pharmaceutics

# Supplementary Materials: Polymer Nanomedicines with Ph-Sensitive Release of Dexamethasone for the Localized Treatment of Inflammation

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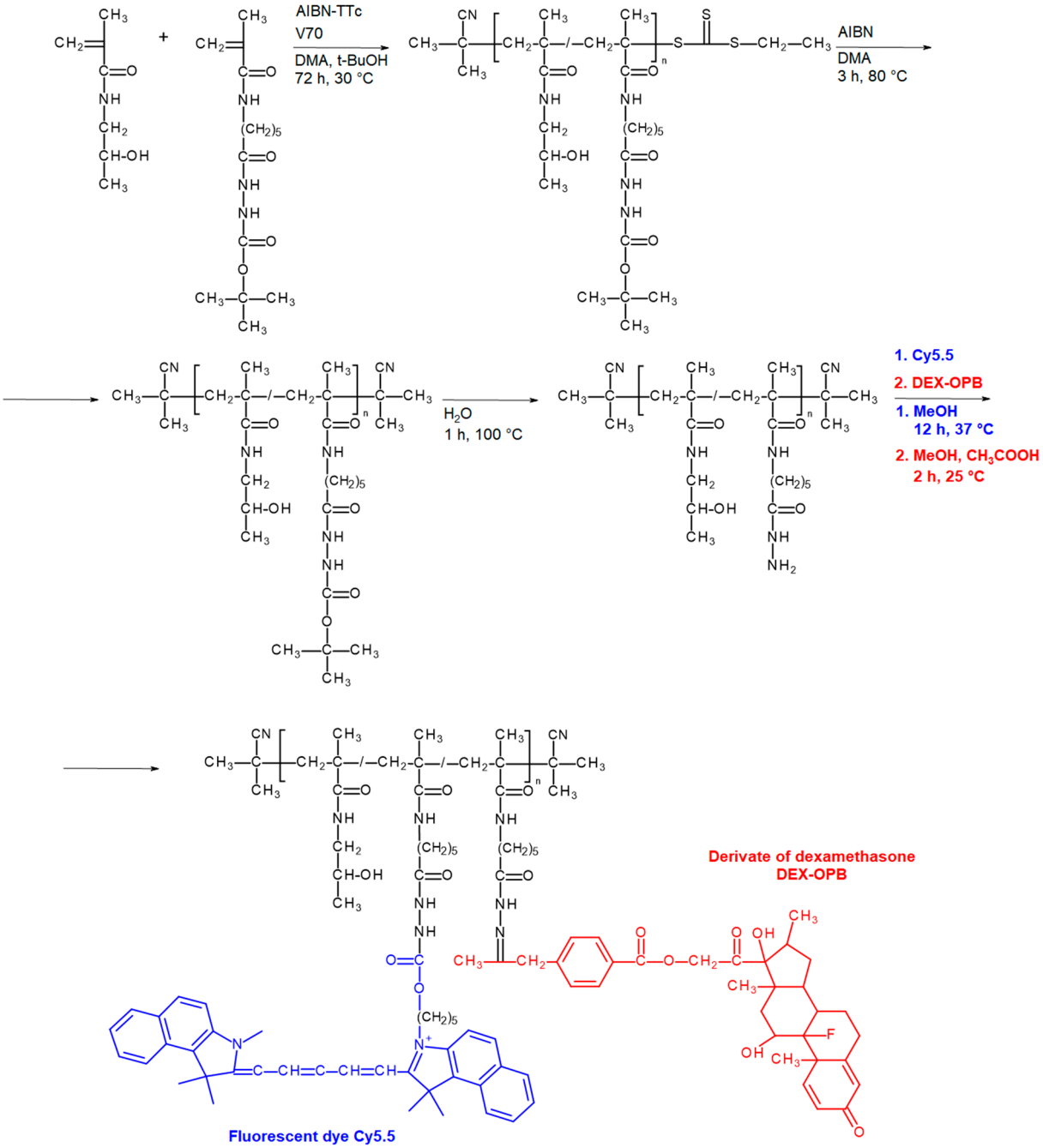


**Figure S1.** Reaction scheme of the preparation of the polymer conjugate with the fluorescent dye (Cy5.5) and derivate of dexamethasone (DEX-OPB).

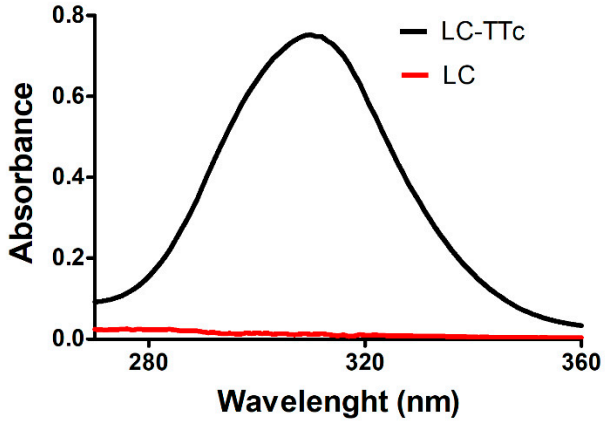


**Figure S2.** Disappearance of the UV/VIS signal of the TTc functional group of the polymer precursor LC-TTc (black) and the polymer precursor LC after reaction with AIBN (red).

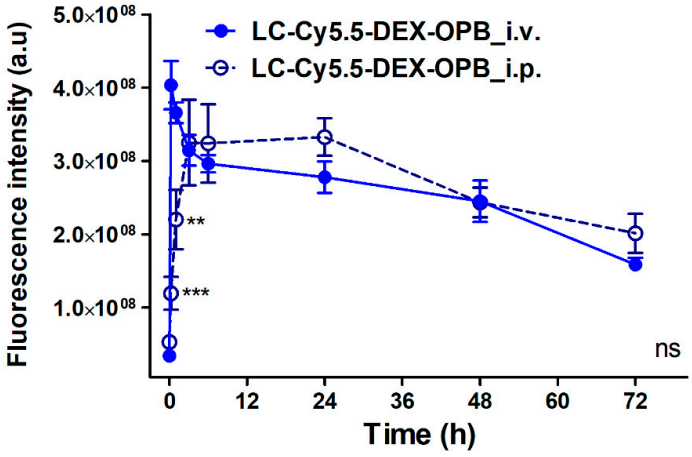


**Figure S3.** In vivo fluorescence data from the healthy leg of the linear copolymer with the fluorescent dye and the dexamethasone injected i.v (LC-Cy5.5-DEX-OPB_i.v.) or i.p (LC-Cy5.5-DEX-OPB_i.p.). Injection of the 0.1 mg Cy5.5 eq/kg, $n = 6$.

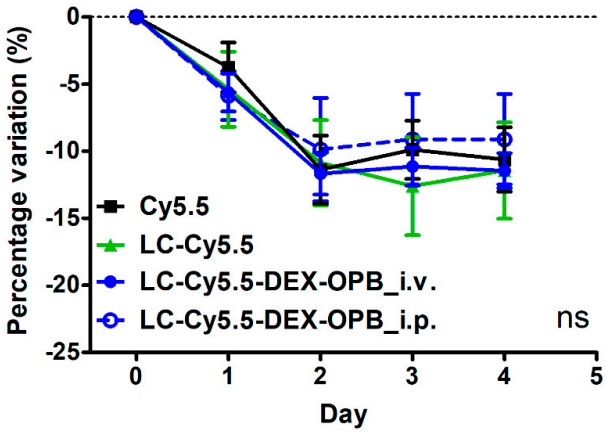


**Figure S4.** Decrease in the weight of the mice during the biodistribution experiment. Injection of the 0. 1 mg Cy5.5 eq/kg, $n = 6$.

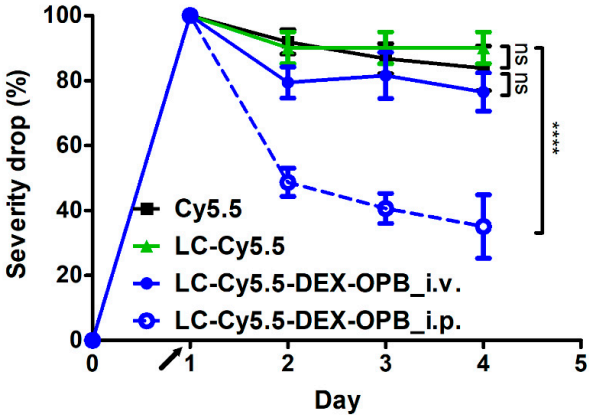


**Figure S5.** Decrease of the clinical severity (CS) of the inflammation of the mice, tarsus, during the biodistribution experiment. Injection of the 0.1 mg Cy5.5 eq/kg, $n = 6$.

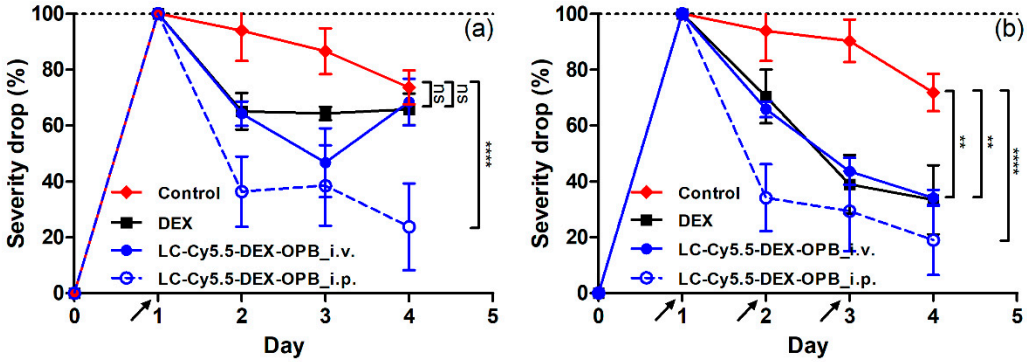


**Figure S6.** Decrease of the CS of the inflammation of mice, tarsus, in the therapeutic experiment of the DEX and the LC-Cy.5.5-DEX-OPB injected i.v. and i.p. in the experiment with two different dosings 1 × 3 mg DEX eq/kg (**a**) and 3 × 1 mg DEX eq/kg. (**b**) $n = 6$.

## ROS determination

One key feature of the synovial joints in RA is a higher ROS content detected in affected tissues compared to normal tissue [1]. The excessive amount of ROS promotes the production of cytokines, such as TNF-$\alpha$, IL-1$\beta$, and IL-6, and the interactions between inflammatory cytokines and ROS would accelerate RA progression [2]. Moreover, elevated levels of ROS in RA can activate metalloproteinases, inhibit cartilage proteoglycan synthesis, promote chondrocyte apoptosis, ultimately leading to disruption of the cartilage and bone tissue [2].

The measurement of the bioluminescence originating from the reaction of the luminol with ROS of neutrophils was performed daily to monitor the therapy course, see Figure SI7. In the one-day dosing scheme, no significant differences between treated groups were observed. However, the daily injection of the DEX showed a significant increase in the signal with a peak after 48 h. This effect could be caused by the increased output of the neutrophils [3], a side effect of the treatment with free DEX. The application of LC-Cy5.5-DEX-OPB (injected i.v. or i.p.) does not exhibit such side effects. The detailed effect of polymer conjugate on healthy tissues will be studied further.

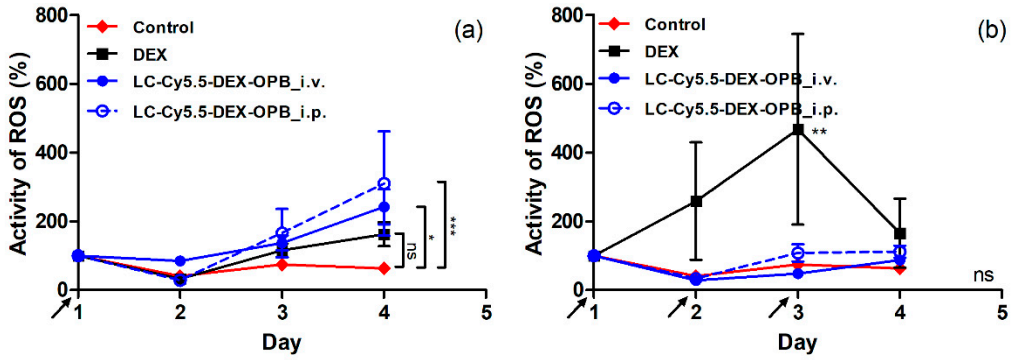


**Figure S7.** Activity of ROS in two experiments with different dosing. (**a**) 1 × 3 mg DEX eq./kg; (**b**) 3 × 1 mg DEX eq/kg. $n = 6$.

## References


1. Davis 4th, R.W.; Snyder, E.; Miller, J.; Carter, S.; Houser, C.; Klampatsa, A.; Albelda, S.M.; Cengel, K.A.; Busch, T.M. Luminol Chemiluminescence Reports Photodynamic Therapy-Generated Neutrophil Activity In Vivo and Serves as a Biomarker of Therapeutic Efficacy. *Photochem. Photobiol.* **2019**, *95*, 430–438, doi:10.1111/php.13040.
2. Ni, R.; Song, G.; Fu, X.; Song, R.; Li, L.; Pu, W.; Gao, J.; Hu, J.; Liu, Q.; He, F.; et al. Reactive oxygen species-responsive dexamethasone-loaded nanoparticles for targeted treatment of rheumatoid arthritis via suppressing the iRhom2/TNF-α/BAFF signaling pathway. *Biomaterials* **2020**, *232*, 119730, doi:10.1016/j.biomaterials.2019.119730.
3. Ohkaru, Y.; Arai, N.; Ohno, H.; Sato, S.; Sakakibara, Y.; Suzuki, H.; Aritoshi, S.; Akimoto, S.; Ban, T.; Tanihata, J.; et al. Acute and subacute effects of dexamethasone on the number of white blood cells in rats. *J. Heal. Sci.* **2010**, *56*, 215–220, doi:10.1248/jhs.56.215.