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# Design of micellized α-cyclodextrin based supramolecular hydrogel system

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In recent years supramolecular structures built from macrocyclic compounds have attracted tremendous interest due to the unique properties derived from dynamic self-assembly. Our study proposed a two-step mechanism to form supramolecular hydrogel system; (1) formation of micelle, (2) micelle association with α-cyclodextrin (α-CD) due to threading of PEGMA in α-CD cavity forming inclusion complexes. With this purpose, a supramolecular hydrogel made from a tri-component copolymer PLLA/DMAEMA/PEGMA and α-CD was fabricated for the first time and characterized in terms of structural, morphological, and rheological properties.

## Introduction

Supramolecular hydrogels hold great potential as novel delivery systems[1]. This attributes from the dynamic, intermolecular non-covalent (via hydrogen bonds, π–π stacking and van der Waals) interactions between two or more molecular entities to induce the formation of a supramolecular network system[2].

Supramolecular structures built from macrocyclic compounds have attracted tremendous interest not only as models for understanding natural supramolecular self-assembly and molecular recognition, but also as precursors for designing novel nanomaterials for electronics, biomedical and pharmaceutical applications[3-12]. One such model is cyclodextrin-based (CD-based) supramolecular hydrogels. First reported in 1994[13], hydrogels formed based on the polymer–cyclodextrin inclusion complexes have been used as a non-covalent binding motif for the development of a wide variety of dynamic polymeric networks and assemblies in aqueous media.

Cyclodextrins are cyclic oligosaccharides, the most common consisting of 6, 7, and 8 glucopyranose units linked by α-1,4 glucosidic bonds; they are called α –cyclodextrin (α-CD), *β*-cyclodextrin (*β*-CD) and *γ*-cyclodextrin (*γ*-CD), respectively. They present a truncated cone shape with a hydrophilic exterior composed of -OH groups, and hydrophobic interior which can accommodate nonpolar compounds[14-16].

Supramolecular hydrogels formed by threading α-CD rings onto PEG-grafted copolymers have been reported[17-20], including more complicated polymers, such as PEG-grafted dextran[21], chitosan[22], PEG brushes[23], and star PEG polymers[24]. Physically cross-linked hydrogels of this type are temperature sensitive, with a sol-gel transition occurring at certain temperatures, which are influenced by the polymer structure and mixture composition[13, 21]. Such hydrogels are shear-thinning[17] and their potential use as injectable drug delivery systems has been proposed. Recently, Chee et al. studied the release of proteins from such supramolecular hydrogels[25]. In this system, the release kinetics of proteins was too fast and gel exhibited unstable characteristics due to fast degradation time.

Our study provides a potential solution by formulating new gel architecture for controlled release and better gel stability. We hypothesize that if the system has 2 degrees of association; micelle and cyclodextrin, a more stable gel network with characteristics for controlled release of actives will be developed. To our knowledge, there has been no report on polymers with micelle architecture for association with cyclodextrin to induce supramolecular network. Polymeric micelle architecture plays a role in supramolecular formation[26]. Having an amphiphilic block copolymer in an aqueous solution forms a hydrophobic core and hydrophilic corona. Micelle association with cyclodextrin promotes self-assembly to form supramolecular network.

Hence, a two-step mechanism to form supramolecular hydrogel system was developed in this study; (1) formation of micelle, (2) micelle association with α-cyclodextrin (Fig. 1). This mechanism exploits the design of reversible, self-assembled hydrogels for applications involving release of actives in a sustained/controlled manner; intravenous encapsulation, delivery vehicle and injectable materials[27-29].

The aim of this work is to utilize the proposed mechanism in understanding the supramolecular hydrogel system. Specifically, random triblock polymer of PLLA-DMAEMA-PEGMA synthesized by ATRP formed supramolecular hydrogel with α-cyclodextrin.

Poly(L-lactide) (PLLA) possesses excellent biodegradability, biocompatibility and therefore has important applications in biomedical field. PLLA has been widely used as biodegradable sutures, implantable screws, drug delivery devices, and as temporary scaffolds for tissue[30-32]. However, lack of controlled degradation due to high degree of crystallinity and hydrophobicity[33-36] made it necessary to modify its structure and physical properties. One of the approaches was by introducing hydrophilic units[37-39], to improve the biodegradability and hydrophilicity of PLLA.

In this study, PLLA is combined with hydrophilic (2-dimethylamino)ethyl methacrylate (DMAEMA) and poly[(ethylene glycol) monomethyl ether methacylate] block (PEGMA) blocks to form amphiphilic polymer that self-assemble to polymeric micelles in solution. The PLLA block forms a central hydrophobic core, while the DMAEMA and PEGMA blocks form hydrophilic corona layer. Threading of PEGMA in α-cyclodextrin cavity forms inclusion complex, resulting in micelle association involving intermolecular non-covalent interactions. This drives the spontaneous formation of a three-dimensional physically crosslinked macromolecular network, or hydrogel.

An alternative mechanism would be that the micelle formation is the result of the delicate balance of opposite forces[40]: (1) CD threading stretches the polymer chains increasing the effective volume fraction of the micelles, minimizing the contact with water and (2) and inducing the gel formation by repulsive interactions, maximizing the contact with water (colloidal glass). Studies have been done to observe structural elucidation of the polymeric network in its hydrated/non-hydrated states[41, 42].

First reported by Ren et al.[43], reports on pH and temperature dual-responsive physical hydrogels from pH-sensitive polymers and a-CDs have been documented. Supramolecular hydrogels formed with CDs containing ionizable copolymers grafted with PEG segments will be responsive not only to temperature but also to pH[44]. Studies have shown the synthesis of PLLA-PDMAEMA[45] and PLLA-PEGMA[46] amphiphilic block copolymers as vehicles in drug delivery system.

Our study of PLLA-DMAEMA-PEGMA and interaction with α-cyclodextrin reveals understanding in the design and formation of micellized supramolecular hydrogel system with pH and temperature dual-responsive behaviour. This study explored PLLA, coupling with different ratios of hydrophilic PEGMA and DMAEMA forming supramolecular gel by incorporation of α-CD in the system.

## Experimental

### Materials and Methods

Poly(L-lactide), 2-bromoisobutyryl terminated (PLLA-Br) $M_n$ 10, 000 g/mol was supplied by Aldrich. (2-dimethylamino)ethyl methacrylate (DMAEMA) was supplied by Aldrich and purified by passing through inhibitor remover before use. Poly[(ethylene glycol) monomethyl ether methacylate] block (PEGMA), $M_n$ 1,100 g/mol was supplied by Fluka and purified by dissolving in tetrahydrofuran followed by passing through inhibitor remover before use. Copper(I) bromide (99.9%), anhydrous 1,4-dioxane (99.8%), 1,1,4,7,10,10-Hexamethyltriethylenetetramine (97%), aluminium oxide (activated, basic, Brockmann I), benzyl bromide (Fluka) were purchased from Aldrich and used as received. α-cyclodextrin (98%) (TCI) were purchased from Tee Hai Chem and used as received. Phosphate buffered saline (pH 7.4) was supplied by Vivantis and diluted 10x.

**Synthesis of poly(PLLA/PEGMA/DMAEMA) triblock polymers.** Poly(PLLA/PEGMA/DMAEMA) triblock polymer was synthesized at random via Atom Transfer Radical Polymerization (ATRP) reaction, using PLLA-Br as a macroinitiator. The synthesis of the triblock copolymers was performed in anhydrous 1,4-dioxane at 50 °C in the presence of CuBr/HMTETA catalyst system and under nitrogen atmosphere. Molar ratio of catalyst and ligand added were in the ratio [PLLA-Br]/[CuBr]/[HMTETA] of 1:4 :4.8. The synthetic scheme shown in Fig. 2.

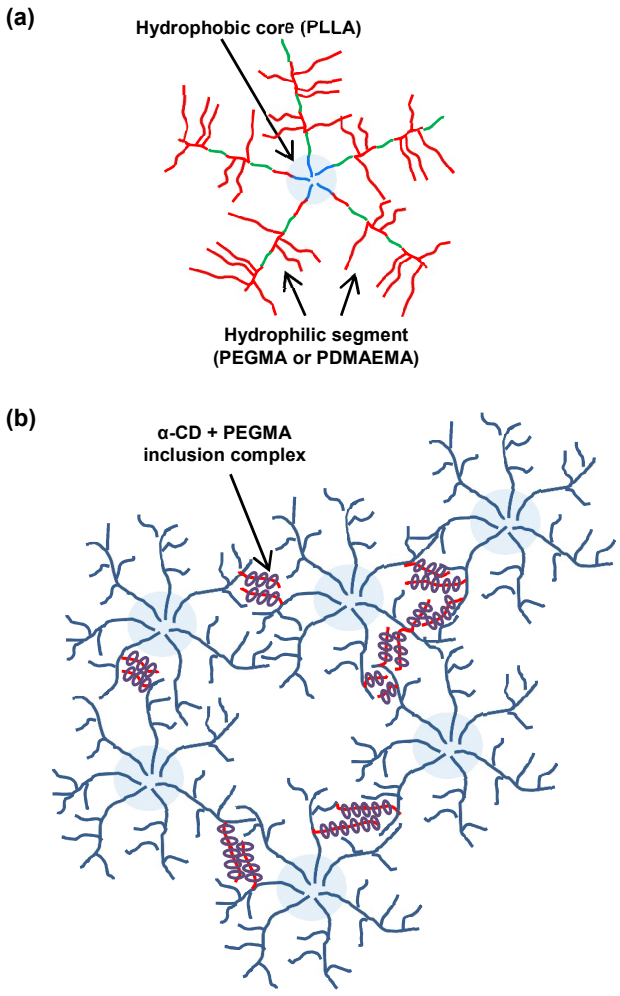


**Fig. 1** (a) Step 1: Micelle formation, (b) Step 2: Supramolecular hydrogel structure due to micelle association with α-cyclodextrin

A typical ATRP procedure was as follows: in a 50 mL rbf, PLLA-Br (0.5 g, 0.05 mmol), DMAEMA (4 g, 25.5 mmol), PEGMA (4 g, 3.64 mmol), and HMTETA (55.0 mg, 0.24 mmol) were dissolved in 20 mL anhydrous 1,4-dioxane. Reaction mixture was degassed by bubbling nitrogen gas for 30 min. CuBr (29.0 mg, 0.20 mmol) was added and the reaction mixture was stirred at 50 °C for 24 h under nitrogen atmosphere. The reaction was quenched by diluting with THF and left to stir at room temperature for 1 h. The copper catalyst was removed by passing the resultant polymer solution through a column of basic aluminium oxide. Excess THF removed from the polymer filtrate and copolymer was recovered by precipitation in hexane. The synthesized copolymer was re-dissolved in THF and re-precipitated in hexane:diethyl ether (70%:30%). The resultant polymer was dried under reduced pressure at 40 °C overnight. The yield was 60% and above after isolation and purification. PDI ranges from 1.33-1.44.

A series of random poly(PLLA/PEGMA/DMAEMA) triblock polymers with varying hydrophilic segments, PEGMA and DMAEMA, were prepared. The amount of hydrophobic PLLA-Br was kept constant for all compositions. Their composition and respective number-average molecular weight values are given in Table 1. $^1$H NMR ($CDCl_3$) of poly(PLLA/PEGMA/DMAEMA) PLPE8PD8: δ (ppm) 3.64 (-O($CH_2$)$_2$-) of PEGMA, 4.10 (-O($CH_2$)-) of DMAEMA, 5.15 (-CH) of PLLA.

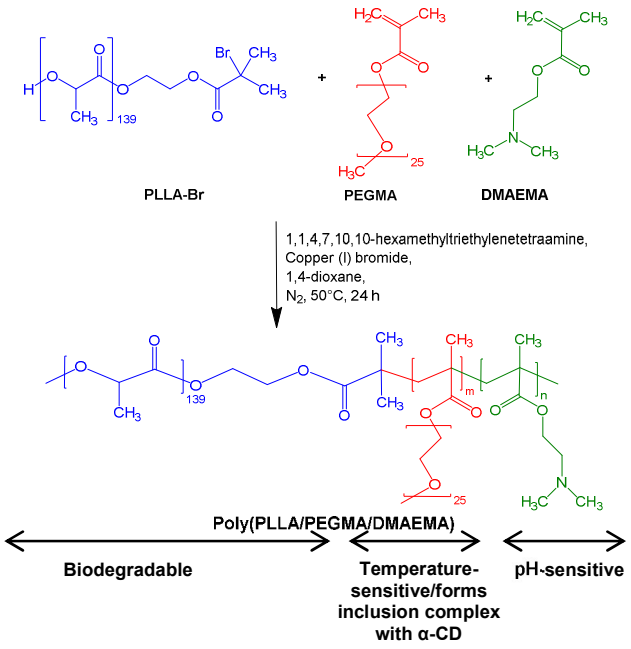


**Fig. 2** Synthesis of random Poly(PLLA/DMAEMA/PEGMA) triblock polymers

**Molecular characterization.** The $^1$H NMR (400 MHz) spectra were recorded on a Bruker AV-400 NMR spectrometer at room temperature. The $^1$H NMR measurements were carried out with an acquisition time of 3.2 s, a pulse repetition time of 2.0 s, a 30° pulse width, 5208 Hz spectral width, and 16 K data points. Chemical shift was referred to the solvent peaks (δ = 7.3 ppm for $CHCl_3$). Fourier transform infrafed (FTIR) spectra of the polymer films dissolved in chloroform, coated on NaCl plate were recorded on a Spectrum 2000 Perkin Elmer FT-IR spectrophotometer; 16 scans were signal-averaged with a resolution of 1 cm$^{-1}$ at room temperature.

**Preparation of poly(PLLA/PEGMA/DMAEMA)/α-cyclodextrin hydrogel.** The most common cyclodextrins are α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) which consist of six, seven and eight glucopyranose units respectively. The chemical and physical properties of the 3 most common cyclodextrins are given in Table S1† (see ESI)[47]. The formation of the inclusion complex and micelle association depends on the chemical property and size of both polymer compounds and cyclodextrin.Stock solutions of the different polymer compositions and α-CD were prepared separately by dissolving in phosphate buffer solution (PBS), pH 7.4. Each sample of a given concentration was prepared by mixing both solutions proportionally. The resultant mixture was left to stand at 25°C and observed for gel formation up to 24 h by a test tube inverting method as shown in Fig. 3. The sol-gel transition was determined by plotting graphs of α-CD concentration versus polymer concentration at different compositions. The range of polymer and α-CD solutions tested was (1-25% w/v) and critical gelation concentration (CGC) was inferred from the graphs.

**Fluorescence Spectroscopy.** The amphiphilic nature of poly(PLLA/PEGMA/DMAEMA) triblock polymer promotes self-assembly into micelles when introduced into aqueous environment. The minimum concentration in which micelles formed was determined by CMC determination on these polymers. The CMC values were determined by using dye solubilisation method[48]. The hydrophobic dye 1,6-diphenyl-1,3,5-hexatriene (DPH) was dissolved in methanol with a concentration of 6 mM. 60$\mu$l of this solution was mixed with 3.0 mL of the copolymer aqueous solution with concentrations ranging from 0.0001 to 0.5 wt% and equilibrated overnight at 4 °C. A UV-vis spectrophotometer was used to obtain the UV-vis spectra in the range of 320-500 nm at 25°C. The CMC value was determined by plotting graph of the difference in absorbance at 378 nm and at 400 nm ($A_{378}$ - $A_{400}$) versus logarithmic concentration.

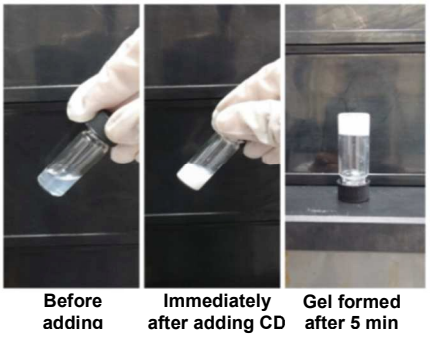


**Fig. 3** Observation on hydrogel formation and determination by test tube inverting method

**Rheological studies.** Rheological behaviors of supramolecular hydrogels were investigated by Discovery DHR-3 hybrid rheometer using a flat plate geometry, and a diameter of 20 mm in steady and dynamic modes, respectively. Stress amplitude sweeps were performed to ensure that subsequent data was collected in the linear viscoelastic regime. Storage modulus (G') and loss modulus (G") were measured as a function of the frequency under oscillatory shear at a strain of 1%, which is within the linear viscoelastic region, as determined by the stress amplitude sweeps experiments, in the frequency range 0.01–100 Hz. All tests were performed at 37.0 °C to mimic human body temperature. Reversibility of hydrogel was determined by amplitude sweep experiments at 2 points (0.1% and 50% strain), 300 s each strain point, for 10 cycles. Temperature sweep experiment was performed at a strain of 1%, in the temperature range of 10 °C-50 °C, ramp rate of 5 °C/min.

**Morphology of the Hydrogels.** The synthesized random triblock poly (PLLA/PEGMA/DMAEMA)/α-CD hydrogels were kept for 12 h after formation, then frozen quickly at -25 °C and freeze-dried in a Labconco Freeze-Drier under vacuum at -54 °C for 2 days. The interior morphology of the hydrogels was studied by a scanning electron microscope (SEM).

## Results and Discussion

### Synthesis and Characterization of random triblock poly(PLLA/PEGMA/DMAEMA)

Poly(PLLA/PEGMA/DMAEMA) triblock polymers were prepared by randomly combining PLLA, PEGMA and DMAEMA segment blocks using HMTETA ligand and CuBr catalyst in ATRP reaction synthesis (Fig. 2). The composition and respective number-average molecular weight values of triblock polymers are given in Table 1. By varying the hydrophilic segments, PEGMA and DMAEMA, the properties of each composition were studied. The chemical structure of random triblock poly(PLLA/PEGMA/DMAEMA) was verified by $^1$H NMR (Fig. 4). The compositions of the triblock polymers were determined from the integration ratio of resonances corresponding to the different blocks within the limits of $^1$H NMR precision, and the results are shown in Table 1.

**Table 1** Molecular Characteristics of Poly(PLLA/DMAEMA/PEGMA) triblock polymers

| Feed ratio –Theoretical (wt%) | | | Composition in copolymer ratio – Experimental (wt%)[b] | | | Copolymer characteristics | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| PLLA | PEGMA | DMAEMA | PLLA | PEGMA | DMAEMA | $M_n$ (x10$^3$)[b] | CMC (g/ml)[c] | G' (kPa)[d] | $M_c$ (kDa)[d] | PDI[e] |
| 5.9 | 47.1 | 47.1 | 8.0 | 52.0 | 40.1 | 125.6 | 3.15 x 10$^{-4}$ | 17.7±0.8 | 14.6±0.8 | 1.35 |
| 3.0 | 48.5 | 48.5 | 2.7 | 41.6 | 55.7 | 365.1 | 4.27 x 10$^{-4}$ | 7.68±1.2 | 33.6±1.2 | 1.44 |
| 3.0 | 90.9 | 6.1 | 3.0 | 89.3 | 7.7 | 332.2 | 4.21 x 10$^{-4}$ | 12.9±0.6 | 20.0±0.6 | 1.33 |

[a]Poly(PLLA/DMAEMA/PEGMA) triblock polymers are denoted PLPED, PL for PLLA, PE for PEGMA and PD for DMAEMA. The $M_n$ of DMAEMA, PEGMA and PLLA used for the copolymer synthesis was 157, 1100, 10000 g mol$^{-1}$, respectively. [b]Calculated from $^1$H NMR results. [c]Determined by UV-vis spectrophotometer. [d]Determined by dynamic mechanical rheology and G' = $\rho RT/M_c$. [d]Determined by GPC

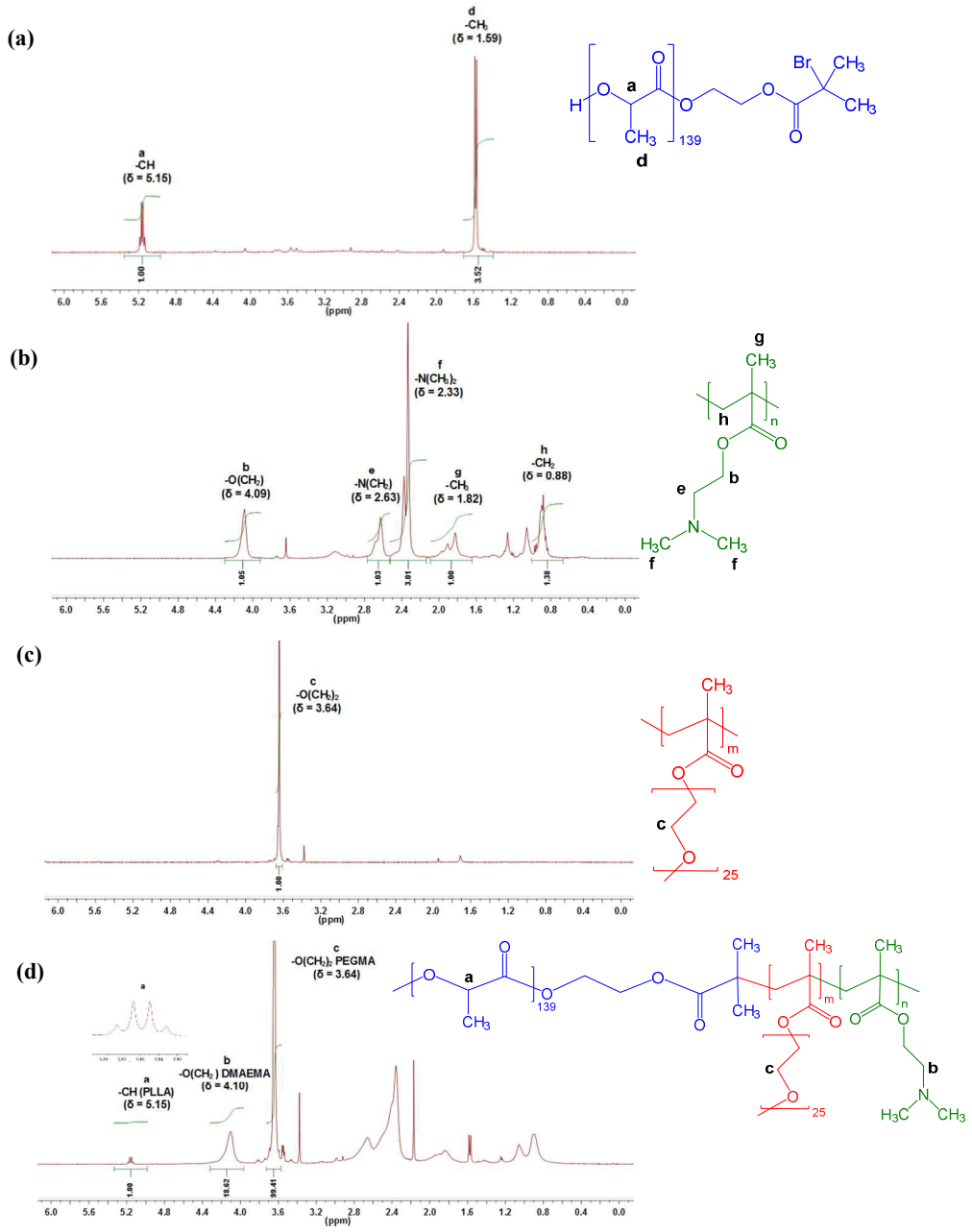


**Fig. 4** 400 MHz $^1$H NMR spectra of (a) PLLA, (b) PDMAEMA, (c) PEGMA and (d) PLPED2 in $CDCl_3$.

FTIR is useful in the characterization of the functional groups present in the polymer. As a typical example, Fig. 5 shows FTIR spectra of PLPE8D8 and its PLLA, PEGMA and DMAEMA precursors. The peaks at 1716 $cm^{-1}$, 1730 $cm^{-1}$ and 1757 $cm^{-1}$ are due to symmetrical stretching vibration of –C=O of PEGMA, DMAEMA, PLLA respectively., The peak at 1153 $cm^{-1}$ represents the –C-N vibration of DMAEMA. It can be seen by comparing the profiles of PLPED1, PLPED2 and PLPED3, they have different ratios of block polymer compositon. PLPED3 had the least DMAEMA -C=N vibration signal at 1153 $cm^{-1}$ indicating low content of DMAEMA segment in the polymer. Hence, FTIR provides preliminary evidence on the ratio of PEGMA and DMAEMA present in each polymer sample, which is further confirmed by $^1$H NMR integration ratio in Fig. 4 and ESI Fig. S1†.

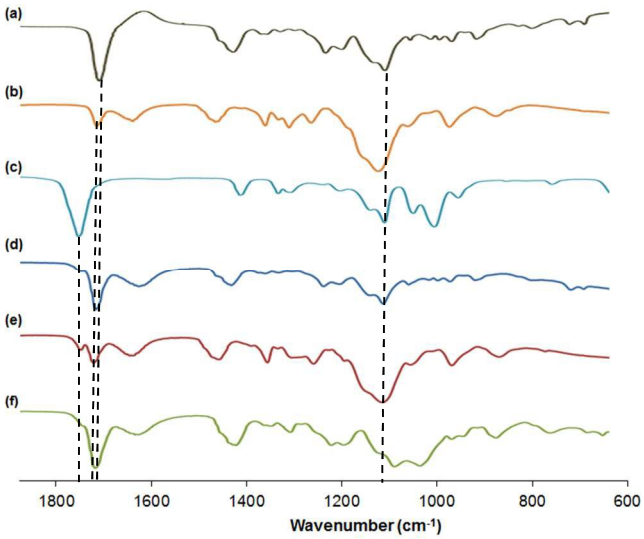


**Fig. 5** FTIR spectra of PLPED1, PLPED2, PLPED3 and its DMAEMA, PEGMA and PLLA precursors: (a) DMAEMA ($M_n$ 157); (b) PEGMA ($M_n$ 1100); (c) PLLA-Br ($M_n$ 10000); (d) PLPED1; (e) PLPED2, (f) PLPED3.

### Proposed hydrogel formation mechanism

Supramolecular hydrogel network can be formed in two-step process; (a) micelle formation resulting from self-assembly of the hydrophobic and hydrophilic segments of polymer in aqueous solution, and (b) micelle association with α-CD due to threading of PEGMA in α-CD cavity forming inclusion complexes (IC)s (Fig. 1). These associations create physical junctions between main polymer chains creating supramolecular network of micelles, physically inducing hydrogel formation.

Controls were done to support the theory of two-step mechanism in forming supramolecular hydrogel, shown in ESI (Fig. S2†). Micelle architecture and micelle association due to inclusion complex formation between α-CD and PEGMA segment are important in inducing the formation of supramolecular network. Homopolymers P(PEGMA) and PDMAEMA are unable to form micelles due to absence of hydrophobic segment. P(PEGMA) do form inclusion complex with α-CD as white precipitate observed. PLLA-PDMAEMA and PLLA-PEGMA polymers do have micelle architectures due to presence of both hydrophobic PLLA and hydrophilic DMAEMA/PEGMA. However, PLLA-DMAEMA polymers did not form gel due to absence of PEGMA segment to form inclusion complex with α-CD. PLLA-PEGMA polymer satisfies the two requirements necessary to form a supramolecular hydrogel network, and as observed, forms a gel.

### Morphology of Hydrogels and Cavity size comparison

The morphological structures of the freeze-dried hydrogels were investigated by SEM. The morphologies of the hydrogels formed by poly(PLLA/PEGMA/DMAEMA) triblock polymers of different compositions and α-CD are shown in Fig. 6. Owing to the amphiphilic property of the triblock polymer as well as the micellization of the triblock polymer in aqueous solution, phase-separation process occurs during the complexation of poly(PLLA/PEGMA/DMAEMA) and α-CD[49]. The hydrogels consisting of poly(PLLA/PEGMA/DMAEMA)/α-CD have porous structures. The pore size of the hydrogels varies with the content of hydrophilic and hydrophobic segments. The pore size decreases with the increasing amount of hydrophobic moiety in the triblock polymer. For hydrogel with higher ratio of hydrophobic polymer segment (PLPED1), the pore size is smaller, while those with lesser ratio of hydrophilic segment (PLPED2, PLPED3), the pore size is bigger. The reason could be that the micellization would aid to the formation of hydrogels and the hydrophobic interactions are stronger for the triblock polymers with the longer hydrophobic segments, rendering smaller pore size.

Supramolecular structures involving cyclodextrin macrocyclic compounds have been documented[47, 50, 51]. However, formation of inclusion complex greatly depends on the chemical property and size of polymer compound and cyclodextrins, as shown in ESI (Table S1 and Fig. S3†). The most common cyclodextrins are α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) which consist of six, seven and eight glucopyranose units respectively. Due to steric factors, cyclodextrins having fewer than six glucopyranose units cannot exist, cyclodextrins containing nine, ten, eleven, twelve and thirteen glucopyranose units, which are designated δ-, ε-, ζ-, η- and θ- cyclodextrin, respectively have been reported[47]. The molecular structures of these glucose derivatives generates a hydrophilic exterior surface and a hydrophobic cavity interior with diameters through which some polymers can penetrate and form inclusion complexes[52-54]. It was shown that only α-CD could induce formation of inclusion complex (see Supporting Information) with the synthesized random triblock poly(PLLA/PEGMA/DMAEMA). β-CD is of limited aqueous solubility compared to the other two, meaning that complexes caused by interaction of hydrophobic PEGMA with these cyclodextrins of limited solubility lead to the precipitation of solid β-CD complexes from aqueous systems. Polymer interaction with γ-CD does show inclusion complex formation due to threading of PEGMA and γ-CD, but because the diameter of γ-CD is almost twice of α-CD, there is a possibility that requires two strands of PEGMA in a ring of γ-CD. The probability of such interactions are much less in a dynamic aqueous solution, hence additive non-covalent interactions are too low to induce formation of gel.

### Critical Gelation Concentration (CGC) determination

To study the effects of polymer and α-CD concentrations on the gelling behavior, hydrogel formation over a range of polymer and α-CD solutions were tested (1-25% w/v). By plotting graphs of concentration of α-CD versus concentration of polymers (Fig. 7), it is observed that hydrogel can be formed at low polymer concentration of 1% (w/v). All compositions showed that hydrogel can be formed at optimum concentration of approximately 5% (w/v) polymer and 5% (w/v) α-CD.

### Critical Micelle Concentration (CMC) determination

These amphiphilic triblock polymers are able to form micelles in aqueous solution. Dye absorption technology was used to detect the

formation of micelles. The CMC values of the poly(PLLA/PEGMA/DMAEMA) triblock polymers in aqueous solutions were confirmed by absorbance spectra of DPH as a function[48]. This experiment was conducted by varying the aqueous polymer concentration in the range of 0.0001 to 0.5 wt%, while keeping the concentration of DPH constant. DPH shows a higher absorption coefficient in a hydrophobic environment than in water. Thus, with increasing polymer concentration, the absorbances at 344, 358 and 378 nm increased, as shown in ESI (Fig. S4†). The point where the absorbance suddenly increases corresponds to the concentration at which micelles are formed. When the micelle is formed, DPH partitions preferentially into the hydrophobic core formed in the aqueous solution[55-57]. The absorbance at 378 nm minus the absorbance at 400 nm ($A_{378} - A_{400}$) was plotted as a function of the logarithmic concentration of the triblock polymer to determine the CMC (Fig. 8). The CMC values for the water-soluble copolymers are tabulated in Table 1 and are in the range of 3.15 x $10^{-4}$ to 4.27 x $10^{-4}$ g $mL^{-1}$. In general, the CMC would decline with higher ratio of hydrophobic segment because longer hydrophobic blocks are easily removed from the aqueous environment in order to achieve a state of minimum free energy[58]. In this study, with the ratio of hydrophobic PLLA increasing, the CMC of the poly(PLLA/PEGMA/DMAEMA) triblock copolymers decreases, which is consistent with the mechanism mentioned above.

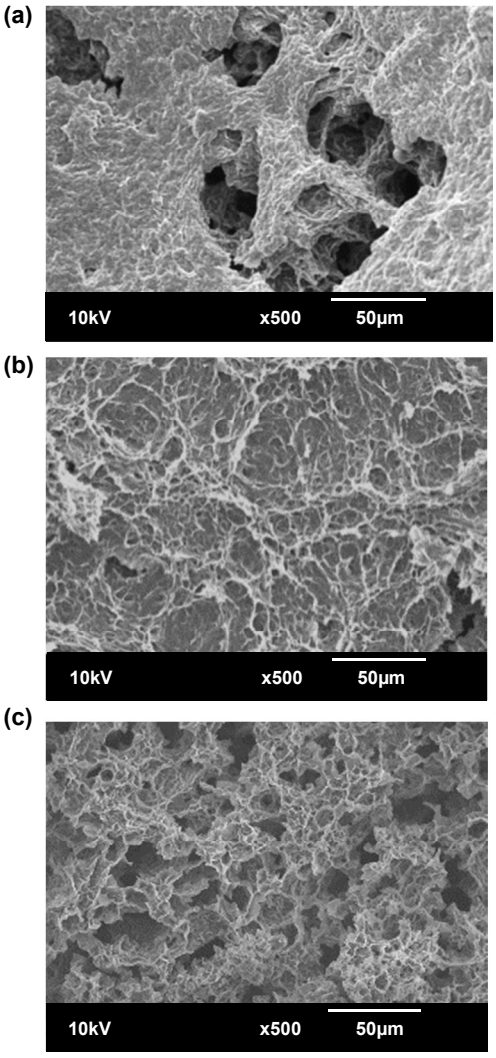


**Fig. 6** SEM micrographs of different compositions of poly(PLLA/PEGMA/DMAEMA) 10% (w/v) / α-CD (10% w/v) hydrogels. (a) PLPED1 (b) PLPED2 (c) PLPED3.

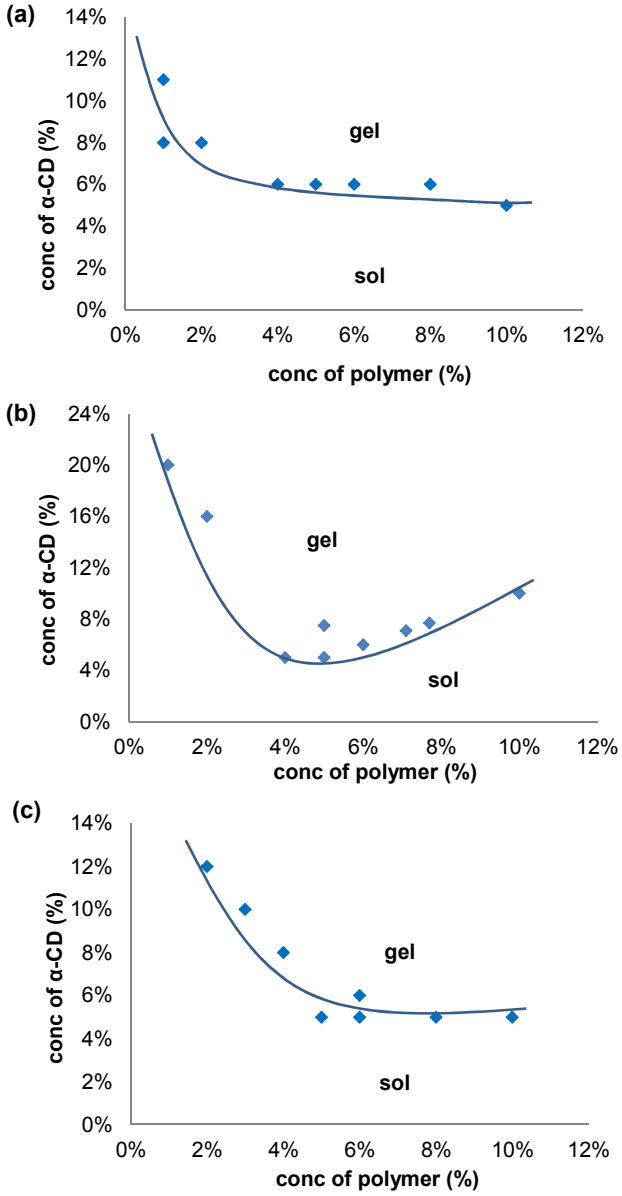


**Fig. 7** Sol-gel transition graphs of different compositions of polymers/α-CD hydrogels: (a) PLPED1; (b) PLPED2; (c) PLPED3

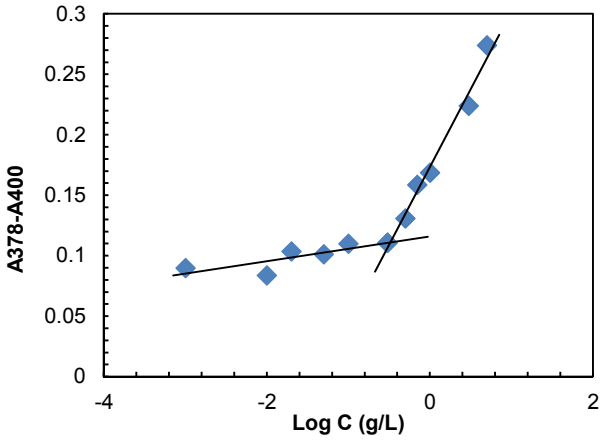


**Fig. 8** CMC determination of PLPED2 by extrapolation of the difference in absorbance at 378 and 400 nm.

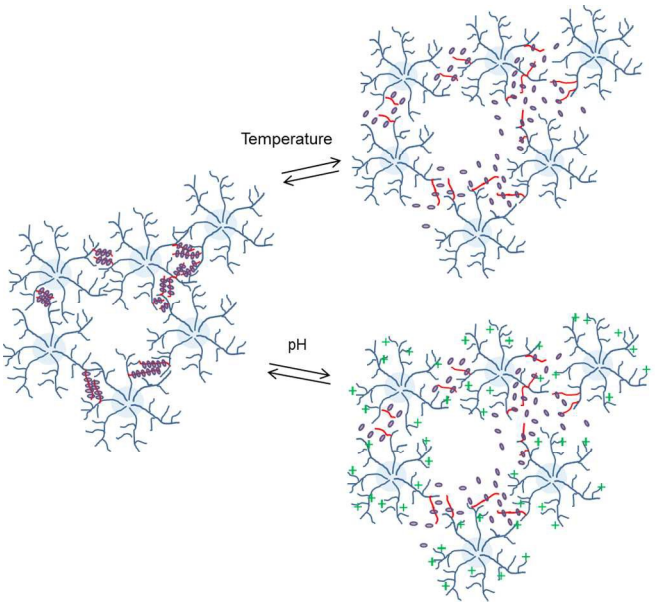


**Fig. 9** Sketch of dual stimuli-responsive mechanism of hydrogel.

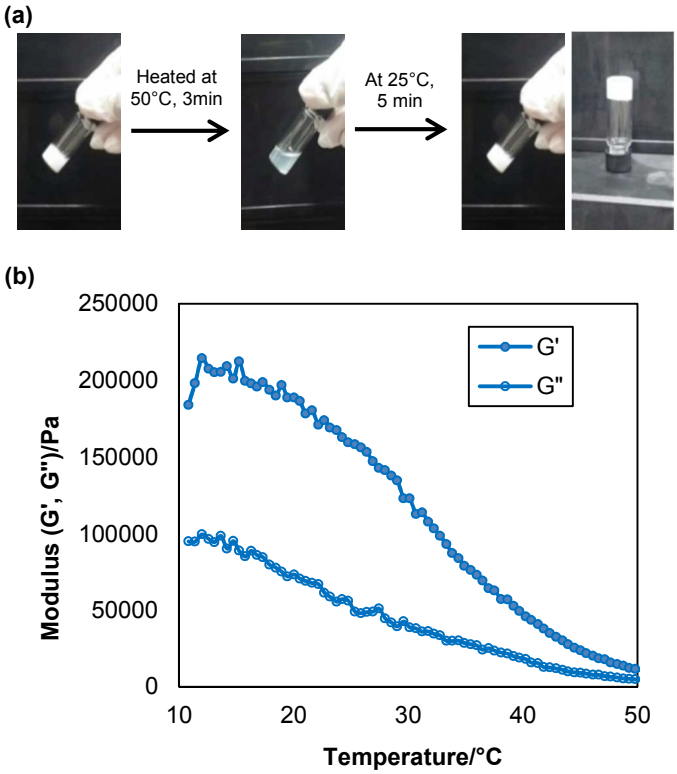


**Fig. 10** Temperature response on PLPED2 10% (w/v) / α-CD 10% (w/v) hydrogel (a) Temperature response observation on physical state of hydrogel (b) Temperature sweep data on hydrogel; Storage modulus (G') and loss modulus (G") measured as a function of temperature, constant strain at 1%, temperature range 10 °C-50 °C, ramp rate 5 °C/min.

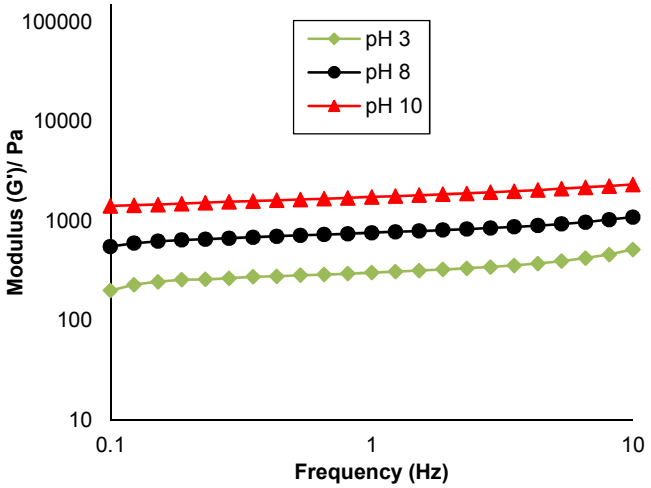


**Fig.11** Frequency sweep data on PLPED2 10% (w/v) / α-CD 10% (w/v) hydrogel at pH 3, 8, and 10 Storage modulus (G') was measured as a function of the frequency under oscillatory shear at a strain of 1%, in the frequency range 0.1–10 Hz, at 37 °C.

**Mechanical property of hydrogels**

Samples of 10% (w/v) triblock polymers of different compositions and 10% (w/v) α-CD were prepared. All samples were characterized by dynamic mechanical rheology. Fig. 12 shows G' and G" versus frequency over a range of 0.01 – 10 Hz at 37°C. Biomaterials with moduli in the kPa range are of widespread interest since many native tissues have moduli in this range, although most have nonlinear response to strain. For example, human nasal cartilage (234727 kPa)[59, 60], bovine articular cartilage (990750 kPa)[59, 60], pig thoracic aorta (43.2715 kPa)[61], pig adventitial layer (4.7271.7 kPa)[61], right lobe of human liver (270710 kPa)[62], canine kidney cortex and medulla (B10 kPa)[63], and nucleus pulposus and eye lens (B103 Pa)[63]have moduli in this range. Moreover, for scaffolding applications, it is often desirable to ''match'' mechanical properties of the polymer matrix to those of the surrounding tissue[64]. Based on the storage modulus, G', the molar weight between the effective crosslinks, $M_c$, was calculated based on the eqn (1)[65, 66].

$$G' = \rho RT/M_c$$

where ρ is the polymer concentration (g m-3), R is the molar gas constant and T is the absolute temperature. The mesh size values are related to the composition of hydrophilic and hydrophobic segments. By increasing the ratio of hydrophilic segments to hydrophobic segments, the mesh size increases due to weaker hydrophobic interactions among the copolymer chains. Based on the mesh size, the formulation can be tuned for desired controlled/sustained release of actives.

**Self-healing property of hydrogels**

Physical interactions in supramolecular hydrogels have important consequence on the self-healing property of the material. The additive weak non-covalent interactions present in the network allows for association strong enough to form gel, yet weak enough for the network to be disrupted when exposed to external stimuli such as mechanical stress. When this external stimulus is removed,

the material will self-assemble or heal to its original gel structure by the same physical interactions. Dynamic rheology study was performed to support this theory (Fig. 13). Varying the strain % at 0.1% and 50% instantaneously for 10 cycles showed that the material was able to recover back to its original gel structure almost immediately after subjected to high strain.

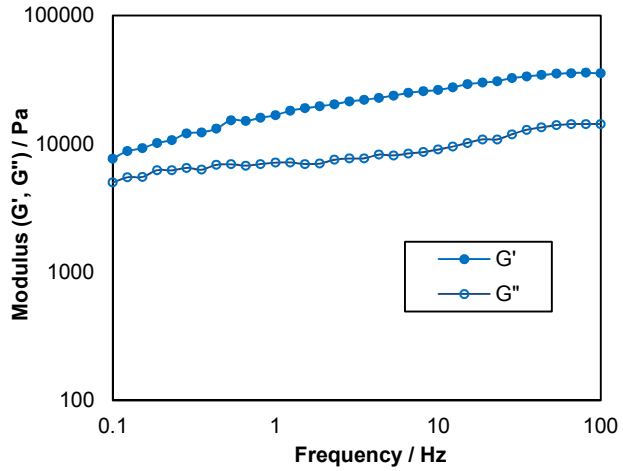


**Fig. 12** Frequency sweep data on PLPED2 10% (w/v) / α-CD 10% (w/v) hydrogel. Storage modulus (G') and loss modulus (G") were measured as a function of the frequency under oscillatory shear at a strain of 1%, in the frequency range 0.1-100 Hz, at 37 °C.

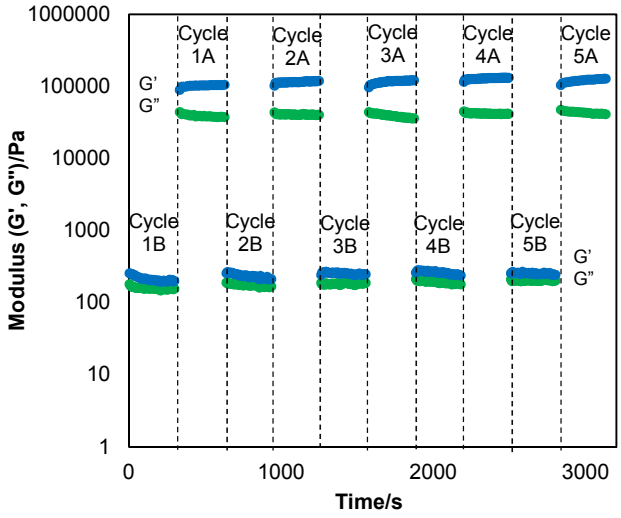


**Fig. 13** PLED2 10% (w/v) / α-CD 10% (w/v) hydrogel subjected to self-healing cycle amplitude sweep test at 2 strain points (A) 0.1% (B) 50%, subjected to 300 s each strain point, at 37 °C.

## Conclusions

A new supramolecular hydrogel system consisted of poly(PLLA/PEGMA/DMAEMA) triblock and α-CD was developed in this study. Poly(PLLA/PEGMA/DMAEMA) triblock polymers of varying hydrophilic compositions were synthesized by ATRP. Their chemical structure and molecular characteristics were determined by $^1$H NMR, GPC and FTIR, which confirmed the architecture of the random block poly(PLLA/PEGMA/DMAEMA).

From the experimental data of the micellar and gelation studies, design of novel supramolecular hydrogel system was proposed in two steps; (a) micelle formation resulting from self-assembly of the hydrophobic and hydrophilic segments of polymer in aqueous solution, and (b) micelle association with α-CD due to threading of PEGMA in α-CD cavity forming inclusion complexes (IC)s.

This finding provides foundation in understanding micelle architecture and cyclodextrin association for supramolecular hydrogel formation to engineer injectable gel depot for controlled/sustained release of actives.

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## Notes and references

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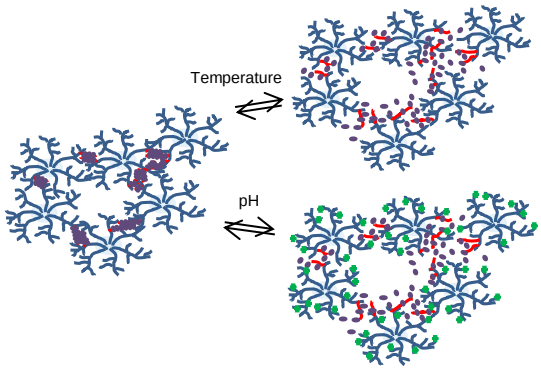


This study fabricated for the first time two-step mechanism of supramolecular hydrogel system to engineer injectable gel depot for controlled/sustained release of actives.