Supporting Information

Shell and Core Cross-Linked Poly(L-lysine)/Poly(acrylic acid) Complex Micelles

Yi-Hsuan Hsieh, Yung-Tse Hsiao and Jeng-Shiung Jan*

Department of Chemical Engineering National Cheng Kung University No 1, University Rd., Tainan, Taiwan 70101 (Taiwan)

Experimental section

Cytotoxicity Assay of cross-linked PIC micelles

The cytotoxicity of the cross-linked PIC micelles was examined by MTT assay.\(^1\) \(^2\) The cross-linked PAA\(_{50}/PLL\(_{50}\) micelles prepared at F\(_{PLL}= 55\) wt\% (80 wt\%) and CF\(_{Cys} = 0.075\) (CF\(_{Gen} = 0.075\)) were selected for evaluation. The fibroblast 3T3 cells were cultured onto a 96-well plate (1x10\(^4\) cells/mL) using Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10\% BS (bovine serum, Gibco) under a humidified atmosphere of 5\% CO\(_2\) at 37 °C. After culturing for 24 h, the medium was replaced with the culture medium containing cross-linked PAA\(_{50}/PLL\(_{50}\) micelles (0-0.2 mg/mL). Then the viability of adherent cells was determined after culturing for another 24 hr. By adding 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to the cells, the tetrazolium salt was converted into an insoluble purple formazan salt. After 4 hr incubation, the formazan salt was dissolved by a detergent solution. The resulting solution was measured at 570 nm using an ELISA plate reader (Sunrise, Tecan). The number of viable cells can be quantified by measuring the absorbance intensity at 570 nm.
The cell viability was calculated by the ratio between the absorbance of the cell culture with and without polypeptide.

**Evaluation of pyranine Release in vitro.** 0.3 mg of pyranine and 2.7 mg of PAA were dissolved in 3 mL of water. 7 mL of PLL solution (1 mg/mL) was added dropwise to the solution under sonication (power: 80 W). The resultant solution was then stirred for 15 min. The excess pyranine was removed by using a Vivaspin 6 centrifugal filter unit with nominal molecular weight limit of 30 kDa (Sartorius, USA) at 6000 rpm for 10 min. For preparing the pyranine-loaded, cross-linked particle solution, genipin with different molar ratios of genipin to lysine was added to the pyranine-loaded particle solution and the resultant solution was stirred for another 2 hr. The excess cross-linking agent was removed by using a Vivaspin 6 centrifugal filter unit with nominal molecular weight limit of 30 kDa (Sartorius, USA) at 6000 rpm for 10 min. Pyranine concentrations in the filtrate was analyzed by fluorescence spectroscopy (Hitachi FL-4500) at the emission wavelength of 510 nm (excitation wavelength: 460 nm) with reference to a calibration curve. The amount of pyranine encapsulated in the micelles was quantified by subtracting the excess model drug. The loading efficiency (EE) was calculated to be 95.9\%\pm2.0\%. Each experiment was performed in triplicate. For the release experiments, the pyranine-loaded particle solution (2 mL) was placed in a dialysis tube (MWCO 3500 g/mL) and dialyzed against 30 mL of PBS (pH 7.4 or 4.7, 0.01 N) at 37 °C with a shaken rate of 100 rpm. The dialyzate was taken out at designated time intervals and measured by fluorescence spectroscopy at the emission wavelength of 510 nm (excitation wavelength: 460 nm) with reference to a calibration curve. Each experiment was performed in triplicate.

**Reference**

Table S1. Characterization of poly(Z-L-lysine) (PZLL) and poly(tert-butyl acrylate) (PtBA).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_n$</th>
<th>$M_w$</th>
<th>$M_n/M_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PtBA$_{50}$</td>
<td>6,200</td>
<td>6,700</td>
<td>1.08</td>
</tr>
<tr>
<td>PtBA$_{180}$</td>
<td>23,100</td>
<td>25,300</td>
<td>1.10</td>
</tr>
<tr>
<td>PZLL$_{50}$</td>
<td>17,400</td>
<td>19,100</td>
<td>1.10</td>
</tr>
<tr>
<td>PZLL$_{100}$</td>
<td>25,400</td>
<td>32,700</td>
<td>1.29</td>
</tr>
<tr>
<td>PZLL$_{250}$</td>
<td>66,000</td>
<td>91,000</td>
<td>1.38</td>
</tr>
</tbody>
</table>
Figure S1. The size and Zeta potential of PAA$_{180}$/PLL particles as a function of PLL mixing weight percentage ($F_{PLL}$).
Figure S2. The polydispersity index (PDI) of (a) PAA$_{50}$/PLL and (b) PAA$_{180}$/PLL particles as a function of PLL mixing weight percentage ($F_{PLL}$).
Figure S3. CD spectra of PAA_{180}/PLL_{100} particles prepared at different F_{PLL} values.
Figure S4. The size distributions of the positively charged PAA$_{50}$/PLL$_{50}$ micelles (F$_{PLL}$ = 70 wt%) (a) without cross-linking and (b) cross-linked by genipin at CF$_{Gen}$=0.075 upon dilution.
Figure S5. The size distributions of the negatively charged PAA$_{50}$/PLL$_{50}$ micelles ($F_{PLL} = 55$ wt%) (a) without cross-linking and (b) cross-linked by cystamine at $CF_{Cys}=0.125$ upon dilution.
Figure S6. Zeta potential of positively charged PAA_{50}/PLL_{50} micelles (F_{PLL} = 80 wt%) cross-linked by genipin at different CF_{Gen} values.
Figure S7. FTIR spectra of negatively charged PAA$_{50}$/PLL$_{50}$ particles ($F_{PLL} = 55$ wt$\%$) cross-linked by cystamine at $CF_{Cys} = 0$ and 0.075.
**Figure S8.** FTIR spectrum of the core cross-linked PAA$_{100}$/PLL$_{50}$ particles ($F_{PLL}= 55$ wt%) by silica deposition.
Figure S9. Cytotoxicity of the shell cross-linked PAA$_{100}$/PLL$_{50}$ particles (a) at F$_{PLL}= 55$ wt% cross-linked by cystamine (CF$_{Cys}= 0.075$) and (b) at F$_{PLL}= 80$ wt% cross-linked by genipin (CF$_{Gen}= 0.075$) (n=6). The PAA$_{100}$/PLL$_{50}$ particle concentration ranged between 0 and 0.2 mg/mL.
Figure S10. Cumulative release of pyranine-loaded, genipin-cross-linked PAA_{50}/PLL_{50} micelles (F_{PLL} = 70 wt\%) at pH 4.7 and 7.4 in tris buffer. The release experiments were conducted at 37 °C.