

iGEM TU/e 2018
Biomedical Engineering

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Synthesis of Dextran Hydrogel

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Synthesis
of Dextran
Hydrogel

1 Synthesis of Methacrylated Dextran

Estimated bench time: 60 minutes

Estimated total time: 1 week

Purpose: Methacrylation of dextran polymer

1.1 Materials

- Dextran T500
- DMSO
- DMAP
- GMA
- HCl (1M)
- Round bottom flask
- Stirring bean & plate
- SnakeSkin Dialysis bag (cutoff 10 kDa)
- NMR tube
- D2O

1.2 Setup & Protocol

Dextran T500 (500 kDa) (4 grams, ~ 0.0247 mol as glucopyranose unit) was dissolved in 36 ml DMSO and degassed with argon for 15 minutes. After dissolving 800 mg DMAP (0,006548 mol), 2.4 mL (0.0181 mol) GMA was added. The solution was stirred at room temperature for 44h and the reaction was stopped by adding 6.6 mL of 1M HCl (an equimolar amount) to neutralize the DMAP. The reaction mixture was transferred to SnakeSkin™ Dialysis bag (cutoff 10 kDa) and dialyzed against demineralised water at RT with changing water every 3 hours (during office hours) for 72 hours.

Next, the dex-MA solution was lyophilized and the dex-MA was stored at -30 °C before use. The degree of substitution (DS) of dex-MA was determined by $^1\text{H-NMR}$ spectroscopy (D2O) as $(100x)/y$, where x is the average integral of the protons at the double bond at δ 5.7 and δ 6.1 ppm and y is the integral of the anomeric proton at δ 4.8 ppm (see Results for NMR).

2 Synthesis of the Hydrogel

Estimated bench time: 60 minutes

Estimated total time: 10 hours

Purpose: Synthesize the dextran hydrogels.

2.1 Materials

- Dex-MA
- Ammonium persulfate
- TEMED
- Mold (desired shape)
- NaOH (0.05 M)
- Round bottom flask
- Stirring bean & plate

2.2 Setup & Protocol

An aqueous solution of dex-MA (5% w/v, 200 mg dex-MA in 4 mL H₂O) and ammonium persulfate (4.5 mg), was degassed with argon, stirred, and cooled in an ice bath for 15 min. From here on, all actions were performed at 4 °C. After the addition of 5 µL TEMED, the solution was mixed rigorously for 4-5 seconds and poured into the mold for freezing at -20 °C. After being kept frozen at -20 °C overnight, one frozen monolith was thawed at room temperature and washed with 20 mL milliQ and allowed to swell for 5 min. Next, the monolith was washed with 20 mL 0.05M NaOH and finally with 20 mL milliQ and stored in the refrigerator in a falcon tube with fresh demineralized water.