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

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Introduction

Depending on the practical task at hand, different materials can be prepared including fibers, micro- and nanoparticles, films, and porous scaffolds.[S1-S3] Incorporation of synthetic and mineral fillers is an established approach for material reinforcement.[S4] In turn, fillers derivable from renewable resources should, in general, maintain the ecologically friendly character of the material. The application of natural polymers is rapidly growing due to their availability from renewable resources in accordance with the Principles of Green Chemistry.[S5-S7] Cellulose is a good candidate due to its low cost and availability from different sources.[S8] The ability to influence mineralization is one of the key properties for materials considered as scaffolds for bone tissue engineering. To induce matrix mineralization, hydroxyapatite or ceramic nanoparticles are usually used as filler for PLA-based matrices.[S9,S10]

Experimental part

NCC was a product of Blue Goose Biorefineries, Inc. (Canada). Shimadzu LC-10 system (Japan) equipped with refractometric detector and a Phenomenex PolySep-GFC-P 4000 column were used for size-exclusion chromatography. NMR and IR-spectroscopy were carried out using an AVANCE II-500 WB Bruker spectrometer (Germany) and an IRAffinity-1S Shimadzu instrument (Japan), respectively. For polymer thermal stability study a DTG-60 Shimadzu analyzer was applied. Mechanical tests were carried out using an AG-100kNX Plus Shimadzu universal mechanical system (Japan) at the extension speed of 10 mm·min^{-1}. The hydrodynamic diameter of NCC was measured using a Zetasizer Nano-ZS Malvern Instrument Ltd. system (UK).

NCC oxidation

NCC suspension (8 wt%) was diluted 10 times with water and cooled to 4 °C. Then solution of $NaIO_4$ in water was added to the NCC suspension to reach the concentrations ratio *[glucose unit]/[$NaIO_4$]* = 5. The reaction was carried out at 4 °C for 24 h. The oxidized NCC was purified by dialysis against water for 2 d using the membrane bag with MWCO 1000 Da. The product was stored as suspension at 4 °C.

Activation of carboxylic groups of PGlu

The threefold molar excess of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) regarding to monomeric units of PGlu was added to the solution of PGlu in 0.01 M MES buffer and reaction medium was incubated at 4 °C for 10 min. Then, threefold excess of *N*-hydroxybenzotriazole (HOBt) was added to the reaction medium and reaction was left for 20 min at 4 °C. After the reaction time was over, PGlu was transferred into water. Finally, the suspension was concentrated *via* spin-column ultrafiltration through the membrane with MWCO 1000 Da.

Modification of NCC with PGlu

i. 250 mg in 10 ml of activated PGlu in water was added to 100 ml of suspension containing 1.0 g of NCC in 0.01 M sodium borate buffer (pH 8.5). The reaction was carried out at 22 °C for 3 h under stirring.

ii. 250 mg in 10 ml of PGlu in 0.01 M sodium borate buffer (pH 8.5) was added to 100 ml of suspension containing 1.0 g of oxidized NCC in 0.01 M sodium borate buffer (pH 8.5). The reaction was carried out at 22 °C for 3 h under stirring. To reduce imine bonds and free aldehyde groups, 200 mg of sodium borohydride was added to reaction medium that was left for 1 h.

iii. 2 ml of *N*-Boc-ethylene diamine (BocEDA) in 0.01 M sodium borate buffer (pH 8.5) was added to 100 ml of suspension containing 1.0 g of oxidized NCC in 0.01 M sodium borate buffer (pH 8.5). The reaction was carried out for 3 h at 22 °C under stirring. To reduce imine bonds and free aldehyde groups 200 mg of sodium borohydride was added to reaction medium that was left for 1 h. Then, the liquid was evaporated up to 1/3 and modified NCC was purified by dialysis against water for 2 d. After that, the suspension was diluted with water up to 100 ml and 5 ml of trifluoracteic acid was introduced into the reaction medium to remove Boc-groups. The reaction was run at 22 °C for 3 h. The product (NCC–EDA) was dialyzed firstly against water up to neutral pH and then against 0.01 M sodium borate buffer (pH 8.5). Finally, 250 mg of activated PGlu in 10 ml of 0.01 M sodium borate buffer (pH 8.5) was added to 100 ml of suspension containing 1.0 g of NCC–EDA in the same buffer. The reaction was performed at 22 °C for 3 h under stirring.

In all cases, the final suspension was dialyzed against water and lyophilized for storage.

Synthesis of PLLA

PLLA was synthesized using the ring-opening polymerization of L-lactide. The synthesis was carried out in bulk at 130 °C for 24 h using the following ratios for the components: *[M]:[MeOH]* = 10000 and *[MeOH]:[Sn(Oct)$_2$]* = 2.

Manufacturing of films

Cellophane, moistened with water, was fixed on the glass cylinder (i.d. 75 mm) and then dried for 24 h. 5% PLLA solution in chloroform (6.5 ml) was poured inside the glass cylinder and left for 12 h on air for chloroform evaporation. After that cellophane was removed and the obtained PLLA films were dried in air thermostat at 50 °C until the constant weight (3 d). To prepare composite films, pure or modified NCC was dispersed in polymer solution in chloroform under ultrasonication for 30 min. Other manipulations were the same as described for the preparation of PLLA films.

Results

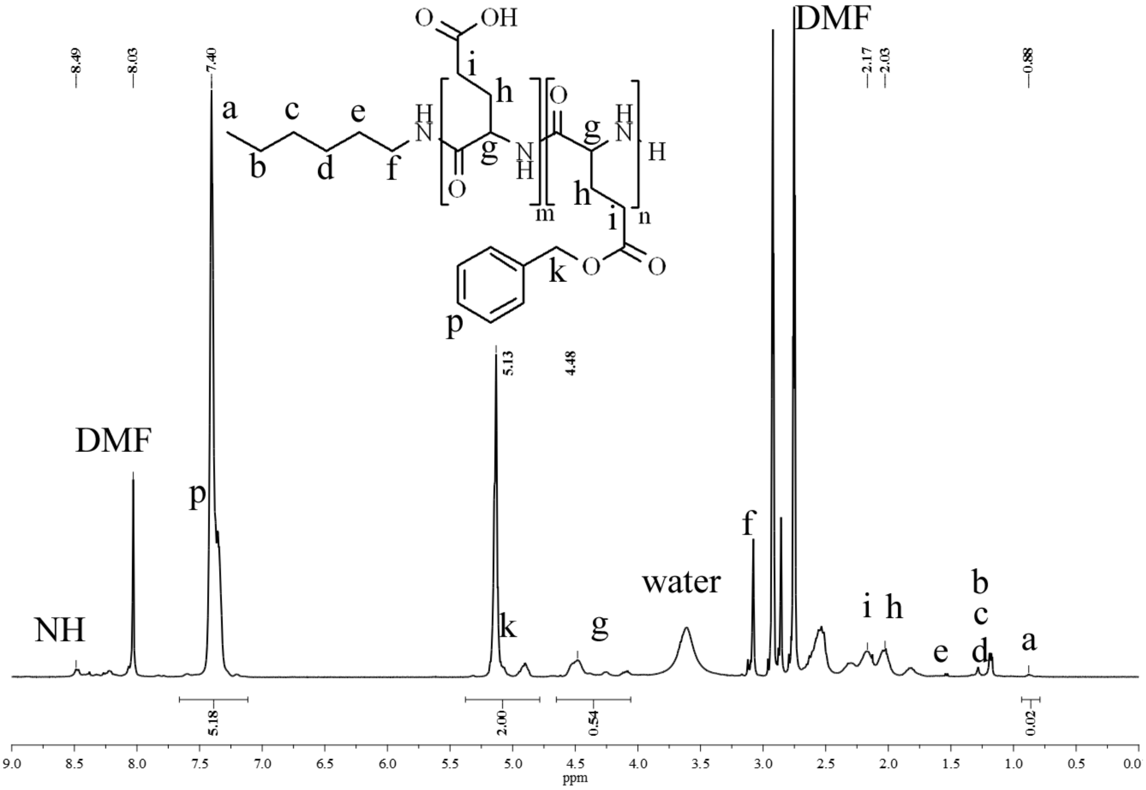


Figure S1 ^{1}H NMR spectrum of high molecular weight poly(glutamic acid). Degree of polymerization was calculated as the ratio of areas $\boldsymbol{g/\left(\frac{1}{3}a\right)}$. The amount of residual benzyl-groups was determined as the ratio of areas $\boldsymbol{g/\left(\frac{1}{2}k\right)}$.

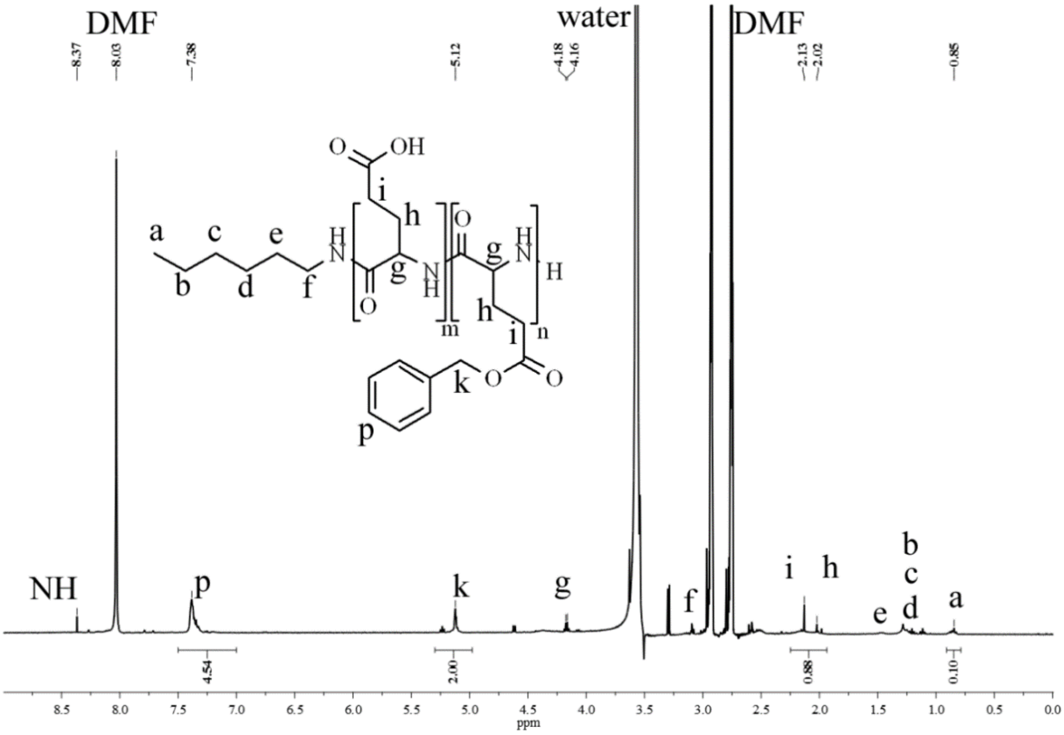
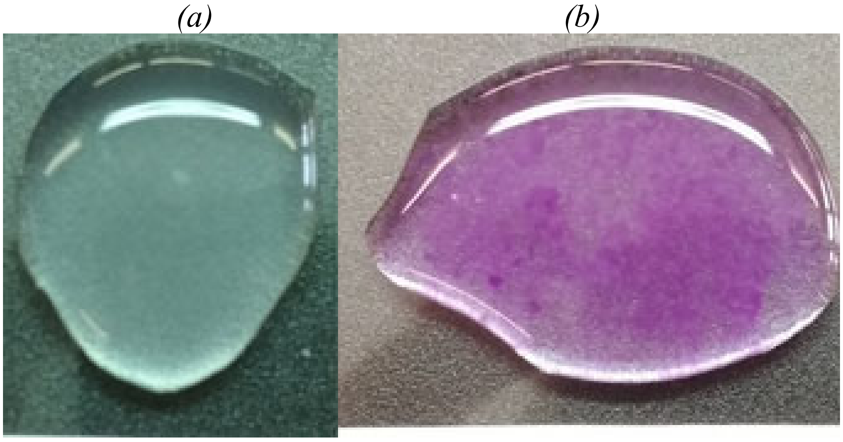


Figure S2 ^{1}H NMR spectrum of low molecular weight poly(glutamic acid). The degree of polymerization was calculated as the ratio of areas $\left[\frac{1}{4}(\boldsymbol{h}+\boldsymbol{i})\right]/\left(\frac{1}{3}\boldsymbol{a}\right)$. The amount of residual benzyl-groups was determined as the ratio of areas $\left[\frac{1}{4}(\boldsymbol{h}+\boldsymbol{i})\right]/\left(\frac{1}{2}\boldsymbol{k}\right)$.



(a) *(b)*

Figure S3 Suspension of nanocrystalline cellulose stained with Schiff's reagent (specific reaction for aldehydes): (*a*) NCC before oxidation and (*b*) NCC after oxidation with sodium periodate. The procedures for glucose oxidation with sodium periodate and staining of adehydes with Schiff's reagent can be found in our previous work.[S11]

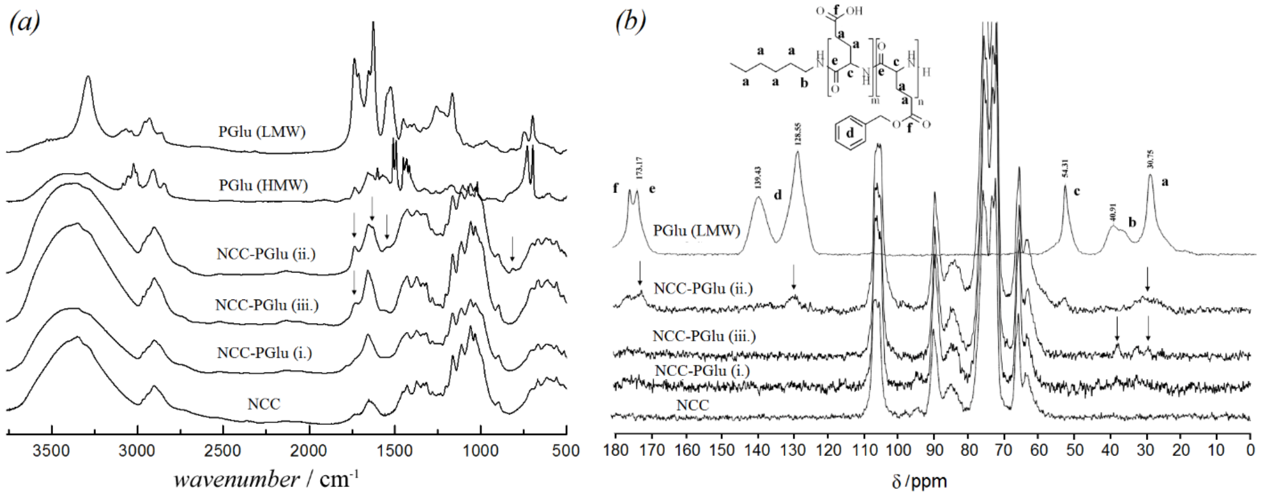


Figure S4 (*a*) FTIR and (*b*) ^{13}C NMR spectra registered for starting polymers and NCC modified with PGlu *via* different routes. The routes are indicated as in the text and Scheme 1 (main text) as i, ii and iii.

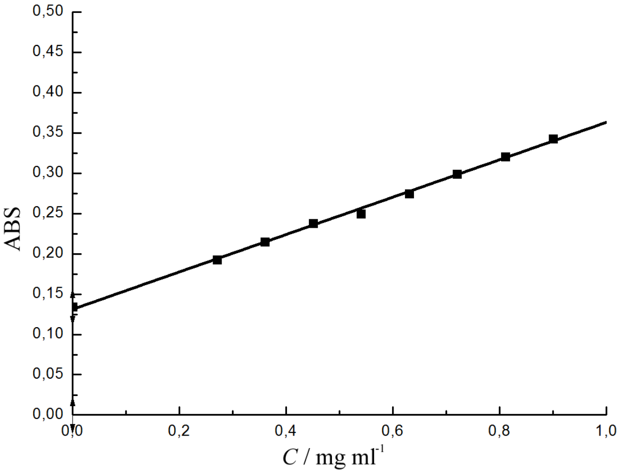


Figure S5 Calibration curve of poly(glutamic acid) (specimen 2) in DMF–0.1 M borate buffer pH 8.5 (75:25 vol.) at wavelength 265 nm.

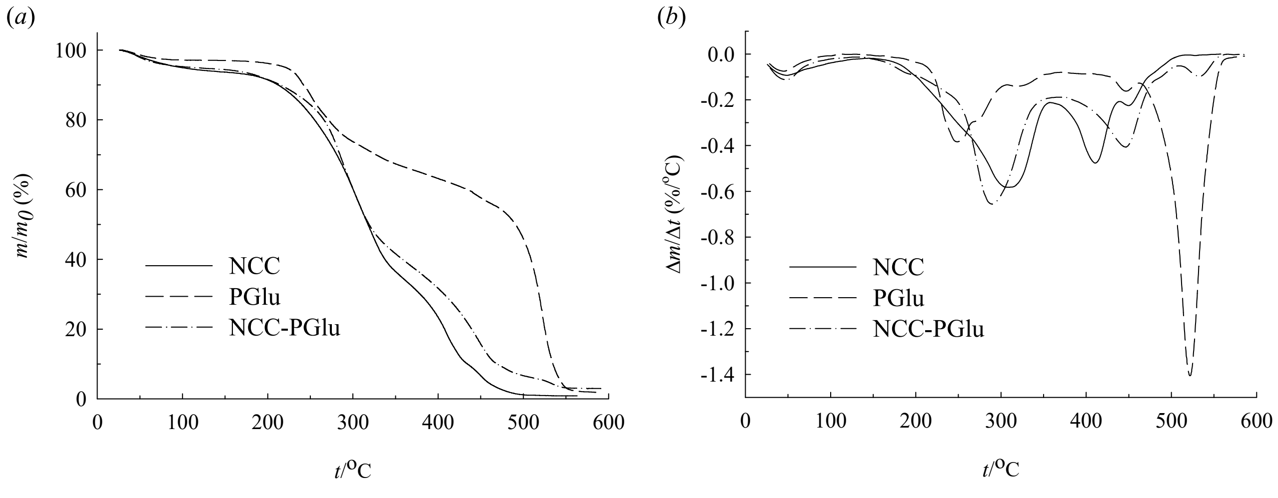


Figure S6 (*a*) TGA and (*b*) DTG curves for NCC–PGlu, NCC and PGlu (LMW) samples.

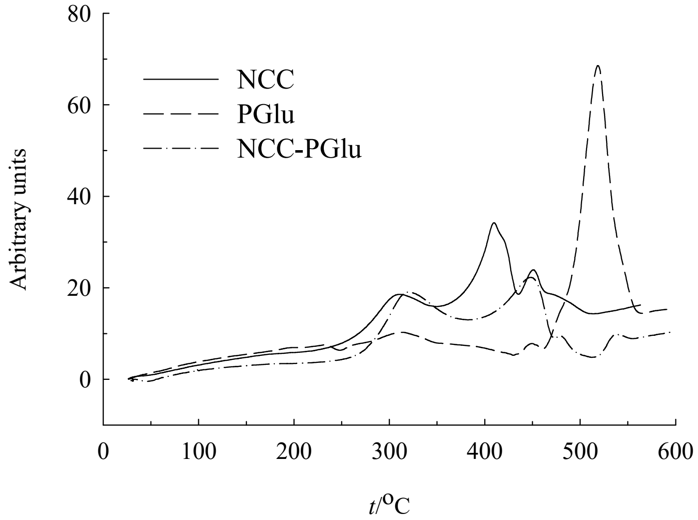


Figure S7 Differential thermal analysis (DTA) for NCC, PGlu and NCC modified by PGlu.

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