# Supporting Information for "Cyclodextrin/dextran based drug carriers for a controlled release of hydrophobic drug in zebrafish embryos"

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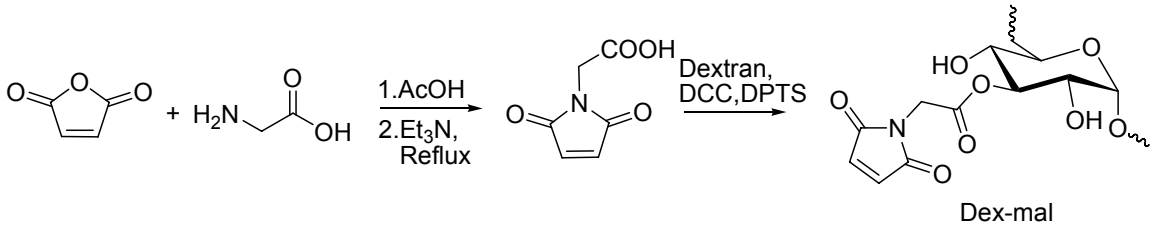
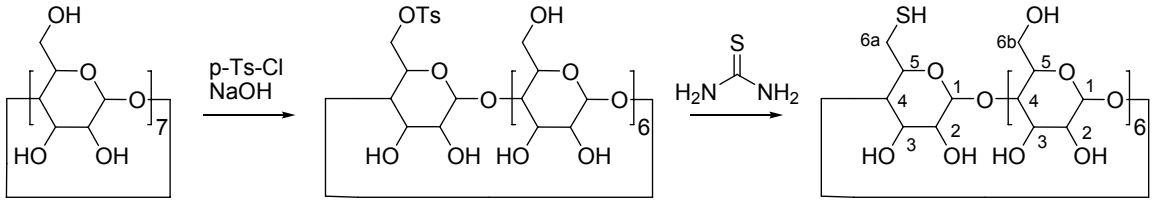
**Synthesis.**

**Preparation of the maleimide modified dextran (Dex-mal).**



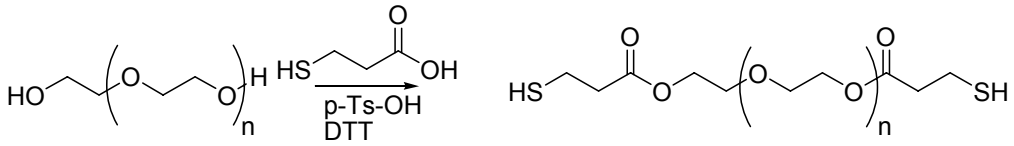

Maleimide modified dextran (Dex-mal) with a degree of substitution (DS) of 14 was synthesized according to our previously reported procedure.[1] $^1$H NMR (400 MHz, $D_2O$): δ 3.2-4.0 (m, dextran glucosidic protons), 4.4 (maleimide), 4.9 (dextran anomeric proton), 6.9 (maleimide).

**Preparation of the mono-6-thio-β-cyclodextrin (MSCD).**




Mono-6-thio-β-cyclodextrin (MSCD) was obtained by a two step reaction according to literature.[2] $^1$H NMR (400 MHz, DMSO-$d_6$): δ 2.1 (t, SH), 2.7–3.2 (m, 2H, H-6a), 3.2–3.5 (m, overlapping with HDO, H-2, H-4), 3.5–3.8 (m, 26H, H-3, H-5, H-6b), 4.8 (br d, 7H, H-1), 5.7 (br, OH).

**Preparation of the dithiol poly(ethylene glycol) (DSPEG).**




α–ω Di-thiol modified polyethylene glycol (DSPEG) with a molecular weight of 2,000 was prepared according to the reported procedure. $^1$H NMR ($CDCl_3$): δ 1.7 (t, 2H,–

$CH_2CH_2SH$), 2.7-2.8 (m, 8H, –$CH_2CH_2$SH), 3.6-3.7 (PEG backbone) 4.3 (t, 4H,–$CH_2$OC(O)–). The Ellman test showed a thiol functionality of 90%.

**Macroscopic hydrogel**

Hydrogels were obtained by mixing solutions of Dex-mal and PSCD or DSPEG. CD-Gel was prepared by mixing Dex-mal (23 wt%) and PSCD (4 wt%) in phosphate buffered saline (PBS) solutions. PEG-Gel and CD-PEG-Gel were obtained by mixing the DSPEG solution (19 wt%) with either maleimide modified dextran (Dex-mal) or β-CD attached Dex-mal (CD-Dex-mal) solutions (22 wt%). Hydrogels were obtained within a minute. (In the resulting mixtures maleimide and thiol groups were contained in the ratio of 1:1.) Hydrogels were formed *in situ* via Michael addition between maleimide and thiol groups.

### *in vitro* release from macroscopic hydrogel

The *in vitro* release behaviors of a hydrophobic drug from the hydrogels were examined by using all-*trans* retinoic acid (RA) as a model compound. Hydrogels that carried RA were prepared and the RA released from the hydrogels was determined by HPLC. (see reference 15)

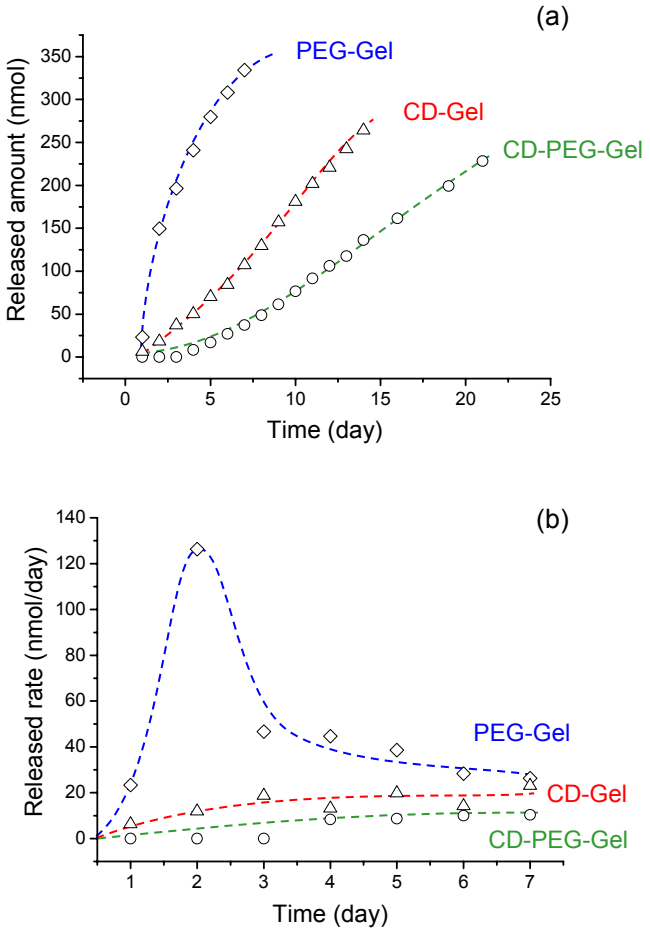



**Figure S1.** Release profiles of RA from the RA loaded hydrogels: (a) Cumulative release amount; (b) release rate of the RA per 24 hours. CD functionalized hydrogels exhibited a controlled release without showing an initial rapid release phase. CD moiety acted as a binding site for the hydrophobic drug and played an essential role to control the release of the hydrophobic drug.

**Viscosity measurement of the injectable hydrogel.**

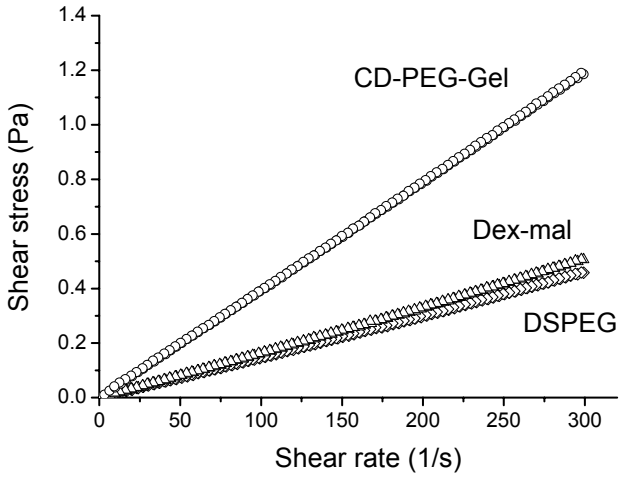



**Figure S2.** Viscosity measurements of the DSPEG and Dex-mal solution before and after mixing. The viscosity of the resulting CD-PEG-Gel particles solution was 3.9 mPa·s which was in the same range of initial solutions (1.7 and 1.5 mPa·s for Dex-mal and DSPEG, respectively). The linear relationship between the shear rate and the stress showed the absence of an extended polymer network.

**Other SEM images of the injectable hydrogels.**

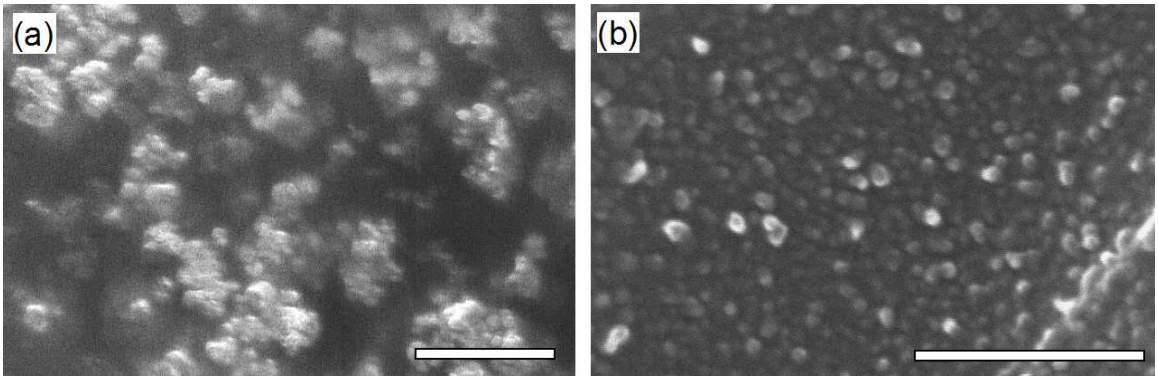



**Figure S3.** SEM images of the CD-Gel (a) and CD-PEG-Gel (b) prepared in pure water. Scale bars in (a) and (b) represent 2 μm and 200 nm, respectively.

**Position of the hydrogel inside the embryos.**

In order to track the position of the injected hydrogel inside zebrafish embryos, the CD-PEG-Gel particles labeled with fluorescein-5-maleimide was injected. As shown in Figure S4, after 30 hours from injection into the yolk of embryos at the one cell stage, the fluorescent gel particles were situated in the yolk sac.

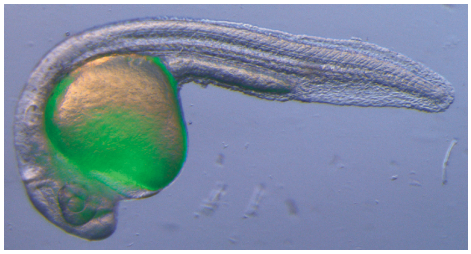


**Figure S4**. The fluorescent image is superimposed onto the bright-field image. The injected carriers are localized in the yolk sac. The embryo is shown in lateral view, anterior to the left.