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Chemical modification of nanocrystalline cellulose for improved interfacial compatibility with poly(lactic acid)

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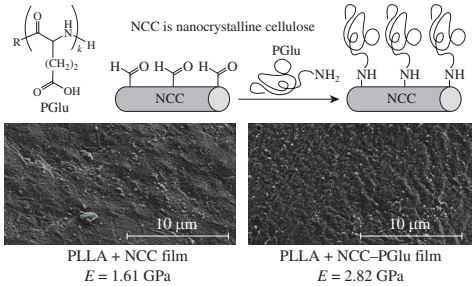
Several methods for chemical modification of nanocrystalline cellulose by poly(glutamic acid) were tested. The best one was based on partial oxidation of cellulose followed by reaction of the resulting aldehyde groups with terminal amino groups of poly(glutamic acid). The modified cellulose filler had an improved compatibility with hydrophobic poly(L-lactic acid) matrix for the preparation of composite materials.



Biodegradable aliphatic polyesters and materials derived from them have found an application in food industry, surgery, drug delivery, regenerative medicine and other areas.[1] Examples of such polyesters are poly(lactic acid) (PLA)[2] and its copolymer with poly(glycolic acid),[3] widely used in medicine.[4] For specific medical needs, the biopolymer properties, including mechanical characteristics, biocompatibility and biological effect, can be tuned by filling with nanoparticles or *via* modification with other (macro)molecules.[5–7]

As an effective nanofiller, nanocrystalline cellulose (NCC) has been proposed in the last years.[8–10] However, the use of hydrophilic NCC with hydrophobic PLA-based matrix is hampered by their poor interfacial compatibility that can be overcome by the chemical modification of NCC surface.[11] For example, the adsorption of poly(ethylene glycol)[12] or grafting of PLA[13] on NCC surface[13] was reported.

Polymeric scaffolds for bone tissue engineering should have robust mechanical characteristics, biocompatibility, appropriate degradation rate and negligible inflammatory response *in vivo*, as well as be suitable for processing. Poly(lactic acid) meets these criteria and therefore has been extensively investigated for application in bone regeneration and orthopedic area.[14] For example, modification of PLA–poly(lactic-co-glycolic acid) nanofibers with glutamic acid-containing EEGGC peptide induces effective calcium phosphate nucleation and osteogenic differentiation of marrow stromal cells.[15] In this work, we synthesized new PLA-based biocompatible and biodegradable composite material with poly(glutamic acid) (PGlu)-modified nanocrystalline cellulose filler.

Poly(glutamic acid) was synthesized by the ring-opening polymerization of γ-benzyl-L-glutamic acid *N*-carboxyanhydride, as described in our earlier work.[16] Two specimens with different molecular weight were prepared using the monomer–*n*-hexylamine initiator ratio of 100 : 1 for specimen 1 and 10 : 1 for specimen 2, their molecular weight distribution characteristics were determined by size-exclusion chromatography (SEC) and ^{1}H NMR spectroscopy (Table 1). The benzyl protective groups were partially removed by trifluoromethanesulfonic acid–trifluoroacetic acid mixture (1 : 10, v/v) at 22 °C for 2 and 3 h for specimens 1 and 2, respectively. The remaining benzyl groups provided amphiphilic nature to hydrophilic PGlu and were intended to achieve a uniform distribution of the hydrophilic PGlu-modified NCC inside the hydrophobic PLA matrix. The amount of residual benzyl groups was determined by ^{1}H NMR spectroscopy as 54 and 22% for high-molecular-weight (HMW) and low-molecular-weight (LMW) specimens 1 and 2, respectively (Figures S1 and S2, see Online Supplementary Materials).

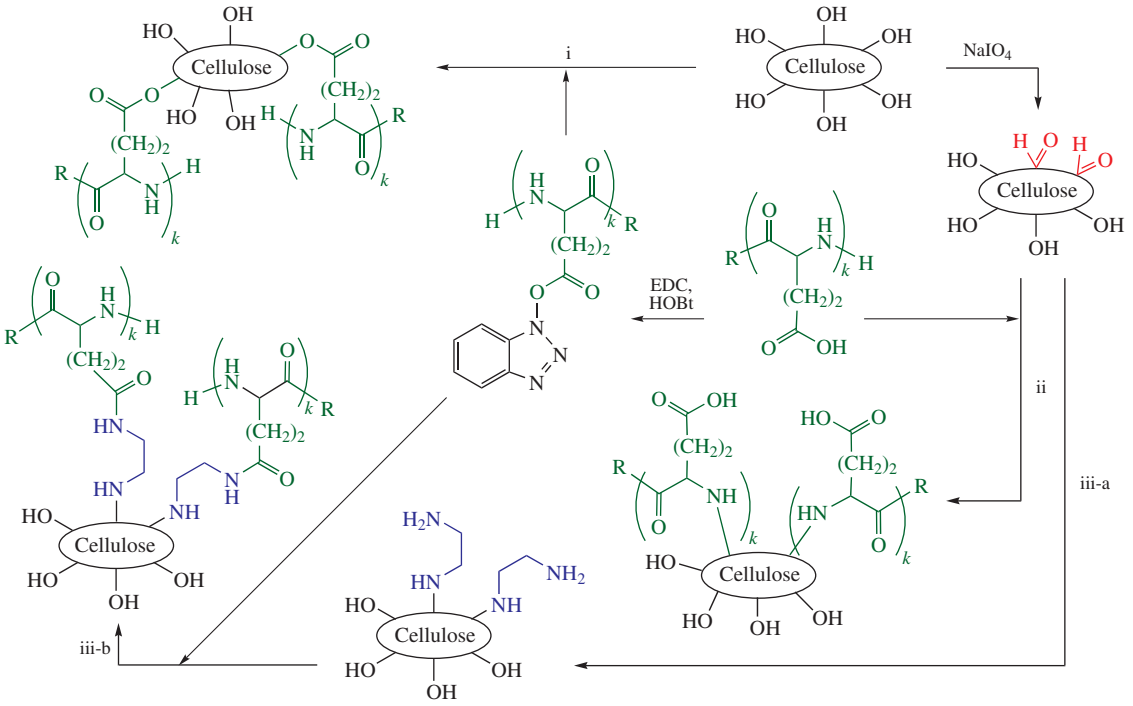
To find the most efficient pathway for NCC modification by the synthesized PGlu, three different routes were tested (Scheme 1). The experimental details can be found in Online Supplementary Materials.

The first route comprised the reaction of preliminary activated carboxyl groups of PGlu with the cellulose hydroxyl groups. In the second and third routes, cellulose was preliminary partially oxidized by sodium periodate to form aldehyde groups, and their formation was confirmed using Schiff's reagent (Figure S3).[17] After the aldehyde groups were generated, their reaction with

Table 1 Characteristics of poly(glutamic acid) samples for NCC modification.

Specimen	SEC			^{1}H NMR		
	M_w[a]	M_n[b]	$Đ$[c]	M_n	DP[d]	Residual benzyl groups (%)
1 (HMW)	10400	7900	1.32	14310	80	54
2 (LMW)	2100	2000	1.05	1005	6	22

[a] Weight-average molecular weight. [b] Number-average molecular weight. [c] Dispersity. [d] Degree of polymerization.



Scheme 1 *Reagents and conditions*: i, sodium borate buffer (pH 8.5), 22 °C, 3 h; ii, sodium borate buffer (pH 8.5), 22 °C, 3 h, then $NaBH_4$, 1 h; iii-a, *N*-Boc-ethylenediamine, sodium borate buffer (pH 8.5), 22 °C, 3 h, then $NaBH_4$, 1 h, then TFA, H_2O, 22 °C, 3 h; iii-b, sodium borate buffer (pH 8.5), 22 °C, 3 h.

terminal amino groups of PGlu (route ii) or with Boc-ethylene diamine (route iii, step iii-a) was carried out. For the third route, the subsequent removal of Boc protective groups by trifluoroacetic acid enabled further reaction with the preliminary activated carboxyl groups of PGlu (step iii-b). The structure of the products obtained was confirmed using solid-state ^{13}C NMR and IR spectroscopy.

The IR and ^{13}C NMR spectra revealed the modification route ii as the most efficient one (Figure S4). In the IR spectrum of the corresponding product, a band at 1737 cm^{-1} originated from the acid C=O group stretching as well as a shoulder at 1630 cm^{-1} and a small band at 1546 cm^{-1} corresponding to the amide group and aromatic C=C group stretching, respectively, were present. In the ^{13}C NMR spectrum, signals originated from carboxylic acid at 172 ppm, aromatic ring carbon atom at 128 ppm and amide carbon atom at 30 ppm were observed only for the product of route ii. Thus, the route ii based on oxidation of the cellulose vicinal diol moieties to aldehyde groups followed by their reaction with amino groups of PGlu and subsequent borohydride reduction was chosen for further investigation.

The amount of PGlu bound to NCC was calculated as a difference between the initial amount of PGlu and its amount remaining unreacted that was determined *via* UV spectroscopy at 265 nm (Figure S5). The bound amount achieved using route ii was found to be 138 and 224 mg g^{-1} for HMW (specimen 1) and LMW (specimen 2) PGlu, respectively. These values corresponded to 55 and 90% of the initial PGlu amount taken for the reaction. The poorer covalent attachment of PGlu with longer polymer chain can be explained by two reasons: the lower molar amount of amino groups in the polymer of higher molecular weight under the same sample mass taken for the reaction and poor accessibility of the terminal amino groups of the elongated polymer chain.[18]

Thermogravimetric analysis (TGA) of low-molecular-weight PGlu bound to NCC as well as starting NCC and PGlu LMW samples (Figure S6) demonstrated for all them an initial mass loss with temperature increase from 40 to 120 °C related to the loss of adsorbed and coordinated water. This initial mass loss was found to be 3% for PGlu LMW, 6.5% for NCC and 6% for NCC–PGlu LMW. The parameters τ_5 and τ_{10} characterizing the 5 and 10% loss of polymer mass, respectively, due to decomposition are summarized in Table 2. The values for NCC–PGlu LMW were found to be between those determined for starting NCC and PGlu LMW. Polymer decomposition after 250 °C occurs *via* destruction of glycoside units, removal of benzyl protective groups of PGlu, depolymerization, oxidation and finally burning of the carbonaceous residue with formation of carbon oxides. The differential thermal analysis (DTA) curves for the samples were obtained (Figure S7).

Table 2 Thermal parameters τ_5 and τ_{10} for the 5 and 10% loss of polymer mass.

Samples	τ_5/°C	τ_{10}/°C
NCC	219	241
PGlu LMW	236	250
NCC–PGlu LMW	221	246

Starting, oxidized and modified NCC samples were also analyzed by dynamic light scattering. The hydrodynamic size D_H, polydispersity index PDI and electrokinetic potential (ζ-potential) values are given in Table 3. The values of D_H and ζ-potential of starting NCC increased after its oxidation. This can be rationalized from the ability of aldehyde groups to interact with hydroxyl

Table 3 DLS characteristics of starting, oxidized and modified NCC (in water, 25 °C, $n = 3$).

Samples	Treatment conditions	D_H/nm	PDI	ζ-potential/mV
NCC	commercial suspension	128 ± 7	0.28 ± 0.01	–23.0 ± 1.7
NCC	lyophilized, redispersed	264 ± 18	0.58 ± 0.04	–25.0 ± 0.5
NCC oxidized	lyophilized, redispersed	363 ± 15	0.48 ± 0.13	–17.8 ± 0.4
NCC–PGlu HMW	lyophilized, redispersed	397 ± 34	0.44 ± 0.03	–26.0 ± 0.3
NCC–PGlu LMW	lyophilized, redispersed	434 ± 48	0.49 ± 0.08	–31.0 ± 0.3

Table 4 Young's modulus, tensile strength and elongation to break for PLLA and composite films.

Film	*E*/GPa	σ_b/MPa	ε_b (%)
PLLA	2.99±0.15	58±3	12.0±2.0
PLLA+5 wt% NCC	1.61±0.11	19±2	3.7±0.5
PLLA+5 wt% NCC–PGlu	2.82±0.21	39±2	3.8±0.2

groups to form hemiacetals in an aqueous environment.[19] The modification of oxidized NCC with PGlu did not result in change of particle size. As expected, ζ-potential value became lower after binding with negatively charged PGlu.

To estimate the effect of NCC modification on the properties of composite materials based on poly(L-lactic acid) as a variety of PLA (PLLA), a series of films were prepared from poly(L-lactic acid), PLLA filled with NCC and PLLA filled with NCC–PGlu; PLLA was synthesized using the ring-opening polymerization of L-lactide as described earlier.[20] The product was characterized by size-exclusion chromatography, M_w, M_n and $Đ$ were equal to 99000, 78600 and 1.26, respectively. The films were obtained from solutions in chloroform (see Online Supplementary Materials). Young's modulus *E*, tensile strength σ_b and elongation at break ε_b were determined under uniaxial extension using band-like samples of 2×20 mm size (Table 4).

The mechanical characteristics obtained for the PLLA films are in agreement with published data (*E* ~2.5–4 GPa, σ_b ~59–66 MPa, ε_b ~4–19%).[21–23] The introduction of 5 wt% of NCC into the films caused a drastic decrease in mechanical parameter values in comparison with PLLA as a result of the poor compatibility of hydrophilic NCC with hydrophobic PLLA.[9] Introduction of NCC modified with PGlu bearing hydrophobic residual benzyl groups into PLLA films led to the 1.75-fold increase in Young's modulus compared to the material filled with untreated cellulose as a result of better compatibility of the modified NCC with the PLLA matrix. In spite of lower values of tensile strength and elongation to break for PLLA filled with NCC–PGlu in comparison with pure PLLA films, the composite materials can surpass PLLA in potential applications.

In summary, a new composite material based on poly(L-lactic acid) and filled with nanocrystalline cellulose modified by poly(glutamic acid) was prepared. Three different pathways to modify NCC by PGlu were tested to select the optimal technique. The method of choice was based on cellulose partial oxidation and the reaction of the resulting aldehyde groups with terminal amino groups of PGlu. In comparison with starting NCC, the obtained NCC–PGlu had improved compatibility with hydrophobic poly(L-lactic acid) matrix that was confirmed by higher value of Young's modulus for the PLLA–NCC–PGlu film. The developed composite has the potential of application as scaffold in tissue engineering.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.03.036.

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