

Encapsulation of Nanomaterials within Intermediary Layer Cross-linked Micelles Using a Photo-Cross-linking Agent

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Abstract: A new method for encapsulating nanomaterials within intermediary layer cross-linked (ILCL) polymeric micelles using a bifunctional photo-cross-linking agent was developed. For ILCL polymeric micelles, an amphiphilic triblock copolymer of poly(ethylene glycol)-*b*-poly(2-hydroxyethyl methacrylate)-*b*-poly(methyl methacrylate) (PEG-PHEMA-PMMA) was synthesized via consecutive atom transfer radical polymerization (ATRP). Di(4-hydroxyl benzophenone) dodecanedioate (BPD) was used as a bifunctional photo-cross-linking agent. The PMMA-tethered Au nanoparticles and BPD, or pyrene and BPD were encapsulated in the PEG-PHEMA-PMMA micelles, and their intermediary layers were photo-cross-linked by UV irradiation for 1 h. The HEMA units donated labile hydrogens to the excited-state benzophenone groups in BPD, and they were subsequently cross-linked by BPD through radical-radical combination. The spherical structures of the PEG-PHEMA-PMMA micelles containing the Au nanoparticles or pyrene were unaffected by the photo-cross-linking process.

Keywords: amphiphilic triblock copolymer, intermediary layer cross-linked (ILCL) micelles, photo-cross-linking agent, encapsulation.

Introduction

Recently, we developed a new and convenient method for preparing polymeric core cross-linked (CCL) micelles using a bifunctional photo-cross-linking agent of di(4-hydroxyl benzophenone) dodecanedioate (BPD).¹ An amphiphilic block copolymer of PEG-*b*-poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (PEG-*b*-P(HEMA-*co*-MMA)) was synthesized via activators generated by electron transfer (AGET) atom transfer radical polymerization (ATRP). The core domains of the BPD-containing PEG-*b*-P(HEMA-*co*-MMA) micelles in an aqueous solution were successful photo-cross-linked by UV irradiation for only 30 min. The HEMA units donated labile hydrogens to excited-state benzophenone (BP) groups in BPD, and they were subsequently cross-linked by BPD through radical-radical combination. A sufficient degree of cross-linking was achieved at an equivalent ratio of the BP groups to the HEMA units.

In this study, this photo-cross-linking method was employed to develop a new method for encapsulating nanomaterials within intermediary layer cross-linked (ILCL) polymeric micelles. The ILCL micelles have combined advantages of shell cross-linked (SCL) and CCL micelles.²⁻⁵ The outer block chains can stabilize the micelles, and the micelle core can provide a carrier compartment for various agents. For ILCL

micelles, an amphiphilic triblock copolymer of poly(ethylene glycol)-*b*-poly(2-hydroxyethyl methacrylate)-*b*-poly(methyl methacrylate) (PEG-PHEMA-PMMA) was synthesized via consecutive AGET ATRP using a PEG macroinitiator. The intermediary layers of the PEG-PHEMA-PMMA micelles containing gold (Au) nanoparticles or pyrene were photo-cross-linked with BPD in an aqueous solution. The ILCL PEG-PHEMA-PMMA micelles showed excellent colloidal stability due to the outer PEG blocks.

Experimental

Materials. Methyl methacrylate (MMA, Duksan, 99.8%, Korea) and 2-hydroxyethyl methacrylate (HEMA, Acros, 96%) were passed through a column filled with neutral alumina. They were stirred over calcium hydride (CaH₂) and then distilled under reduced pressure. Anisole (Acros, 99%) and isopropyl alcohol (Duksan, 99.5%) were distilled under vacuum. Tetrahydrofuran (THF, Duksan, 99.5%) was dried over Na and BP. Poly(ethylene glycol) methyl ether (MPEG, number-average molecular weight (M_n)=2,000 g/mol, Fluka), ethyl 2-bromoisobutyrate (Aldrich, 98%), copper(II) bromide (CuBr₂, Acros, 98%), *N,N,N',N',N''*-pentamethyldiethylene-triamine (PMDETA, Acros, 99+%), tin(II) 2-ethylhexanoate (Sn(EH)₂, Aldrich, ~95%), pyrene (Aldrich, 98%), dodecanedioyl dichloride (Aldrich, 98%), 4-hydroxybenzophenone (Aldrich, 98%), triethylamine (TEA, Acros, 99%),

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sodium bicarbonate (NaHCO_3 , Duksan, >99%), hydrogen tetrachloroaurate(III) ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, Aldrich, >99.9%), sodium borohydride (NaBH_4 , Acros, >98%), sodium citrate tribasic dihydrate (Aldrich, $\geq 99.0\%$), methanol (Duksan, 99%), dichloromethane (Duksan, 99%), and *N,N*-dimethylformamide (DMF, Duksan, 99.5%) were all used as received.

Synthesis of BPD. 4-Hydroxybenzophenone (3.97 g, 20.0 mmol) was dissolved in THF (200 mL). TEA (3.49 mL, 25.0 mmol) and dodecanedioyl dichloride (3 mL, 12.0 mmol) were added dropwise to this solution, which was being kept in an ice bath. After stirring for 24 h, the amine salt formed was removed by filtration. After concentrating the solution, dichloromethane was added, and the solution was washed twice with 3% NaHCO_3 aqueous solution, and water. The resulting solution was dried over magnesium sulfate, and concentrated under reduced pressure. The product was purified by recrystallization from dichloromethane. The yield was 1.73 g (37%).

^1H NMR spectrum, (CDCl_3 , ppm): 1.3 (s, 12H, $-\text{CH}_2-$), 1.56 (quint, 4H, $-\text{OCOCH}_2-\text{CH}_2-$), 2.23 (t, 4H $-\text{OCO}-\text{CH}_2-$), 7.17(d, 4H, $-\text{COO}-\text{ArH}-$), 7.83 (d, 4H, $-\text{CO}-\text{ArH}-$), 7.49~7.75 (m, 10H, $-\text{CO}-\text{ArH}$).

Synthesis of the PEG-PHEMA-PMMA Triblock Copolymer. The PEG macroinitiator was synthesized by reacting MPEG with 2-bromoisobutyl bromide. For the synthesis of the PEG-PHEMA-PMMA triblock copolymer, a 100 mL flask was charged with PEG macroinitiator (2.4 g, 0.48 mmol), CuBr_2 (0.05 g, 0.024 mmol), anisole (15 mL), and isopropyl alcohol (5 mL). The reaction mixture was bubbled with N_2 for 10 min, and then PMDETA (0.075 mL, 0.36 mmol), HEMA (1.16 mL, 9.6 mmol), and $\text{Sn}(\text{EH})_2$ (0.116 mL, 0.36 mmol) were added. The mixture was then placed in an oil bath preheated at 80 °C. When the HEMA conversion had reached over 90% (determined by ^1H NMR), degassed MMA (2.6 mL, 24 mmol) was added to the reaction flask. After 24 h, the mixture was diluted with THF and passed through a silica gel column to remove the copper catalyst. After evaporation of all of the solvent, drying in a vacuum oven at room temperature yielded a colorless copolymer.

^1H NMR ($\text{DMSO}-d_6$, ppm): 0.61-1.32 (3H, $-\text{C}-\text{CH}_3$), 1.70-2.15 (2H, $-\text{CH}_2-\text{C}-\text{CH}_3$), 3.51-3.73 (3H, $-\text{O}-\text{CH}_3$), 3.8-4 (2H, $-\text{COO}-\text{CH}_2$), 4.8-4.9 (s, $-\text{OH}$).

Preparation of the PMMA-Tethered Au Nanoparticles. 2-Cyanoethyl dithiobenzoate (CED) was synthesized as a reversible addition-fragmentation chain transfer (RAFT) agent.^{6,7} Dithioester-terminated PMMA was synthesized using CED via RAFT polymerization of MMA at 80 °C for 2 h. The dithioester terminal groups were completely reduced with NaBH_4 to produce thiolated PMMA (PMMA-SH). An aqueous solution of citrate-stabilized Au nanoparticles was added dropwise to a PMMA-SH solution in methanol under vigorous stirring.^{6,8} After 1 week of equilibration at room temperature, the reaction solution was centrifuged at 13,000 rpm for 0.5 h. The supernatant, which contained

the free PMMA chains that were not conjugated to the Au nanoparticles, was removed. The precipitate was dissolved in deionized water and the resulting solution was centrifuged in order to wash away any residual polymers. This washing procedure was repeated two more times. Finally, the resulting PMMA-tethered Au nanoparticles were dissolved in DMF (5 mg/mL). The PMMA-tethered Au nanoparticles were spherically shaped with an average diameter of 3.0 nm.

Preparation of the BPD-Containing PEG-PHEMA-PMMA Triblock Copolymer Micelles. 1 mL of a PEG-PHEMA-PMMA solution in DMF (7.5 mg/mL) and a predetermined volume of a BPD solution in DMF (2 mg/mL) were added to a 50 mL vial. The final volume of the solution was adjusted to be 7.5 mL by adding pure DMF, after which 15 mL of deionized water was gradually added dropwise (1 mL/min) with vigorous stirring. DMF was removed by dialysis against water for 24 h. The final concentration of polymer in each solution was 0.5 mg/mL.

Encapsulation of PMMA-Tethered Au Nanoparticles. To 2.5 mL of a PEG-PHEMA-PMMA solution (2 mg/mL) in DMF were added 1.35 mL of a BPD solution (2.0 mg/mL) in DMF and 0.1 mL of a PMMA-tethered Au nanoparticles solution in DMF while stirring vigorously. 10 mL of deionized water was then gradually added dropwise (0.5 mL/min) while stirring vigorously. The DMF was removed by dialysis against deionized water for 24 h.

Encapsulation of Pyrene. To 1.25 mL of a PEG-PHEMA-PMMA solution (2.0 mg/mL) in DMF were added 0.7 mL of a BPD solution (2 mg/mL) in DMF and 0.5 mL of a pyrene solution (1.2 mg/mL) in DMF while stirring vigorously. 5 mL of deionized water was then gradually added dropwise (0.5 mL/min) while stirring vigorously. The DMF was removed by dialysis against deionized water for 24 h.

Photo-Cross-Linking of the PEG-PHEMA-PMMA Micelles. The intermediary layer of the PEG-PHEMA-PMMA micelles was cross-linked at room temperature for 1 h by UV irradiation using a 500 W high-pressure mercury lamp (Ushio UI-501-C) located 15 cm from the sample surface.

Characterization. The molecular weight and polydispersity of the resulting polymers were determined by a gel permeation chromatography (GPC) system (Young Lin SP930D solvent chromatography) coupled with an RI detector (RI 750F) and two columns (GPC KD-G and KF-806, Shodex). The eluent used was DMF at 40 °C with a flow rate of 1.0 mL/min. Poly(ethylene oxide) standards were used for calibration. ^1H nuclear magnetic resonance (NMR) spectroscopy was performed using a Varian VXR-Unity NMR spectrometer (400 MHz) with CDCl_3 or $\text{DMSO}-d_6$ as the solvent. The transmittance of the aqueous solution was acquired on a Turbiscan LAB-expert and measured at a wavelength of 880 nm. The micellar size and size distribution were determined by dynamic light scattering (DLS) using a BI-200SM particle size analyzer. Each analysis lasted 360 s and was

performed at room temperature with an angle detection of 90°. The concentration of the polymer solution was 0.1 mg/mL. The morphologies of the copolymer micelles were investigated using a JEM-2100F transmission electron microscope operating at an accelerating voltage of 120 kV. A drop of aqueous PEG-PHEMA-PMMA solution (0.5 mg/mL) was deposited onto a 200 mesh copper grid that had been coated with carbon. The size and shape of the micelles were directly determined using transmission electron microscopy (TEM). The encapsulation of pyrene in the PEG-PHEMA-PMMA micelles was confirmed by confocal laser scanning microscopy (CLSM, ZEISS, LSM 510 META). Sample preparation involved depositing a drop of micelle solution containing pyrene on a glass surface. The samples were excited using a multi-argon laser (458/477/488/514 nm).

Results and Discussion

The chemical structures of PEG-PHEMA-PMMA and BPD are shown in Scheme I. PEG-PHEMA-PMMA was synthesized via consecutive AGET ATRP using PEG macroinitiator at 80 °C in a mixed solvent of anisole/isopropyl alcohol (3/1 v/v). The molecular characteristics of PEG-PHEMA-PMMA were determined by a combination of ¹H NMR spectroscopy and GPC. The degrees of polymerization of the PHEMA and PMMA blocks were determined by ¹H NMR spectroscopy to be 13 and 98, respectively, on the basis of the PEG block. The M_n of PEG-PHEMA-PMMA was calculated to be 13,600 g/mol. The M_n of PEG-PHEMA-PMMA determined by GPC was 15,800 g/mol ($M_w/M_n=1.58$).

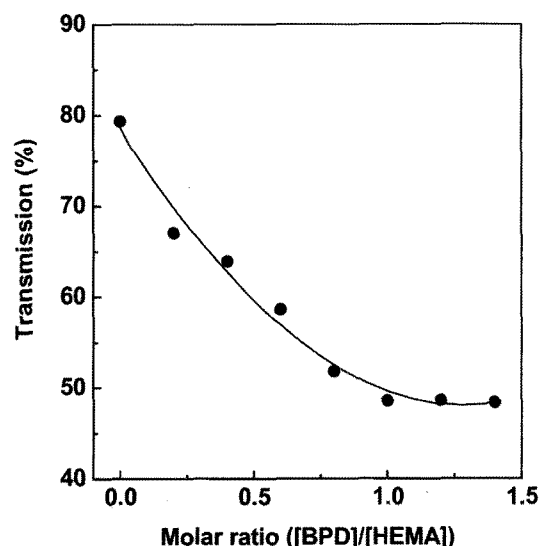
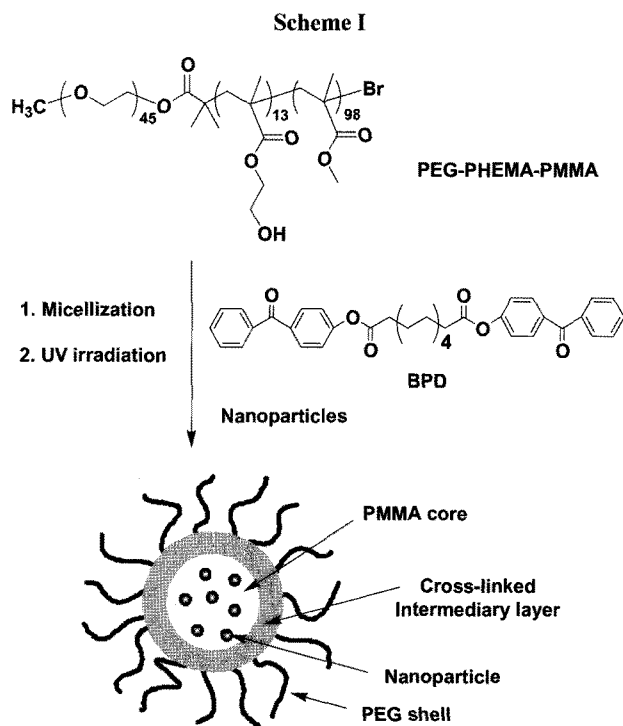


Figure 1. Turbidity of the BPD-containing PEG-PHEMA-PMMA micellar solutions. The micelles were UV irradiated for 1 h, then recovered and dissolved in THF.

The intermediary layers of the BPD-containing PEG-PHEMA-PMMA micelles in aqueous solution were photo-cross-linked by UV irradiation. When BPD is exposed to UV, the carbonyl group in the BP moiety is excited to a triplet excited state, and abstracts a hydrogen atom from the HEMA unit. This reaction produces radicals, and the radical-radical combination results in subsequent chain cross-linking.⁹⁻¹³ The minimal amounts of BPD to obtain stable ILCL PEG-PHEMA-PMMA micelles were determined by judging the stability of the ILCL PEG-PHEMA-PMMA micelles in THF. When the micelles were sufficiently cross-linked, they remained intact in THF. Figure 1 shows the turbidities of the BPD-containing PEG-PHEMA-PMMA micellar solutions. All of the PEG-PHEMA-PMMA micelles containing different amounts of BPD were UV irradiated in an aqueous solution for 1 h. The micelles were then recovered and dissolved in THF. As the molar ratio of BPD to the HEMA units in PEG-PHEMA-PMMA ($[BPD]/[HEMA]$) increased, the micelle solutions became opalescent, indicating that their micellar structures were stabilized by intermediary layer cross-linking. The turbidity value reached a constant value from a molar ratio of $[BPD]/[HEMA]=1.0$, indicating that the ILCL PEG-PHEMA-PMMA micelles could be obtained above this ratio. An equal molar ratio of BPD to the HEMA units was required to produce the stable PEG-PHEMA-PMMA micelles. BPD was present in the core domains of PMMA. The possibility of photo-cross-linking of the core PMMA blocks by BPD was investigated using a PMMA film cast from a solution of PMMA ($M_n=31,000$ g/mol) and BPD in ethyl acetate. The concentration of BPD was 2 mol% to the MMA units. The PMMA film of 25 μ m in thickness was UV irradiated for 1 h under the same UV irra-

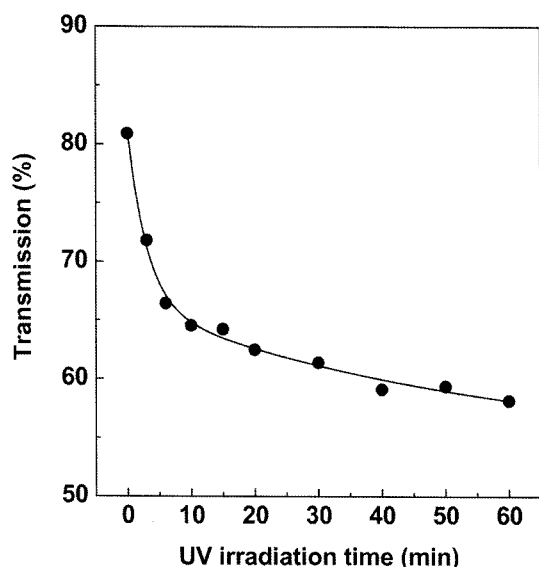


Figure 2. Changes in turbidity of the BPD-containing PEG-PHEMA-PMMA micelles according to UV irradiation time: Molar ratio of [BPD]/[HEMA]=1.0.

diation condition for the ILCL micelles. The M_n of the PMMA was not changed at all after UV irradiation. This result supports that BPD presenting around the HEMA units took part in the photo-cross-linking, and produced the stable ILCL PEG-PHEMA-PMMA micelles. Figure 2 shows the changes in the turbidity of the BPD-containing PEG-PHEMA-PMMA micelles as a function of the UV irradiation time. The molar ratio of [BPD]/[HEMA] was fixed at 1.0. The transmittance decreased rapidly in the beginning of the UV irradiation, indicating that BPD presenting around the HEMA units readily reacted with the HEMA units. After 10 min, the photo-cross-linking reactions gradually proceeded according to the UV irradiation time. Therefore, in order to obtain sufficiently cross-linked micelles, the PEG-PHEMA-PMMA micelles were UV irradiated for 1 h. The micellar characteristics of PEG-PHEMA-PMMA in aqueous solution were investigated using TEM. Figure 3 shows the TEM images of the BPD-containing PEG-PHEMA-PMMA micelles ([BPD]/[HEMA]=1) before and after UV irradiation for 1 h. The micelles were spherically shaped with an average diameter of 79 nm. The cross-linked micelles were recovered and dissolved in THF. The spherical structures of the cross-linked micelles remained intact in THF, indicating that the sufficient photo-cross-linking of the PEG-PHEMA-PMMA micelles was achieved by using BPD.

The PEG-PHEMA-PMMA micelles were used to encapsulate functional nanomaterials. Figure 4 shows the TEM images of the ILCL PEG-PHEMA-PMMA micelles containing Au nanoparticles or pyrene. The PMMA-tethered Au nanoparticles and BPD or pyrene and BPD were encapsulated within the PEG-PHEMA-PMMA micelles, and their intermediary layers were photo-cross-linked by UV irradiation

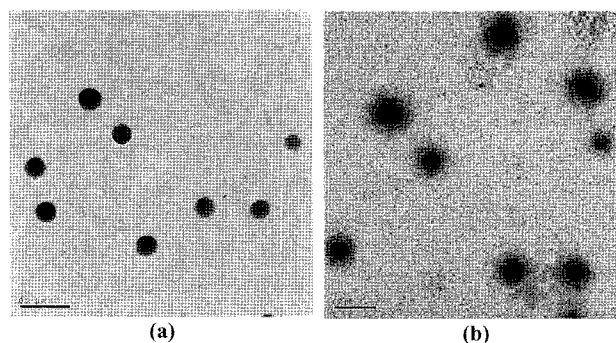


Figure 3. TEM images of the BPD-containing PEG-PHEMA-PMMA micelles (a) before and (b) after UV irradiation. The micelles were UV irradiated for 1 h, then recovered and dissolved in THF.

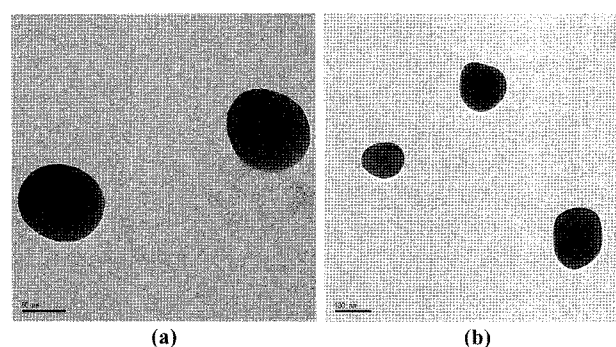


Figure 4. TEM images of the ILCL PEG-PHEMA-PMMA micelles containing (a) Au nanoparticles and (b) pyrene.

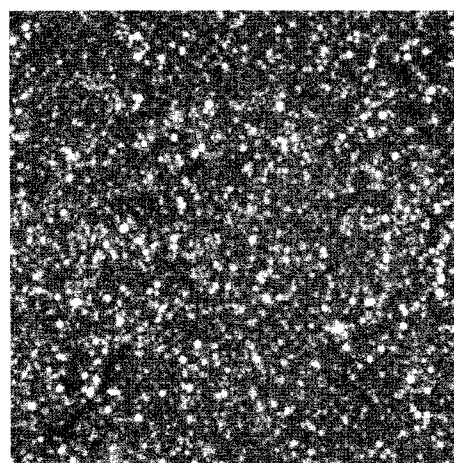


Figure 5. CLSM image of pyrene encapsulated in the ILCL PEG-PHEMA-PMMA micelles.

for 1 h. The molar ratio of [BPD]/[HEMA] was 1.0. The spherical structures of the PEG-PHEMA-PMMA micelles containing the Au nanoparticles or pyrene were not changed by the photo-cross-linking process. The Au nanoparticles were very small compared to the diameters of the PEG-PHEMA-PMMA micelles and the ILCL PEG-PHEMA-PMMA micelles

contained multiple Au nanoparticles. Similar cases of multiple encapsulations of small nanoparticles within polymer surfactant micelles have been reported previously.¹⁴⁻¹⁸ The encapsulation of pyrene in the ILCL PEG-PHEMA-PMMA micelles was confirmed by CLSM (Figure 5). The samples were excited with the 488 nm laser line of the microscope. Bright green circular spots, corresponding to fluorescent light emitted by pyrene molecules, were clearly observed, indicating that pyrene was successfully encapsulated in the ILCL PEG-PHEMA-PMMA micelles.

Conclusions

The PEG-PHEMA-PMMA triblock copolymer ($M_{n,GPC}=15,800$ g/mol, $M_w/M_n=1.58$) was synthesized via consecutive AGET ATRP using PEG macroinitiator at 80 °C. The degrees of polymerization of the PEG, PHEMA, and PMMA blocks were 45, 13, and 98, respectively. The intermediary layers of the PEG-PHEMA-PMMA micelles were successfully photo-cross-linked using BPD at a molar ratio of [BPD]/[HEMA]=1.0. The prepared PMMA-tethered Au nanoparticles were spherically shaped with an average diameter of 3.0 nm. The PMMA-tethered Au nanoparticles and BPD or pyrene and BPD were encapsulated within the PEG-PHEMA-PMMA micelles, and their intermediary layers were photo-cross-linked. The ILCL PEG-PHEMA-PMMA micelles successfully encapsulated multiple Au nanoparticles and pyrene. The encapsulation of pyrene in the PEG-PHEMA-PMMA micelles was confirmed by CLSM. The spherical structures of the ILCL PEG-PHEMA-PMMA were unaffected by the photo-cross-linking process.

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