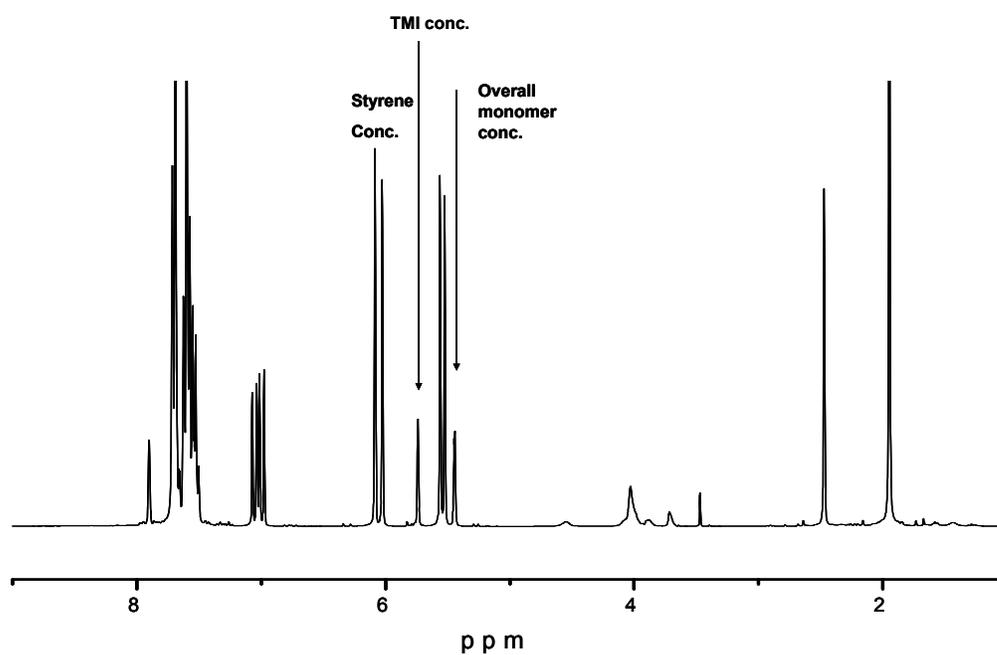


# Micelles with surface conjugated RGD peptide and cross-linked polyurea core via RAFT polymerization

*Hien T.T. Duong, T.L.Uyen Nguyen, Martina H.Stenzel\**

NMR of copolymerization



## Results of chain extension of POEGMA with STY and TMI

Time (h)	STY conversion (%) <sup>a</sup>	M <sub>n</sub> <sup>theo</sup> (STY) <sup>b</sup>	TMI conversion (%) <sup>c</sup>	M <sub>n</sub> <sup>theo</sup> (TMI) <sup>d</sup>	M <sub>n</sub> <sup>theo</sup> (copolymer) <sup>e</sup>	M <sub>n</sub> (GPC) <sup>f</sup>	PDI <sup>f</sup>
2	0.6	5,800	12.53	33,530	67,282	71,700	1.23
4	4.3	41,500	14.62	39,112	80,704	108,500	1.18
7.5	13.19	126,000	16.96	45,375	171,620	127,000	1.35
16.5	26.34	251,700	23.35	62,465	314,489	133,000	1.66
25	41.46	396,100	26.97	72,158	468,265	162,700	1.9
30	45.83	437,900	29.95	80,132	518,067	204,000	2.05

<sup>a</sup> The monomer conversion was calculated from the <sup>1</sup>HNMR of the reaction mixture from the decrease of the intensity of STY vinyl proton peaks (6 ppm)

<sup>b</sup> Theoretical molecular weight of STY was calculated from linear relationship between molecular weight and conversion according to  $M_n = ([M_{STY}]_0/[POEGMAMacroRAFT]_0) * x * M_w_{STY}$  where  $[M]_0$ ,  $[POEGMAMacroRAFT]_0$ ,  $x$ ,  $M_w_{STY}$  are STY, POEGMAMacroRAFT concentration, monomer conversion, molecular weight of STY respectively

<sup>c</sup> The monomer conversion was calculated from the <sup>1</sup>HNMR of the reaction mixture from the decrease of the intensity of TMI vinyl proton peaks (5.4 ppm)

<sup>d</sup> Theoretical molecular weight of TMI was calculated from linear relationship between molecular weight and conversion according to  $M_n = ([M_{TMI}]_0/[POEGMAMacroRAFT]_0) * x * M_w_{TMI}$  where  $[M_{TMI}]_0$ ,  $[POEGMAMacroRAFT]_0$ ,  $x$ ,  $M_w_{TMI}$ , and  $M_w_{macroRAFT}$  are TMI, POEGMAMacroRAFT concentration, monomer conversion, molecular weight of TMI respectively.

<sup>e</sup> Theoretical molecular weight of block copolymer was calculated from  $M_n^{theo}(\text{copolymer}) = M_n^{theo}(\text{STY}) + M_n^{theo}(\text{TMI}) + M_n^{theo}(\text{POEGMAMacroRAFT})$

<sup>f</sup> The experimental  $M_n$  and PDI was measured by GPC using dimethyl acetamide (DMAc) (0.05% w/v BHT and 0.03% w/v LiBr) as eluent and polystyrene standards with the molecular weight ranging from 168 to 10<sup>6</sup> g. mol<sup>-1</sup>)

## Cell assays

Australian Standards protocol for Biological evaluation of medical devices: Tests for *in vitro* cytotoxicity AS/ISO10993.5-2002

### Materials

The fibroblast Earle's L cells-NCTC Clone 929 (Murine) was obtained from the Biomedical Lab, UNSW. The media Eagle's Minimal Essential Medium (EMEM, Sigma) were prepared as instruction from the manufacturers and sterilized by filtering. Other chemicals were used in the experiment include: foetal calf serum (FBS, Invitrogen), antibiotics (5000 u·mL<sup>-1</sup> penicillin and 5000 mg·mL<sup>-1</sup> streptomycin; CSL Biosciences, Melbourne, Australia), Trypsin/ Versene (Sigma), Dulbecco's phosphate buffered saline with no calcium or magnesium (JRH BioSciences) and Osmosol (Lab Aids, Sydney, Australia). Petri dishes were from Crown Scientific, Sydney, Australia.

### Cytotoxicity of uncrosslinked and crosslinked micelles

L-929 cells were harvested from the 75cm<sup>2</sup> flasks. The media was poured out to leave behind cells sticking to the wall of the flask. 3mL of trypsin was placed into the flask and the flask was put back in the incubator for 5 minutes to get the cells to solution. The flask was then examined under microscope to check for the detachment of cells. 6 mL of media was then added into the flask. This solution was subsequently centrifuged at 1000 rpm for 3 minutes. After centrifuging, the supernatant was poured out. Re-suspend cells in 8 mL media by stirring the mixture with a pipette. A haemocytometer was used to count the number of cells.

35mm Petri dishes were used for the cell plating. Each sample was prepared in triplicate. Cells were seeded to plates at the concentration of 50 000 cells/ 1 mL media and each plate contains 2 ml media. Plates were placed into a 5% CO<sub>2</sub> incubator maintained at 37°C ± 2°C. After 24 ± 1 hrs, plates were collected and checked for sign of infection.

On day 2, solution of fluorescein-labeled uncrosslinked and crosslinked micelles was prepared at the final concentration of 1.25 mg mL<sup>-1</sup>. The solution is sterilized by passing

through a 0.2  $\mu\text{m}$  filter. 100  $\mu\text{L}$  of this solution was added to the plates. Plates were then returned to the incubator maintained at 37  $^{\circ}\text{C}$  and 5%  $\text{CO}_2$ .

On day 3, plates were examined under the microscope and counted using ViCell.

- Prepare sample for counting: The media was poured out from plates and cells were washed with saline solution. 1 mL of trypsin was added to the plate and left for 5 minute to allow the detachment of cells 1 mL of saline solution was then added to the plate. 1 mL of this solution was added to a ViCell sample vial for analysis. After counting 1 set of sample, flush the column again and repeat the procedure.
- Prepare sample for fluorescence microscope: The media was poured out from plates and washed 3 times with DPBS-CaMg. 500  $\mu\text{L}$  DPBS-CaMg was added to the plate and cells were examined under microscope (AxioCam HRm)