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First comb-like copolymer of poly(ethyl 2-cyanoacrylate) grafted as a side-chain to dextran

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^{1}H NMR spectra were recorded on a Bruker Avance-400 pulse spectrometer (operating frequency 400.13 MHz) in DMSO-d_6. ^{13}C NMR spectra were recorded in D_2O on a Bruker Avance 500 Spectrometer ("Bruker", Switzerland).

MALDI-TOF mass spectra were recorded using an Ultraflex II (Bruker, Germany) in positive ion reflector mode with an accelerating voltage of 25 kV, desorption was performed with a Nd:YAG laser, wavelength 355 nm using a 1000-fold excess of dihydroxybenzoic acid as the matrix.

Infrared spectra of the obtained compounds were recorded using KBr pellets on a Thermo Scientific Nicolet FT-IR spectrometer (USA) with a resolution of 4 cm^{-1} in the wavenumber range from 400 to 4000 cm^{-1} at room temperature with 128 scans. The results were processed using OMNIC software.

Synthesis of poly(ethyl 2-cyanoacrylate)-dextran comb-like graft copolymer

In a 50 ml conical flask, 1 g (6.2 mmol) of dextran (M_w=35±5 kDa) and 0.01 g (0.05 mmol) of citric acid were dissolved in 10 ml of water while stirring on a magnetic stirrer in an ice bath. After dissolution, 100 µl (0.1 g, 0.8 mmol) of ethyl 2-cyanoacrylate was gradually added dropwise from a dispenser. The reaction was stirred on a magnetic stirrer for 8-12 hours. After that, it was purified by dialysis against water (dialysis membrane M-Cel MWCO 1 kDa, Viskase, USA) until pH=7, then lyophilized.

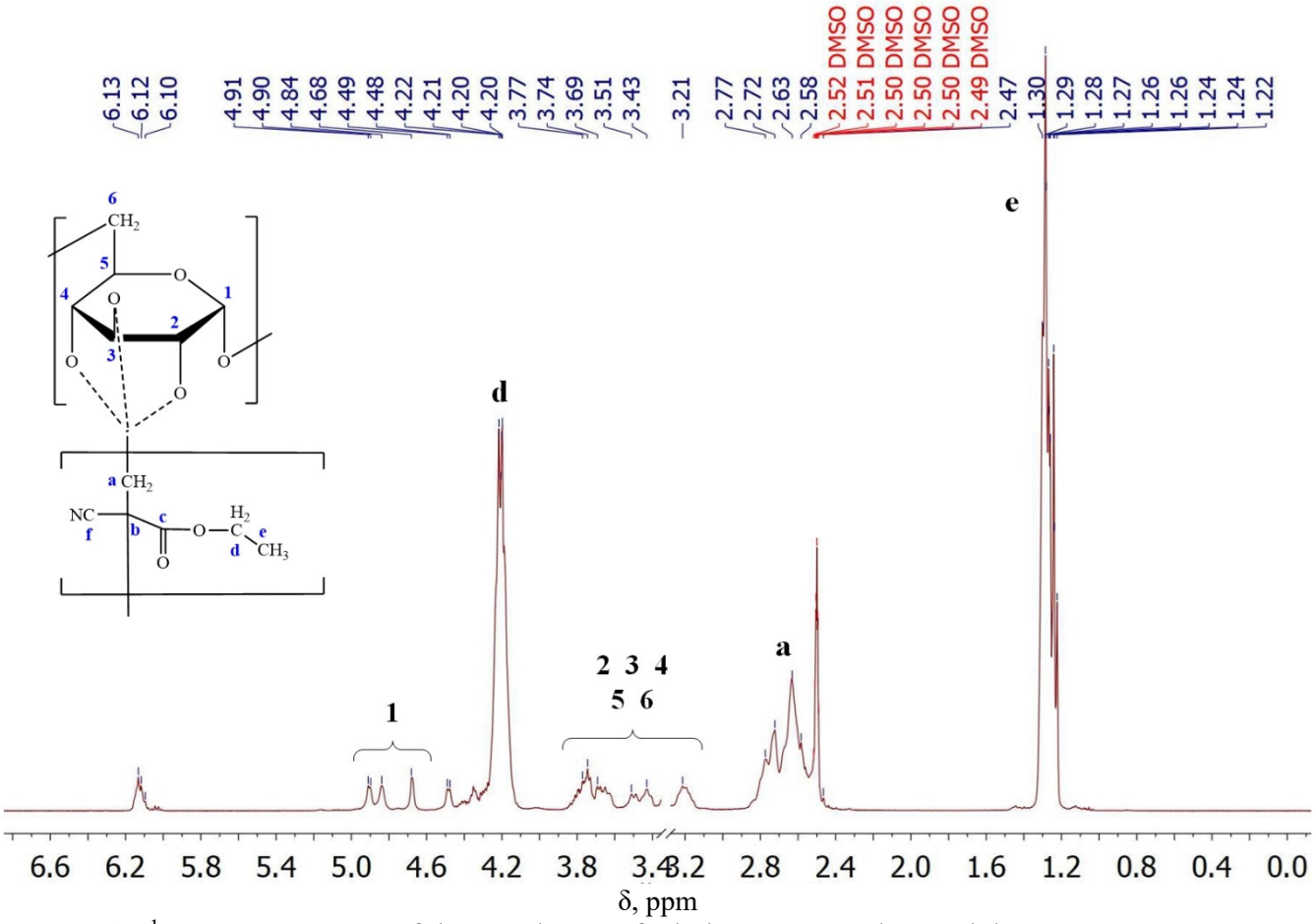

Figure S1. ^{1}H NMR spectrum of the copolymer of ethyl 2-cyanoacrylate and dextran

In the spectrum **S1** of the dextran copolymer with ethyl 2-cyanoacrylate, signals indicate the presence of both components in the copolymer structure. The signals at 4.84 and 4.90 ppm correspond to the anomeric protons at the C^1 carbon atom of the anhydroglucose units. However, the spectrum also shows a signal at 4.68 ppm, which suggests that the attachment of ethyl cyanoacrylate segments to the OH groups of dextran may occur via the hydroxyl group at the C^2 carbon atom of the anhydroglucose ring, resulting in the shift of the anomeric proton signal to the downfield region by 0.12 ppm.

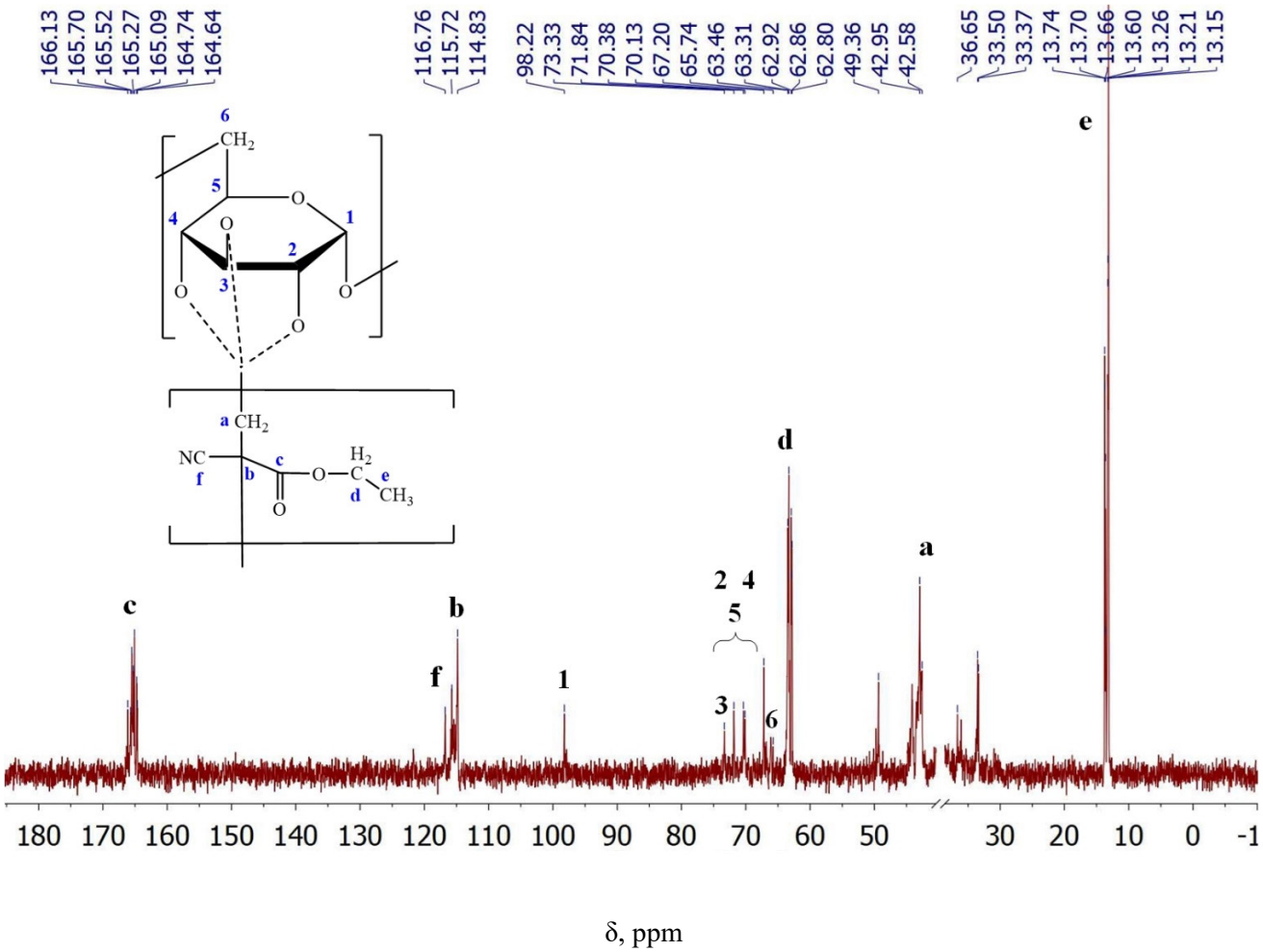

Figure S2. ^{13}C NMR spectrum of the copolymer of ethyl 2-cyanoacrylate and dextran

The ^{13}C NMR spectrum **S2** of the ethyl cyanoacrylate and dextran copolymer (recorded in DMSO-D6) exhibits peaks of carbonyl groups (C=O) in ethyl-2-cyanoacrylate segments, connected in a “head-to-head” and “head-to-tail” manner, in the region of 166.0-167.0 ppm. The signal at 116.16 ppm corresponds to the cyano groups (C≡N) present in the ethyl cyanoacrylate structure. Signals in the region of 98.71 ppm, as well as in the range of 65.0-70.0 ppm, indicate the presence of methoxyl (CH_2-O) and CH-OH groups of dextran. The region of 20.0-50.0 ppm relates to methyl (CH_3) groups of the ethoxy radical and methylene (CH_2) groups of main chain poly(2-cyanoacrylate) and methylene groups (CH_2) of the anhydroglucose units of dextran.

Thus, the spectra of the copolymer exhibit signals from PECA fragments grafted to the polysaccharide chain of dextran. However, IR and NMR spectroscopy data alone are insufficient to prove the comb-like structure of the polymer. The spectra fail to register signals of carbon atoms in anhydroglucose units caused by the attachment of PECA chains, and the terminal group signal of the PECA homopolymer does not differ from the signal of the grafted comb-like block copolymer.

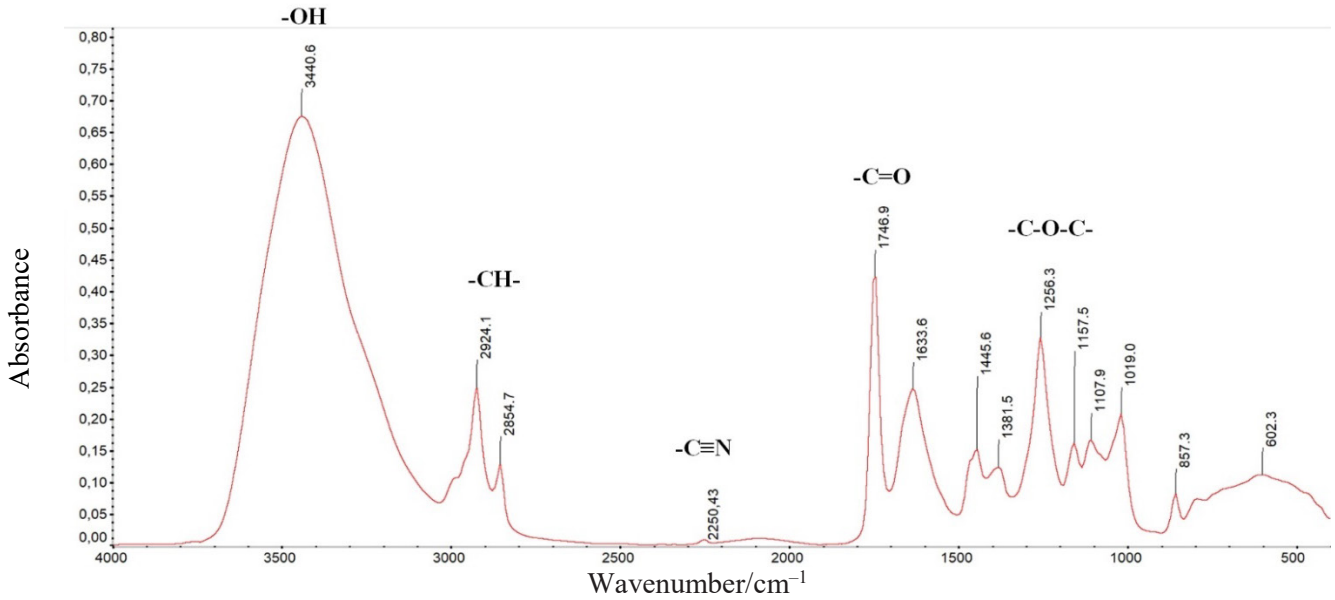

Figure S3. IR spectrum of the copolymer of ethyl 2-cyanoacrylate and dextran

Determination of cytotoxicity of substances by MTT test on human normal cell culture *HEK293*

For the analysis of cytotoxicity of polymer compositions, *HEK 293T* cells were cultured in DMEM medium with high glucose and L-glutamine (Capricorn Scientific GmbH, Germany) supplemented with 10% fetal bovine serum. *HEK 293T* cells were detached from the substrate using a 0.25% trypsin solution, seeded into a 96-well plate at a concentration of 1×10^4 cells per 100 µl, and incubated for 24 hours at 37 °C and 5% CO_2.

After 24 hours of incubation, various concentrations of the test compounds were added to the *HEK293* cell culture by titration (with a two-fold dilution step). Each concentration was tested in triplicate.

After 24 hours of incubation of the *HEK293* cell line with the test substances, 20 µl of the working MTT solution was added to each well. The plates were incubated for 3 hours in a CO_2 incubator. After 3 hours, the MTT solution in the medium was aspirated from the wells, followed by the addition of a DMSO solution. The optical density of each well at 555 nm was determined using an iMark microplate reader from Bio-Rad Lab. Inc., USA, with background absorption measured at 620 nm.

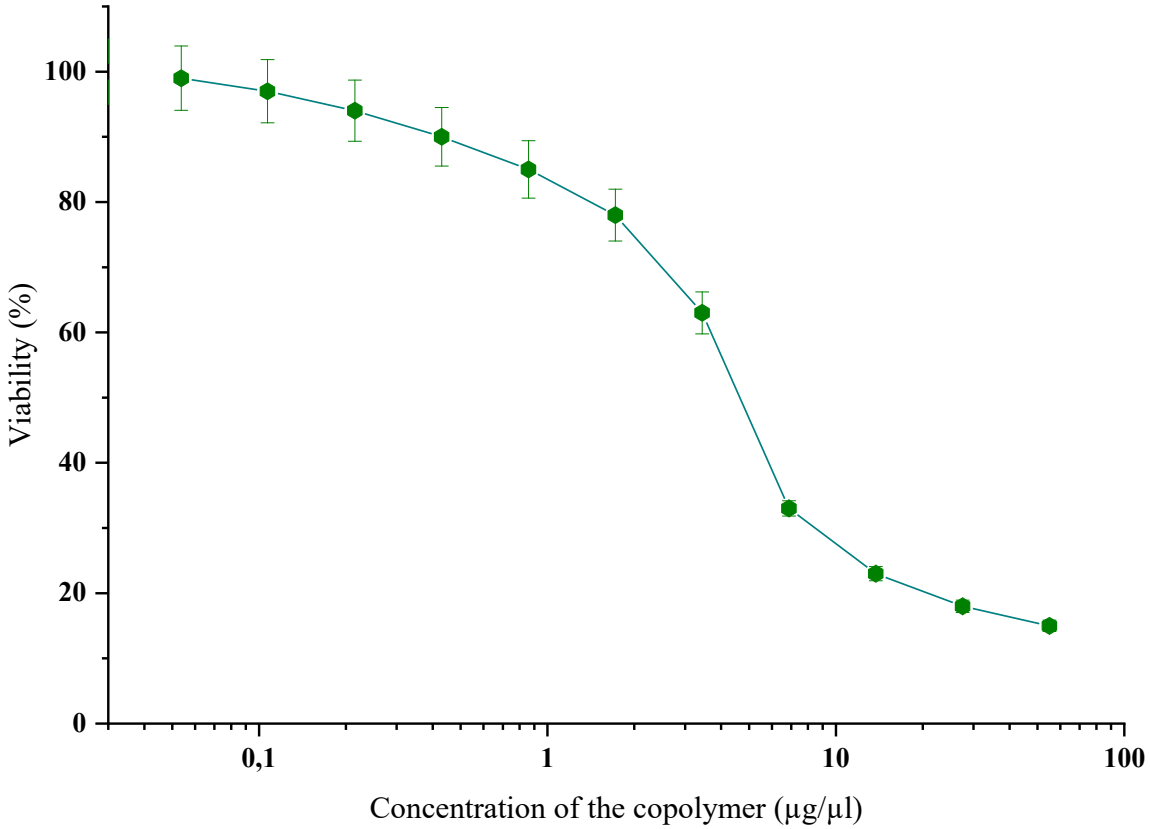

Figure S4. The dependence of cell survival on the concentration of the copolymer of ethyl 2-cyanoacrylate and dextran