		[153]
Chitosan	NC, α-chitin, H(HCl)	24 ± 11	540 ± 250	Film	Solution casting	43	5.3 ± 1.3/2.01 ± 0.36			[154]

Table 2. (Contd.)

Matrix	Filler	*d*, nm	*l*, nm	Item type	Preparation method	Optimum concentration, wt %	Young modulus, composite/matrix, GPa[a]	Tensile strength, composite/matrix, MPa[a]	Tensile elonga-tion, composite/matrix, %	Ref.
Chitosan	NF, α-chitin	20	600–800	Film	Solution casting	1–5	5.1/4.1	150/130	34–38/27	[155]
Chitosan	NC, α-chitin, H(HCl)	15	200–500	Porous matrix	Freeze drying	67	747/77 kPa (dry) 40/3 kPa (wet)			[162]
Chitosan	NC, NF, α-chitin, H(HCl), M	30 (NC) 85 (NF)	200 (NC) 5000 (NF)	Film, porous matrix	Solution casting, freeze drying, covalent cross linking of a suspension	67	5/2.5 (NC) (film) 4/2.5 (NF) (film) 1.00/0.15 MPa (NC) (matrix) 0.95/0.15 MPa (NF) (matrix)			[157]
Chitosan	NC, α-chitin, H(HCl)	14	200	Injection hydrogel	Thermally induced cross linking with glycerophosphate	5% (71% with regard to chitosan)		4/1.3	250/160	[143]
CMC/glycerol (70/30)	NC, α-chitin, APS	15	400–500	Film	Solution casting	10	2.7/0.8	75.9 ± 2.6 40.3 ± 2.4	13.6/38.9	[172]
CMC	NC, α-chitin, IL	20–60	100–400	Film	Sorption of HA on the film surface	26.5 μg/cm^2		24.2/7.1	7.6/2.6	[175]
Cellulose NF	NC, α-chitin, H(HCl)	60	300	Film	Hot pressing	0	–/2.73	–/57	–/7.5	[178]
Ca alginate	NC, α-chitin, H(HCl)	46	343	Fibers	Wet spinning	0.15		10.5/9 cN/fiber	27/18	[191]
Ca alginate	NC, α-chitin, H(H_2SO_4)	20	200–500	Hydrogel	Ionic cross linking	50	13.7/5.1 MPa	3.18/1.39	80.3/75.6	[182]
Starch/glycerol (77/23)	NSph, α-chitin, H(HCl)	50–100	50–100	Film	Solution casting	3.85		7.79/2.84 (50% *RH*)	20/60 (50% *RH*)	[183]
Starch/glycerol (60/40)	NC, α-chitin, H(HCl) NF, α-chitin, M	60 (NC) 90 (NF)	300 (NC) 5000 (NF)	Blade	Extrusion, melt pressing	12 (NC) 12 (NF)	390/85 MPa (NCs) 520/85 MPa (NFs) (50% *RH*)	10.8/4.4 (NC) 15.0/4.4 (NF) (50% *RH*)	12/85 (NC) 8/85 (NF) (50% *RH*)	[185]
Starch/glycerol (70/30)	NC, α-chitin, H(HCl) NF, α-chitin, M	60 (NC) 90 (NF)	300 (NC) 8000–10000 (NF)	Film	Solution casting	14 (NC) 14 (NF)	75/10 MPa (NC) 425/10 MPa (NF) (50% *RH*)	3/1.5 (NC) 11/1.5 (NF) (50% *RH*)	20/75 (NC) 5/75 (NF) (50% *RH*)	[128]
Starch/glycerol (70/30)	NC, α-chitin, H(H_2SO_4)	10–50	100–400	Film	Solution casting	0.7		3.7/1.6 (53% *RH*)	75/75 (53% *RH*)	[186]

NF, nanofibrils; NC, nanocrystals; NSph, nanospheres; Ac-α-chitin, acetylated α-chitin particles; H(HCl), acid hydrolysis with hydrochlorid acid; H(H_2SO_4), acid hydrolysis with sulfuric acid; Ac, acetylation; DA, partial deacetylation; TEMPO, TEMPO oxidation; APS, ammonium persulfate oxidation; M, mechanical dispersion methods (grinder, ultrasound, microfluid methods, etc.); IL, preparation of nanocrystals by precipitation into methanol from a solution of chitin in an ionic liquid.

[a] If no other measurement unit is given.

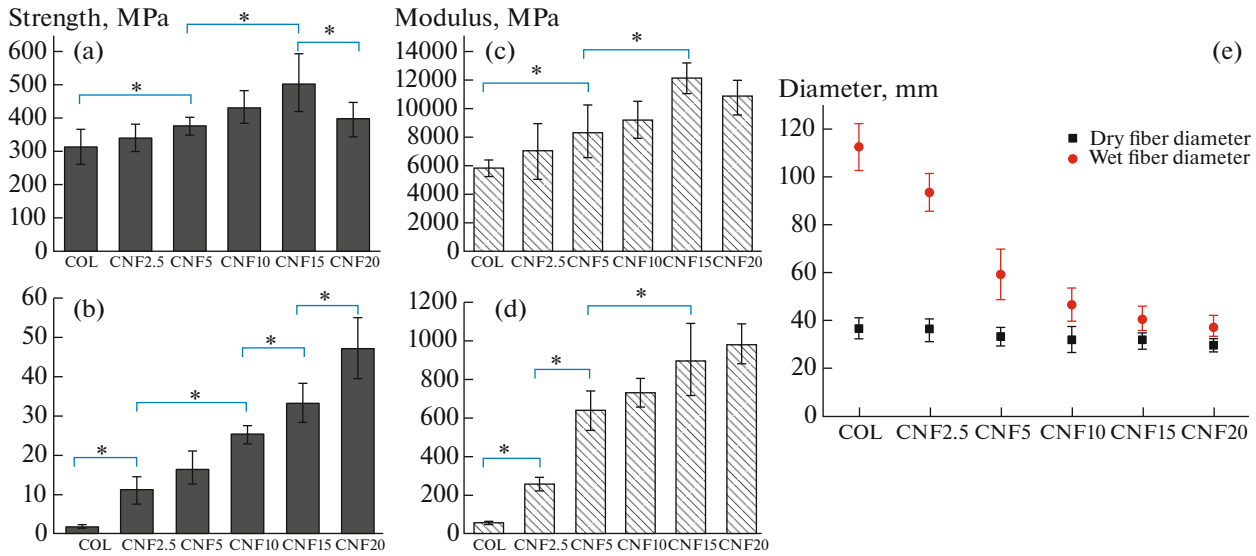

Fig. 7. (Color online) Mechanical properties of pure collagen (COL) and composites filled with α-chitin fibrils (CNFNo., where No. is content of fibrils in composite, wt %): tensile strength of composite fibers in dry state (a) and after holding for 90 min in phosphate buffer (b); Young'a modulus in dry state (c) and after holding in phosphate buffer (d). Fiber diameter of materials in dry state and after 90 min holding in phosphate buffer (e) [115].

2.5 to 20 wt % [115]. For this composite, α-chitin fibrils with a diameter of 10–20 nm and a length of 200–1000 nm were obtained by partial deacetylation [116]. The feature of this method was that the precipitating agent for collagen was a 1% solution of sodium alginate, which enabled mild preparation of fibers without a damage of the collagen triple helix [115]. The addition of chitin nanofibrils favored a considerable improvement in the mechanical properties of the composite relative to pure collagen in both dry and wet states, as well as a decrease in the degree of swelling in phosphate buffer (Fig. 7). The maximum reinforcing effect of polysaccharide particles in a dry state was observed at their content of 15 wt %. But the most pronounced reinforcing effect of chitin nanofibrils was observed after keeping the fibers with 20 wt % filling for 90 min in phosphate buffer. For example, the tensile strength of the filler-free fiber in a wet state was 1.9 MPa and the modulus was less than 100 MPa, while the tensile strength of the composite was 47.3 MPa and the modulus was 1000 MPa. Note that the diameter of the pure collagen fiber increased three-fold upon swelling, whereas composites with a chitin content of ≥10 wt % swelled insignificantly. The cytotoxicity with regard to fibroblasts was estimated by MTT assay. The cell viability on the composite material differed insignificantly from that on pure collagen.

There are also works where the main material of composite is chitin and collagen covers the chitin framework to increase the biocompatibility of the material and to favor an improvement in the proliferation and differentiation of cultured cells. For example, it was shown in [117] that commercially available chitin membrane (Hunan Yinghua Biomedical Co.), which is a nonwoven material obtained by electrospinning (the fiber diameter was 10–15 μm), became a comfortable medium for culturing of murine epidermal stem cells upon treatment with a solution of collagen. On the basis of this material, a matrix including cell components of skin was designed for more efficient healing of mice wounds. Note that the matrix based on collagen-covered chitin also accelerated wound healing compared to the control group, where injuries were treated with pure collagen.

3.1.2. Silk fibroin. Silk fibroin is a protein extracted from *Bombyx mori* butterfly kells and some spiders, which has long been used as a suture material. However, its application with detailed characterization can be associated with adverse immune response, which is attributed first of all to insufficient purification from sericin and silk industrial wastes [118, 119]. After corresponding purification, fibroin demonstrate the biocompatibility level comparable with that of PLA and collagen [120]. Mesenchymal stem cells [120], endothelial cells [121], keratinocytes, fibroblasts [124, 125], and osteoblasts [126] are cultured with success on fibroin-based materials, which make them promising for tissue engineering. Silk fibers also feature high tensile and impact strengths in combination with elasticity [118]. However, upon processing fibroin losses its supramolecular packing and, in attempting to restore the β-fold structure by a mild method, such as solvent annealing, the material undergoes shrinkage. This phenomenon is especially typical of high-porosity materials, such as sponges [127]. The addition of reinforcing fillers can influence this effect.

In [127], a material based on α-chitin nanocrystals and silk fibroin was obtained by freeze drying. An aqueous solution of fibroin obtained by dialysis from a solution of fibroin in an ethanol/$CaCl_2$ mixture was mixed with a suspension of nanocrystals, frozen, and lyophilized. The content of chitin in the sponge varied from 11 to 50 wt %. Then, to restore the structure of fibroin the sponges were placed in a 90% solution of methanol and, after washing in water, lyophilized again. The addition of chitin nanocrystals deceased the shrinkage of the material upon solvent annealing; e.g., the volume of pure fibroin sponge after treatment in methanol decreased by 37%, while that of sponges filled with 20–50 wt % of the polysaccharide decreased by less than 10%. The study of mechanical properties of the material under compression showed that, upon normalizing of the modulus to the density of the material, the modulus monotonically increases with an increase in the filling; the specific modulus of the composite filled with 50% of nanocrystals exceeds 7.5-fold that of the nonfilled matrix. The study of biological properties of the matrices showed that, upon culturing of L929 murine fibroblasts, the level of adhesion of fibroblasts on the composite sponges does not differ from that on the material made of pure silk fibroin. However, 24 h after culturing on the sponge filled with 50 wt % of chitin, the portion of fibroblasts having typical flattened and spread-eagled shape was 61%, while that on the pure fibroin material was only 31%. These data show that chitin nanocrystals as a part of silk fibroin sponges favor propagation of fibroblasts over the material.

3.2. Natural Polysaccharides

Among advantages of application of polysaccharides as a matrix for chitin particles-filled composites is their structural similarity, which can provide a strong interaction between composite components and, correspondingly, a considerably reinforcing effect of nanocrystals and/or nanofibrils [128]. In addition, natural polysaccharides hold a leading position in terms of availability; e.g., cellulose, chitin, and starch are the most common polymers occurring in nature [104]. For this reason, a great number of works, as it will be noted below, are dedicated to composites based on polysaccharides filled with chitin nanocrystals and nanofibrils.

3.2.1. Chitosan and its derivatives. Chitosan is the *N*-deacetylated derivative of chitin used in different areas of medicine, such as bone tissue repair, dentistry [129–131], drug delivery systems [132, 133], nerve tissue remodeling [134, 135], therapy of wounds and burn injuries, and tissue engineering [136–138]. A great interest in chitosan is due to the fact that it combines such properties as biodegradability, biocompatibility, nontoxicity, and antibacterial activity [131]. However, due to the hydrophilic nature chitosan possesses insufficient mechanical properties in a wet state and, for this reason, chitosan is applied often using chemical cross linking agents or mixing with other polymers or reinforcing filler [10, 139].

Composite materials based on chitosan and CNPs can be obtained in the form of hydrogels [140–143], fibrous materials [139, 144–147], films [148–157], porous matrices [157–162], and microparticles [163–165]. Composites are obtained using aqueous solutions and suspensions: films are obtained by casting [148–157]; fibrous materials are obtained by electrospinning from a solution [144–147] or wet spinning of a suspension into a solution of precipitating agent [139]; porous matrices are obtained by freeze drying of a prefrozen suspension [157–162]. To obtain stable composite hydrogels, physical and covalent entanglement nodes should be created. Covalent nodes in hydrogels based on chitosan and CNPs are formed using different cross-linking agents: hexamethylene-1,6-diaminocarboxysulfonate [140] and dextran dialdehyde (DDA) [142]. Covalent cross linking is also used to stabilize the structure and/or to limit the degree of swelling of composite films (glutaraldehyde (GA) [149] and genipin [159–161]), porous matrices (genipin [157, 159–161]), nonwoven material (GA [147]), and microparticles (GA [163, 164]) based on chitosan and chitin. The most of cross linking agents use free amino groups for cross linking and glutaraldehyde additional uses imide and hydroxyl groups; therefore, covalent bonds are formed both between the molecules of chitosan and between chitosan and particles of chitin [140, 149, 157]. The formation of physical entanglement nodes is applied upon preparation of thermally-induced composite hydrogels. For this purpose, glycerophosphate [143] is added or hydroxybutylchitosan (HBC), the chitosan derivative capable of reversible sol-gel transition upon temperature increase to physiological values, is used as the matrix [141]. The addition of CNPs to the composition of thermally induced hydrogels can increase the rate [143], decrease the gelation temperature, and favor retention of the integrity of hydrogel-based wound coating [141]. The aqueous stability of materials obtained from chitin and chitosan also can be increased through high-temperature treatment of the composite with aqueous vapor, which decreases the equilibrium degree of swelling of the material and the portion of the outwashed polymer upon keeping in water [148].

One or more other components are often added to chitin and chitosan. For example, fiber-forming polymers, such as PEG [144–146] and PVA [147], are added upon preparation of chitin/chitosan composites by electrospinning. In the most of works on the preparation of chitin- and chitosan-based composites by electrospinning, high concentrations of fiber-forming additives with regard to chitosan, 50 wt % of PEG [144, 145] and 30 wt % of PVA [147], were used. High-molecular-weight additives of PEG and PVA, despite their nontoxicity, do not degrade in a body [166, 167],

which is a limitation in the case of biomedical materials. In [146], the mixture composition was optimized to prepare a composite nonwoven material based on chitosan and α-chitin nanofibrils with addition of PEG. The authors found that, even a small amount of chitin nanofibrils (about 1% with respect to chitosan) considerably decreases the surface tension of a suspension [146], which is important for the preparation of material by electrospinning. In addition, at a concentration of about 20% with respect to chitosan (~1 wt % in a suspension), clusters of nanofibrils are produced in a suspension, which are preserved and oriented upon electrospinning. This results in strong differences in the mechanical properties of the jet in the longitudinal and transverse directions and favors the separation of jet into microjets and the formation of almost defectless nonwoven material with slight addition of PEG (10 wt % with respect to chitosan). Nevertheless note that wet spinning makes it possible to prepare fibers consisting of only chitosan and chitin nanofibrils [139]. Also, different functional additives, epithelial cell growth factor, linezolid antibiotic [141], HAP [159], gelatin [161] are added to the composition of materials based on chitosan and CNPs. Composite microparticles were loaded with cisplatin [163] and methothrexate [164]. Both works on the preparation of microparticles note that, with an increase in the content of chitin in the microparticle, the drug loading also increases, which reaches 180 (mg of Pt)/mg for cisplatin [163] and more than 50 wt % for methothrexate [164]. The addition of chitin to microparticles retarded the release of cisplatin and also suppressed the initial explosive increase in the concentration of drug substance [163].

Since CNPs are anisometric and possess high elastic modulus, they can have a reinforcing effect, which was studied in many works dedicated to chitosan/chitin composites, where chitin nanocrystals and nanofibrils increase the Young modulus and/or tensile strength of the material. In the most of works, these parameters increase monotonically with an increase in the concentration of the filler in the studied range [140, 142, 150, 152, 153, 155, 157, 162]. This effect was shown in highly filled materials where the concentration of CNPs reaches 67 [153], 70 [152], and even 90 wt % [150]. However, in some works the modulus and/or tensile strength passes through maximum at a relatively low content of chitin: 0.1–0.3 [139], 2.96 [148], 3 [151], and 1–5 wt % [155]. Let us note the work [154] where the Young modulus decrease upon an increase in the concentration of chitin nanocrystals in the composite from 43 to 75 wt %. In addition, the effect of the filler on the relative tensile elongation is ambiguous: in some works the relative tensile elongation of the composite decreases with an increase in the concentration of chitin therein [144, 148, 152]; however, some researchers note an increase in the strain of the material at the optimum content of the filler in the composite [143, 151, 155]. Note that only composites with a low content of CNPs (≤5 wt %) are characterized by an increase in the tensile elongation, which is likely due to the fact that at these concentrations the percolation threshold is not reached [139]. The addition of chitin into the chitosan-based material also can decrease the equilibrium degree of swelling [140, 148, 149, 156], retard the sorption of water [149], and increase the force of hydrogel adhesion to skin [142].

The range of possible applications of composites based on chitosan and CNPs include materials for the therapy of wounds and burns [141, 143, 144, 155], restoration of defects of cartilaginous [158–161, 168] and bone tissues [147, 156, 162], tissue adhesive [142], as well as drug delivery and prolonged-release systems [163, 164]. The studied materials also showed the absence of cytotoxicity with regard to fat tissue, fibroblasts [142–144, 157], murine osteoblasts [147, 162], stem cells of human fat tissue [157, 168] even when the cross-linking agent was glutaraldehyde [147]. Also, materials from chitosan and chitin show a high adhesion of chondrocytes [158], fibroblasts [155, 157], and mesenchymal stem cells of fat tissue [157]. With an increase in the content of chitin in the matrices, the viability and proliferative activity of osteoblasts can increase [147]. It was shown on composite chitin/chitosan films that the use of higher-molecular-weight chitosan and its subsequent neutralization favor the formation of a cytoskeleton of stromal cells of rat bone marrow, more corresponding to the natural one, and increase the level of their proliferation [156]. On the surface of the porous chitosan matrix filled with 33 wt % of α-chitin nanocrystals, the authors of [168] could differentiate mesenchymal stem cells of fat tissue into chorondrospheroids under hypoxia conditions and obtain a chondral extracellular matrix with a high content of sulfated glycosaminoglycan, which is typical of articular cartilage.

Besides the biocompatibility with animal tissue cells, materials based on chitin and chitosan can have an inhibitory effect on the growth of different bacteria (*S. aureus*, *E. coli* [142, 151] and *C. michiganence* [151]) and fungi (*Alternaria alternata* [150], *A. niger* [153]). Some researchers attribute the antifungal activity first of all to the presence of chitosan in the composite [150]. Conversely, other researchers note that the inhibitory effect on the growth of fungi is higher for composites than for pure chitosan material; composites filled with α-chitin nanofibrils demonstrate a stronger inhibitory action on the growth of *A. niger* than those filled with nanocrystals [153]. Some of researchers show that the bactericidal effect is higher in the composite films compared to the pure chitosan films [151]; other researchers demonstrate that the inhibition of bacterial growth is mainly due to the hydrogel cross linking agent DDA [142]. Thus, data on the inhibitory activity against microorganisms are quite contradictory, which is likely due to few studies; therefore, a complex hydrogel including HBC, chitin nanocrysatls and carboxymethylchitosan

nanoparticles (CMC) was used in [141] for the therapy of chronic pains of mice and this hydrogel was additional loaded with linezolid and epithelial cell growth factor. The authors showed that this combination of components provided faster wound healing accompanied by the formation of dense collagen and vascular network [141].

3.2.2. Cellulose and its derivatives. Cellulose is the most common polymer on the Earth; it is a component of cell walls of the most of plants and also forms a part of outer covering of some chordates, tunicates [19]. Cellulose is a highly crystalline polymer insoluble in the most of standard solvents, which complicates its processing and use as a part of composites. However, this problem can be solved using following techniques. For example, cellulose possess a high number of hydroxyl groups convenient for modification, through which its solubility in different solvents, e.g., water, can be improved [169]. In addition, cellulose is characterized by a number of untypical solvents, such as ionic liquids, solutions of some complex compounds in water, melts of inorganic salt hydrates, aqueous solutions of alkali and alkali with urea, thiocyanate-containing solutions, different imidazolium salts, and organic solvents containing lithium salts [169]. Also, to prepare composites cellulose is often used as suspensions of nanocrystals and nanofibrils dispersed by different methods [170]. Such approaches are applicable in the preparation of different composite materials based on cellulose and chitin.

For example, water-soluble carboxymethylcellulose (CMC) can serve as a basis for wound coatings [171]. The authors used glycerol-plasticized CMC as the matrix for composites and filled it with α-chitin nanocrystals obtained by ammonium persulfate oxidation [172], which resulted in the formation of carboxyl groups on the surface of rod-like nanocrystals. Composite films with a filler content of 1–10 wt % were prepared by casting. The study of their mechanical properties showed that the Young modulus and tensile strength increased with an increase in the concentration of the filler and the tensile elongation decreased. For example, the film containing 10% of chitin demonstrated a more than three-fold increase in the modulus and almost two-fold increase in the tensile strength. The comparison of the reinforcing effect of α-chitin nanocrystals obtained by ammonium persulfate oxidation and hydrolysis in concentrated sulfuric acid showed that no significant changes in the effect of filler type were observed at 5% filling [173]. The addition of 5 wt % of wine-stone oil did not deteriorate the studied mechanical properties, but suppressed the growth of gram-negative (*E. coli*) and gram-positive bacteria (*L. monocytogenes*). Also, the authors of [174] used ZnO–Ag nanoparticles, stabilized in the synthesis by α-chitin nanocrystals, as the antibacterial additive. In [175], electrostatic attraction between the acid form of CMC and α-chitin nanocrystals was used to produce composites. Composite films were obtained by sorption of nanocrystals from a suspension onto the CMC film. The density of the resulting layer depended on the content of nanocrystals in a methanol suspension to be 13.9, 17.5, and 26.5 μg/cm^2 at a concentration of 0.75, 1.50, and 3.00 mg/mL in the suspension, respectively. The tensile strength and elongation increased with an increase in the concentration of nanoparticles on the film surface. For example, the tensile strength and elongation was 24.2 MPa and 7.6% for the composite with a chitin content of 26.5 μg/cm^2 and only 7.1 MPa and 2.6% for the CMC film, respectively.

The charge interactions between the carboxyl groups of cellulose nanofibrils obtained by TEMPO oxidation and the amino groups of surface-deacetylated α-chitin nanocrystals were used in [176] to obtain composite fibers by wet spinning with a polysaccharide ratio of 50/50. The precipitating solution was a suspension of chitin nanocrystals, through which a suspension of cellulose nanofibrils was fed through a needle with a diameter of 0.6 mm (Fig. 8). Multilayered carbon nanotubes were used to give conductivity to the composite. The percolation threshold of conductivity was achieved at a nanotube concentration of 14 wt % in the composite, when the conductivity of the composite reached 1.6 mS/cm, which enables power supply of a light-emitting diode at a voltage of 5 V (Fig. 8c). The conductive properties of these materials demonstrate a great application potential of such composites as biocompatible and/or biodegradable conductive materials.

In the design of cellulose-based composites, one can use its ability to reversibly aggregate upon drying [177]; e.g., films with 1–10 wt % of chitin were obtained from cellulose I nanofibrils and α-chitin nanocrystals by hot pressing [178]. These films feature high specific surface area (~70–80 m^2/g) and porosity of about 30%; the content of chitin has no significant effect on these parameters. The addition of nanocrystals had no effect on the tensile strength and Young modulus, but decreased almost two-fold the relative tensile strain. The strain decreased at a α-chitin concentration of 1% and then remained unchanged upon an increase in the content of this polysaccharide. However, the addition of chitin to the film composition favored the growth inhibition of *Aspergillus* sp. fungi, the viability of which monotonically decreased with an increase in the concentration of chitin in the composite film: from 40% for the film made of cellulose nanofibrils to 18% for the film filled with 10% of chitin.

3.2.3. Starch. Starch is the main storage polysaccharide of most plants; it is among three leaders in terms of occurrence of polymer materials on the Earth. Starch consists of glucose residues linked into linear (amylose) and branched chains (amylopectin) [179, 180]. In contrast to many other natural polysaccharides, starch can pass to a thermoplastic state upon

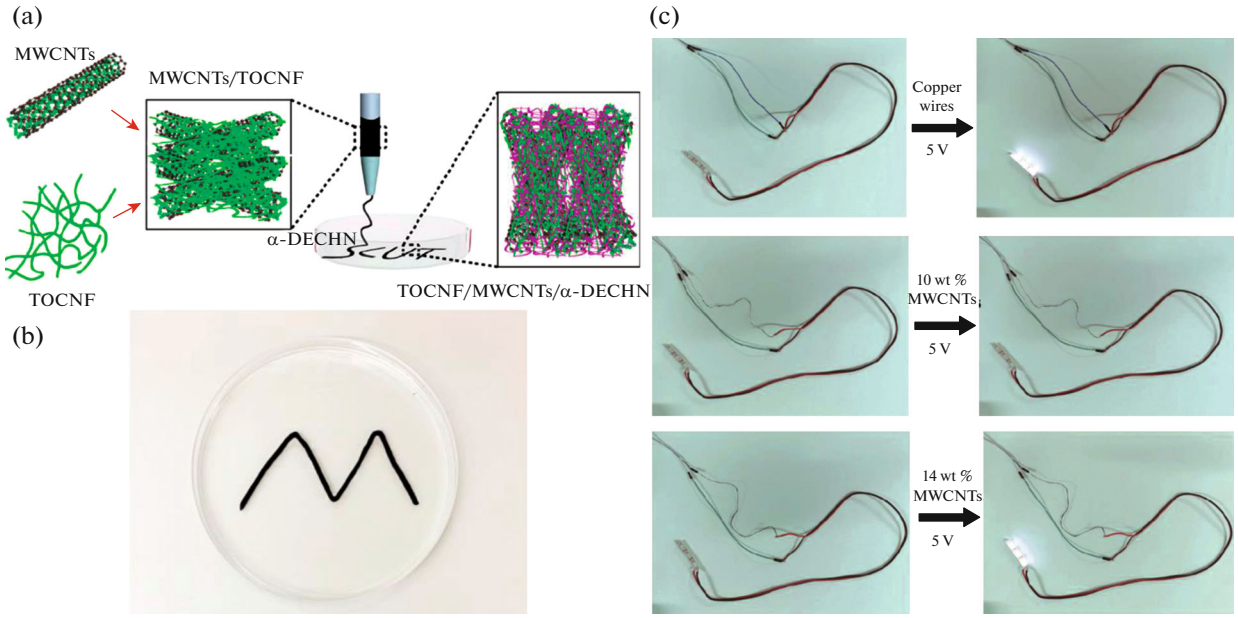

Fig. 8. (Color online) Schematic diagram for preparation of composite fiber (a): MWCNTs, multilayered carbon nanotubes; TOCNF, cellulose nanofibrils obtained by TEMPO oxidation; α-DECHN, surface-deacetylated α-chitin nanocrystals. Photograph of composite fiber in Petri dish immediately after precipitation (b). Photographs of light-emitting diode power supplied through copper wire (top) and composite containing 10 (middle) and 14 wt % (bottom) carbon nanotubes (c) [176].

addition of water, sugars, alditols, glycerol, and ethylene glycol [181]. Due to its biodegradability and biocompatibility, starch has found biomedical application [180]; however, its poor mechanical properties and high hydrophilicity restrict its application as a multicomponent material, giving impetus to the preparation and study of its modification and composite materials [179]. CNPs are suited to solve such problems and demonstrate its efficiency by the example of other polysaccharide matrices [182].

The work [183] was the first one on the preparation and study of starch/chitin composites. To fill the glycerol-thermoplasticized starch, the authors used α-chitin nanoparticles the content of which varied from 0.77 to 3.85 wt % (1–5 wt % with respect to starch). However, despite the use of the common acid hydrolysis method for isolation of nanocrystals [184], the authors did not obtain rod-like nanocrystals. The isolated chitin particles possessed a lower crystallinity than the starting chitin and most likely had a round shape. Nevertheless, with an increase in the content of the filler the tensile strength increased several-fold and the relative tensile elongation decreased.

Nanocomposites based on thermoplasticized starch and α-chitin were studied more comprehensively in [128, 185], where both the filler type (nanocrystals or nanofibrils) and the preparation method of composite (extrusion or solution casting) were varied. In the works, all filler types were shown to result in a monotonic increase in the Young modulus and tensile strength and a decrease in the tensile elongation with an increase in the filler concentration to 12–14 wt % (to 20 wt % with regard to starch). In all studied methods of preparation, nanofibrils possessing a high aspect ratio showed to be a more efficient reinforcing filler. Note that the preparation method has a significant effect on the mechanical properties of both pure plasticized starch and composites. For example, a higher modulus and a higher tensile strength were observed for starch and starch-containing composites obtained by extrusion. However, extrusion processing and subsequent pressure casting resulted in darkening of the materials [185], the composites being colored stronger than the polymer matrix.

In [186], somewhat different pattern of relationship between the mechanical properties of composites and the content of α-chitin nanocrystals was shown. In the study of composites based on thermoplasticized starch in a filler concentration range of 0.35–3.5 wt % (0.5–5 wt % with respect to starch), the highest tensile strength was observed at a filler content of 0.7% (1% with respect to starch). Nevertheless, the tensile elongation and degree of swelling of the composite in water monotonically decreased with an increase in the concentration of α-chitin nanocrystals.

The studied composites show inhibitory action on the growth of *A. niger* fungi [128] and the growth of *E. coli* and *L. monocytogenes* bacteria [186]; the effect increases with an increase in the concentration of chitin.

3.2.4. Alginates. Alginates are linear water-soluble heteropolysaccharides consisting of residues of uronic acids (D-mannuronic and α-L-guluronic acid)

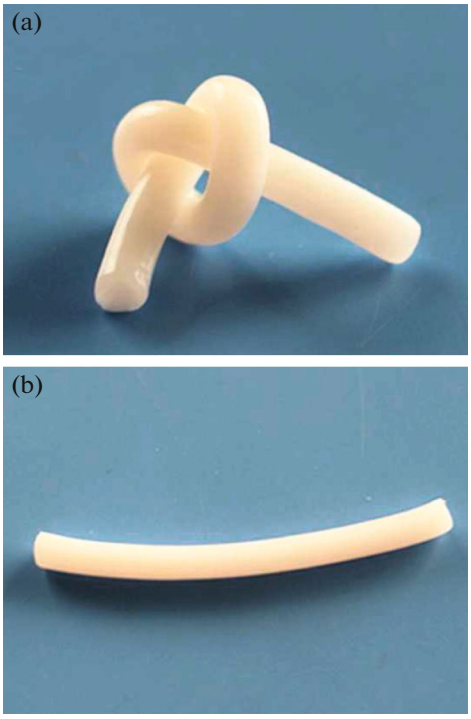

Fig. 9. (Color online) Photographs of knotted (a) and unknotted (b) hydrogel containing 50 wt % of chitin (total amount of polymers in hydrogel was 4%) [182].

extracted mainly from brown algae, such as *Laminaria* sp. and *Ascophyllum* sp. Due to a great number of carboxyl groups, alginates are easily cross linked with divalent metal ions, e.g., Ca^{2+}. This property, as well as the biocompatibility and biodegradability make alginates a promising basis for a wide range of advanced biomedical goods, especially, in the therapy of wounds and burns [187]. Some companies have designed hydrogel (AlgiSite™, Kaltostat™, Tegagel™, etc.) and fibrous (Algosteril™, Kaltostat™, Sorbsan™, etc.) wound coatings [188]. Alginate-based materials can be filled with water-soluble drug substances and enzymes to provide their prolonged action [189, 190]. For functional modification and improvement in the mechanical properties, alginate gels are mixed with other polymers or fillers [187]. CNPs can be used as a reinforcing and functional filler.

One of the first work on the study of composite alginate/chitin fibers was described in [191]. The fibers were obtained by wet spinning from a suspension of α-chitin nanocrystals in a solution of sodium alginate passing it through a precipitating bath with a solution of $CaCl_2$ in 50% methanol. The concentration of nanocrystals varied in a range of 0.05–2.00 wt %. The study of mechanical properties showed that, in a dry state, the composite with a CNP content of 0.15% possessed the highest tensile strength and elongation. Further increase in the concentration resulted in adhesion of the filler and, correspondingly, deterioration in the mechanical characteristics of composite fibers. This effect was observed upon mixing of positively charged CNPs with a negatively charged matrix [182]. In [182], a method was proposed to eliminate this negative effect. The filler was α-chitin nanocrystals obtained by hydrolysis in H_2SO_4 to result in the appearance of certain amount of sulfo groups at the nanocrystal surface. As a result, the filler suspensions become stable not only in the acid medium, but also in the alkaline one where mixing with a solution of alginate is performed. In the alkaline medium, the amino groups of chitin are uncharged; therefore, no precipitation and aggregation occurs upon mixing of chitin particles and a solution of alginate. pH of the medium was changed after fixation of the hydrogel structure with Ca^{2+} ions. This approach enabled preparation of composite alginate hydrogels with a content of chitin nanocrystals from 20 to 56 wt %. The optimum concentration of the filler was 50%, at which the strength of the material increased 2.3-fold compared to the pure polymer matrix and the Young modulus increased 2.7-fold with retention of the relative tensile strain at a level of ~75–80%. The mechanical characteristics of the hydrogel are such that the hydrogel rod with a water content of 96% and a chitin content of 2% can be tied in a knot and untied back (Fig. 9). Note that the addition of chitin nanocrystals decreases the rate and ultimate degree of swelling from 35 to 15-fold. In addition, with an increase in the content of the filler the adhesion and rate of proliferation of MC3T3-E1 murine preosteoblasts increase, which make these composite materials promising as matrices for the treatment of bone tissue defects.

3.2.5. Hyaluronic acid. Hyaluronic acid (HA) is a natural water-soluble linear nonsulfated glucosaminoglycan, the monomeric unit of which is a disaccharide consisting of β-glucuronic acid and β-1,3-*N*-acetylglucosamine residues [192]. This polysaccharide is a component of the intercellular matrix and involved in cell signaling, wound healing, and morphogenesis [193]. Hydrogels based on HA and its derivatives are studied as wound coatings, systems for delivery of drugs, particles, and cells, as well as for tissue engineering [192]. Since HA is a polyanion, it is extensively studied as a component for the preparation of microparticles coupled with polycations [194], because this approach has been shown to be promising compared to the application of single-component drug delivery systems [195]. CNPs can act as a polycation due to the presence of free amino groups on their surface.

In [196], Morganti et al. compared hydrogel microparticles based on HA in combination with chitosan, amorphous chitin, and α-chitin nanofibrils filled with lutein. The method consisted in precipitation of a mixture of cationic polymer with lutein upon dropwise addition to a solution of HA. Composite microparticles based on chitin fibrils showed the high-

est efficiency in terms of the yield of particles (42 vs. 31–33% in remaining materials) and loading of drug substance, lutein (35 vs. 18% in the material with amorphous chitin and 10% in chitosan-containing particles), as well as a slower drug release. The rate of lutein release from these composite particles was almost constant for 45 h. These results suggest these microparticles to be a suitable system for drug delivery, e.g., in the antiageing therapy in cosmetology or skin regeneration. In [197], the chitin/HA composite was also used to design tableted dosage forms of prolonged-release drugs. To obtain tablets, a suspension of surface-deacetylated fibrils of α-chitin was mixed with a solution of HA, freeze dried, and pressed. The model drug substance for loading was famotidine, the content of which in the resulting tablet was 90 wt %. The tablets based on the interpolyelectrolyte chitin/HA complex provide a longer release of drug substance compared to the tablets consisting of chitin nanofibrils only. According to the data on the turbidity of the mixture and X-ray diffraction data, the optimum ratio of nanofibrils and HA is 1 : 1.

Summarizing the chapter on composites based on natural polymers filled with chitin nanocrystals and nanofibrils, let us remark the following. α-Chitin nanocrystals obtained by the acid hydrolysis serve as the most common filler in the preparation of composite materials, which is used in more than 60% of works. Note that, in the works where both nanocrystals and nanofibrils were used, the latters had a more pronounced reinforcing effect on the polymer matrix of chitosan [153] and starch [128, 185], if no additional cross-linking agent is applied. When covalent cross linking is performed, the reinforcing effect of these two fillers becomes comparable [157]. In addition, some works on the preparation of materials based on naturally occurring matrices and chitin use cosolvents to destruct the crystalline structure of chitin and to obtain a mixture of polymers. Such an approach was applied in the preparation of materials based on chitin and collagen [198], cellulose [199], pectin [200], skin fibroin [201–205], and HA [206], but it is not suited for the topics of this review. Note that we failed in finding works on the preparation of composites based on chitin and resilin, albumin, dextran. Only one work [207] deals with the comparative study of the reinforcing effect of cellulose and α-chitin nanocrystals on the matrix of guar and hydroxypropylguar. Also, we found only one work on the preparation of a composite based on carrageenan and chitin, where molecular dynamics was studied by dielectric spectroscopy [208], which also goes beyond the scope of this review. All researchers note excellent characteristics of materials with regard to cell cultures of different mammal tissues: nontoxicity, cytocompatibility, high adhesion and rate of proliferation; in most cases, chitin has a positive effect on these parameters of the composite. In addition, some works showed that the addition of CNPs retards the growth of bacteria and fungi.

CONCLUSIONS

Chitin nanoparticles for the preparation of composites based on biodegradable polymers are extracted from different sources by different methods, but easy-to-use acid hydrolysis in a hydrochloric acid solution is most often applied, which makes it possible to obtain polysaccharide nanocrystals,. Remaining methods, such as partial deacetylation, mechanical dispersion, hydrolysis in sulfuric acid, and surface oxidation, are much less used in research works. Also, α-form is the polymorphic modification of chitin that is most commonly used upon filling of composites. Among methods for the preparation of composites, techniques using a solvent predominate. These methods make it possible to decrease the viscosity of a medium upon mixing with a high-molecular-weight polymer and to keep a high degree of filler fineness thereby favoring its more homogeneous distribution in a material. In the case of application of natural polymers as a matrix, composite could be obtained only using solution methods. Note that the necessity for the design of porous or hydrogel materials from biodegradable composites contemplates the use of solvents. Extrusion and casting methods are more processable, but can be applied only for the composites the polymer matrix of which can pass into a viscoelastic state, such as polyesters and thermoplasticized starch. However, the use of these methods is accompanied by heating of the composite to high temperatures and, correspondingly, possible temperature destruction of polysaccharides; it also prohibits the addition of bioactive substances, such as antibiotics and growth factors. Nevertheless, this technology has a field of application in cases when mechanical properties are foremost, e.g., in the design of screws, pins, and other items for the treatment of compound fractures. The optimum concentration of chitin as a reinforcing filler strongly depends on the hydrophobicity of polymer matrix, since the more hydrophobic is the polymer matrix the lower is the concentration of chitin particles at which they undergoes aggregation and this has a negative effect on the mechanical properties of the composite. The hydrophilicity of natural polymer materials, as well as their structural similarity in being used as the matrix of polysaccharides, makes it possible to achieve a uniform filler distribution even with a high content of nanocrystals and nanofibrils. Good surface interaction between the hydrophilic matrices and chitin particles also result in more efficient transfer of mechanical loads on the material, which directly affects the reinforcing effect of the filler on the polymer.

All studies in which the effect of CNPs on culturing different mammalian cells was investrigated mention a positive or neutral effect of this filler on the bioactivity of the composite material vesus a pure polymer matrix. Some studies note an increased rate and quality of wound healing when chitin-based composites are applied in experiments in vivo. Nanoparticle activ-

ity against fungi and bacteria was demonstrated; however, some data are contradictory.

Thus, the range of composites based on different polymer matrices described in the present work demonstrates the great interest and promising potential of applying CNPs as a reinforcing and functional filler for medical biodegradable materials. Nevertheless, we note that not all known biodegradable polymers applied in medicine have become a basis for chitin-filled composites; it seems they are waiting for researchers to find them.

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AUTHORS' CONTRIBUTIONS

O.I. Bogdanova and A.P. Istomina each contributed equally to this article.

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