Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Thermo-responsive Polyurethane Organogel for Norfloxacin Delivery

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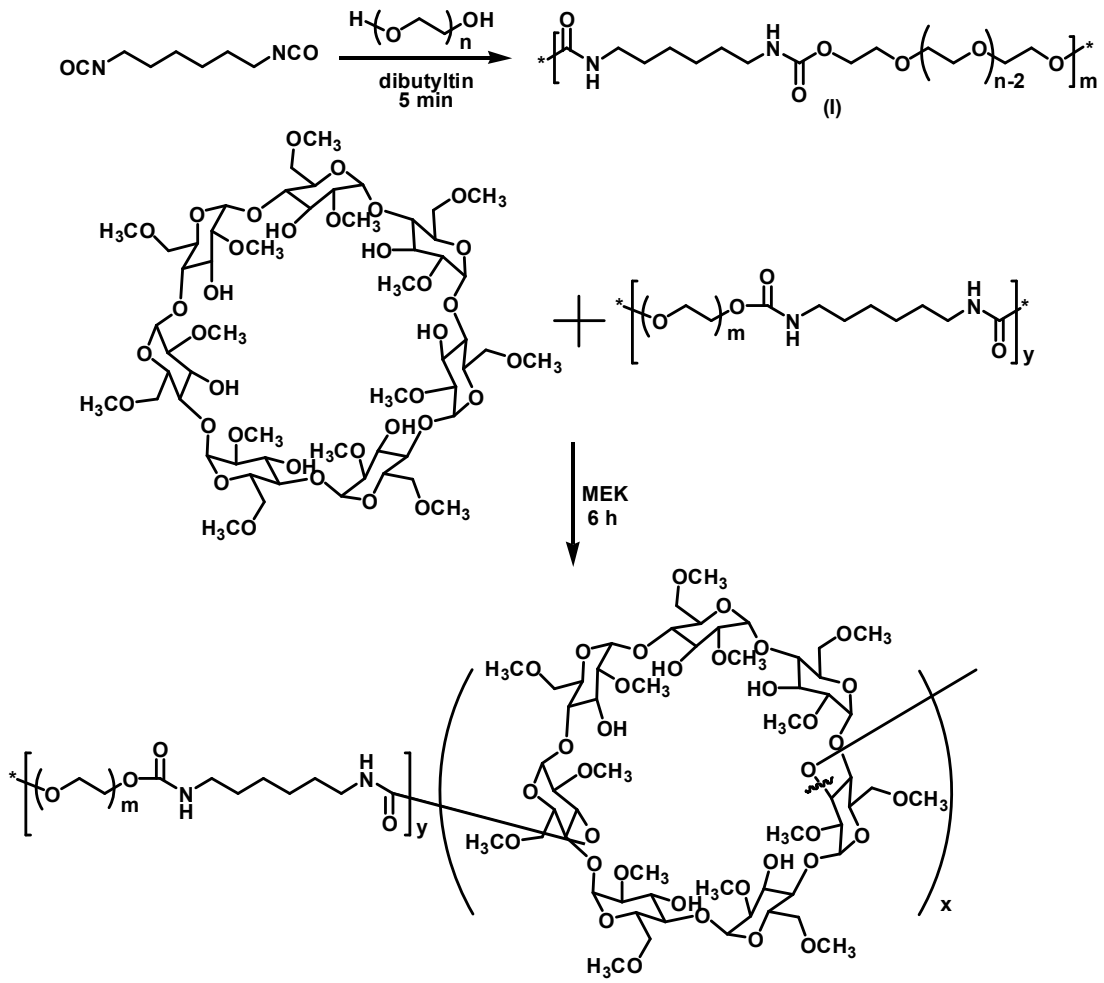
1. Experiments:

1.1 Cell viability assays

Cell viability was detected using Cell Counting Kit-8 (CCK8) assay. L929 cells were cultivated in a humidified 5% carbon dioxide atmosphere at 37 ^{o}C on a 96-well microplate, with 5000 cells immersed in complete growth medium per well. The cells were allowed to attach for 24 h. Subsequently, the PUs with the concentrations of 0.1, 0.2, 0.25, 0.5, and 1.0 mg/mL were added to 96-well plates at 10 μL per well and incubated for 48 h, respectively. The solution was then removed and replaced with 100 μL of RPMI-1640. Then, CCK-8 solution was added to 96-well plates at 10 μL per well and incubated for 0.5 h, and the resulting solution was analyzed at 450 nm by means of a plate reader with a background correction using a Bio-Tek FLx800 Fluorescence Microplate Reader. This process was repeated for eight times in parallel. The results are expressed as the relative cell viability (%) with respect to control wells containing culture medium.

1.2 Mouse wound infection model

To evaluate the *in vivo* antibacterial effect of organogels, the *S. aureus* infection model was built. Hair from the back of each mouse was shaved with a finetooth electric clipper and the exposed skin was burnished with a scraper to ooze blood. The size of the wound was consistently 1×1 cm. Mice were not anaesthetised when the trauma was induced. Each wound was inoculated with 50 μL of the indicated bacteria suspended in the LB broth (the concentration was approximately 5×10^6 CFU/mL). These samples were extracted with 100 mL and coated on a Petri dish after 16 h. Finally, we count the colony of bacteria as the ultimate content after 24 h of incubation. For each study, it included an uninfected group that did not receive any treatment, one group that received singly drug NF, and two groups that received NF-loaded PEG4000-HDI-CD/PEG4000-HDI organogels (100 mg mL^{-1}), respectively, at the same dosages as the NF treated groups. For the high dosage group, the concentrations of NF were 20 μg mL^{-1}, while the concentrations of NF were 10 μg mL^{-1} for the low dosage group. Drugs were applied (0.3 mL) at 4 h after inoculation, twice every day (drug delivery every 12 h). On the third day for postwounding, animals were euthanized by cervical dislocation and the wounds were assessed. Each wounded skin was removed from the animal and then measured for weight, with unwounded skin as the blank control and wounded skin without applying drug taken from the back as the negative control. For each group, tissue samples were removed from the wounds after the rats were sacrificed 4 days upon the treatment for pathology observations. After fixation with 4% phosphate-buffered formaldehyde for at least 24 h, the specimens were embedded in paraffin and sectioned into a thickness of 10 μm. The samples underwent routine histological processing with hematoxylin and eosin. The samples then were observed under a microscope.



Scheme S1. Synthetic scheme of PEG-HDI-CD.

2. Results and Discussion:

2.1 Cell viability assays

The in vitro cytotoxicity of the polymers is an extremely important factor for their potential application as drug delivery vehicles. In this work, the in vitro cytotoxicity of the PUs of PEG4000-HDI and PEG4000-HDI-CD were evaluated against L929 cells as a function of the concentrations using CCK8 assays (Fig. S3). All the samples retained high cell viability (>90%) after 48 h of incubation at all tested concentrations up to 1.0 mg/mL. Some average cell viabilities were a little bit larger than 100% (about 105%) in Fig. S3, probably due to the experimental error of the data, with standard deviation of ~10%. Their low cytotoxicity further highlighted the PUs could be used as biomedical materials.

2.2 In vitro drug release

Pertaining to the distinctive gel to sol states transition of the organogel responsive to

the incubation temperature, it could be assumed that organogel should present a marked promoted drug release from organogel in 1,3-propanediol at 37 °C compared to that at 4 °C. To verify this assumption, the release profile of NF was investigated for the PUs/NF organogel at 4 °C and 37 °C in 1,3-propanediol. The drug loading capacity was determined as 1% for PU-based organogels in this study even though a high drug loading can be realized if the drug solubility in the organic solvent can be improved. In consistent with our speculations, the drug release rate was slow, where merely 35% and 28% of NF for PEG4000-HDI-CD and PEG4000-HDI were confirmed to release at 4 °C upon 72 h post incubation, respectively (Fig. S2). On the contrary, the drug release rate was observed to be accelerated, where the cumulative drug release of 92% and 75% was confirmed for PEG4000-HDI-CD and PEG4000-HDI at 72 h post incubation at 37 °C, markedly higher than that at 4 °C. Apparently, this pronounced temperature-dependent drug release profile is crucial interesting and importance for practical applications, where the minimal release rate at low temperature enables the preservation of organogel in the refrigerator, and the accelerated drug release profile once placed onto the skin enables the performance of the therapeutic potency. During the observation period, PEG4000-HDI-CD exhibited the faster release rate than that of PEG4000-HDI *in vitro*. The large space steric hindrance of CD may disturb the formation of compact physical crosslinking, as a consequence, more NF in CD-based PUs could be released. At the same time the introduction of CD can provide cavities for the effectively escape release from the entangled organogel three-dimensional network. Due to the interesting temperature-responsive transition property, the prepared organogels can be used as drug carriers with extended preservation time at low temperatures.

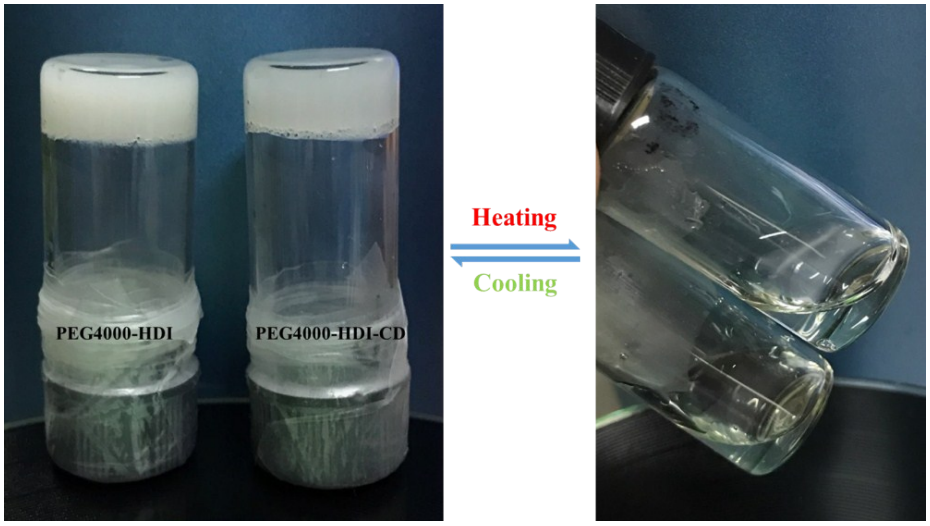


Fig. S1 The macroscopic images of organogel in 1,3-propanediol (100 mg/mL) at 4 °C and 37 °C.

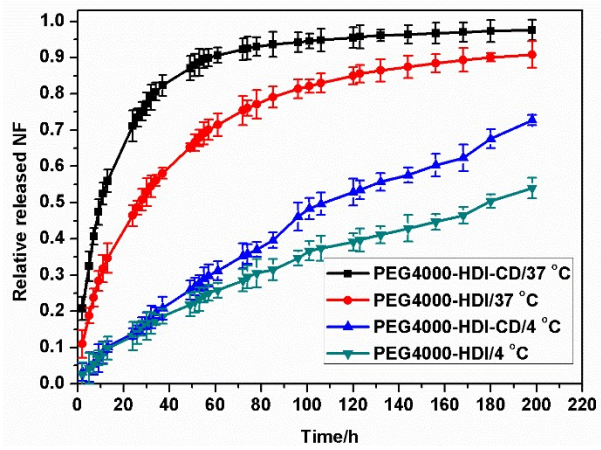


Fig. S2 Cumulative release profiles of NF *in vitro* from PEG4000-HDI and PEG4000-HDI-CD based organogel at 4 °C and 37 °C.

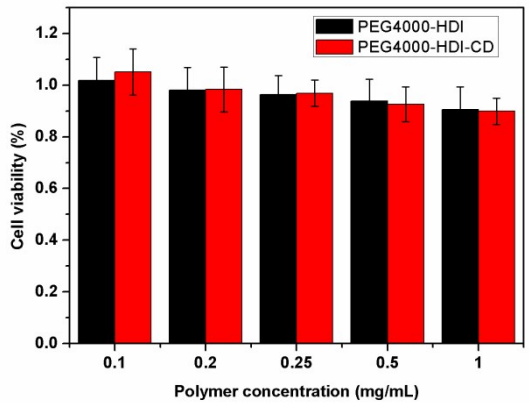


Fig. S3 Cell viabilities of different polymers against L929 cells. The cells were treated with increasing concentrations of polymers, incubated for 48 h before analysis by CCK8 assay.

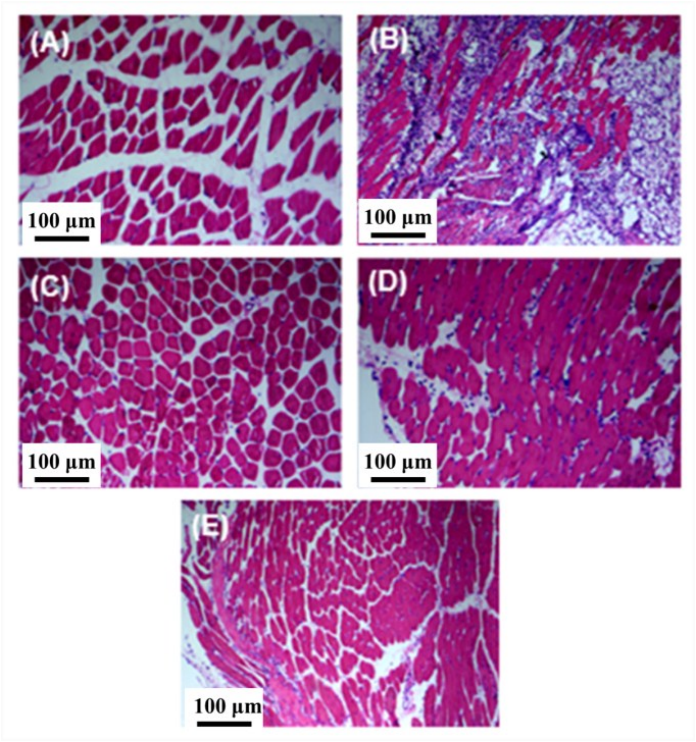


Fig. S4 Study on the effects of *S. aureus*-induced wound infections for 3 days (n = 7) *in vivo*: (A) normal mice group, (B) blank control group, (C) free NF group, (D) PEG4000-HDI-CD/NF inclusion complex group and (E) PEG4000-HDI/NF inclusion complex group.

Table S1. Gelation behavior of PUs in a variety of solvents.

solvent	PUs without CD	PEG2000-HDI-CD	PEG4000-HDI-CD
n-Pentane	P	P	P
Hexane	P	P	P
Benzene	S	S	S
Toluene	I	I	I
Dioxane-1,4	S	S	S
Dichloromethane	S	S	S
Ether	I	I	I
Petroleum ether	I	I	I
Ethyl Oleate	P	P	P
Ethyl acetate	P	P	P
Ethanol	S	S	S
Octanol	G	G	G
Ethylene glycol	G	G	G
1,3-Propanediol	G	G	G
Oleic acid	G	G	G

I, insoluble; S, soluble; P, precipitation; G, gelation.