**Supporting Information**

# Self-Assembly of Partially Alkylated Dextran-*graft*-Poly[(2-dimethylamino)ethyl methacrylate] Copolymer Facilitating Hydrophobic/Hydrophilic Drug Delivery and Improving Conetwork Hydrogel Properties

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**Synthesis of Dex-*g*-PDMA Copolymer**

First ATRP initiator viz. Dex-Br macro-initiator was prepared.[1] 2-bromoisobutyric acid (4.2 g, 0.0251 mol) was dissolved in dried DMSO (10 mL), and to this solution, CDI (3 g, 0.0185 mol, separately dissolved in 10 mL DMSO) was added through a syringe. The reaction mixture was allowed to stir for 5 h at 30 $^{o}$C. After 5 h of reaction, this solution was added drop-wise to the dextran solution (dextran $M_n$~10000, 2 g, separately dissolved in 10 mL of dried DMSO). The reaction was allowed to run with continuous stirring for 6 h at 60 $^{o}$C. After 6 h, the reaction mixture was precipitated in excess acetone. The precipitated mass was washed with hexane thrice and then dissolved in deionised water. The solution was dialysed against water using dialysis bag of MWCO 2000. The separated transparent aqueous solution was then lyophilized to collect the product and characterised by $^{1}$H NMR spectroscopy (Figure S1). $^{1}$H NMR spectrum ($D_2O$, δ/ppm): 3.4−4.0 (C<u>H</u>−O and C<u>$H_2$</u>−O) of glucose unit of Dex, and 1.93 (C−Br)−(C<u>$H_3$</u>)$_2$ of initiator fragment.

Dex-*g*-PDMA copolymer was synthesised by the atom transfer radical polymerization (ATRP). A typical example is as follows.[1] Dex-Br macroinitiator (0.4 g, 0.00004 mol) was dissolved in deionised water (5 mL) and then methanol was added to it (3 mL). The mixture was allowed to stir for 20 min. Next, (DMA, 3 mL, 0.0178 mol) was added to this solution. The mixture was purged with nitrogen for 5 min. CuBr (0.045 g, 0.00031 mol) was added to the reaction mixture under nitrogen atmosphere and sealed with the rubber septum. 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 0.1 mL, 0.00037 mol) was then added to the above reaction mixture via the Hamilton syringe under the nitrogen atmosphere. The reaction was allowed to run 15 min at 30 $^{o}$C. The reaction was stopped by the addition of THF and opened in the air. THF was removed by rotary evaporator. The remaining mixture was diluted with deionized water and dialysed against deionized water using dialysis membrane (MWCO 12000) to remove copper catalyst for 48 h. The dialyzed mass was

precipitated in excess methanol. The mass was then extracted with toluene for 6 h twice. It was then dried under rotary evaporator. The obtained product was characterised by $^1$H NMR spectroscopy (Figure S1). The monomer conversion was calculated to be 21%. This copolymer was abbreviated as (Dex-*g*-PDMA-1). A similar procedure was used to synthesise copolymer Dex-*g*-PDMA-2 by increasing the ATRP time to 45 min. The monomer conversion was 47%. $^1$H NMR spectrum ($D_2O$, δ/ppm): 3.4−4.0 (CH−O and $CH_2$−O) of glucose unit of Dex, 4.2 (-COOC$\underline{H}_2$-), 2.67 (-COOCH$_2$C$\underline{H}_2$-), 2.28 (N-(C$\underline{H}_3$)$_2$, 0.89 (C-C$\underline{H}_3$), and 1.83 (C-C$\underline{H}_2$), of PDMA.

**Quaternization of Dex-*b*-PDMA-1 and Dex-*b*-PDMA-2 with Octadecyl Bromide**

The graft copolymer was quaternized as follows. The Dex-*g*-PDMA-1 copolymer (0.6 g, 0.0027 mol of DMA unit) was dissolved in 5 mL of DMF in a round bottom flask. 1-bromooctadecane (0.124 g, 0.000371 mol) was added to the above solution. The reaction was allowed to continue for 24 h at room temperature. After that, the DMF was removed by rotary evaporator. The reaction mixture was then diluted with methanol and precipitated in hexane. The obtained product was characterised by $^1$H NMR spectroscopy (Figure S1). This copolymer was abbreviated as Dex-*g*-PDMA-QPDMA-1A. Similarly, the copolymer abbreviated as Dex-*g*-PDMA-QPDMA-1B (0.6 g, 0.0027 mol of DMA unit) was prepared by the reacting Dex-*g*-PDMA-1 with 1-bromooctadecane (0.248 g, 0.000743 mol). $^1$H NMR ($D_2O$, δ/ppm): 0.89 (C-C$\underline{H}_3$), 1.83 (C-C$H_2$) of PDMA backbone, 2.28 (N)-(C$\underline{H}_3$)$_2$, 2.67 (N-C$\underline{H}_2$) of PDMA side chain, 3.17 ($N^+$-C$\underline{H}_3$), 3.42 ($N^+$-C$H_2$) of quaternized PDMA and 4.12 (-COOC$\underline{H}_2$).

**Synthesis of PDMA Homopolymer by RAFT and Quaternization**

The PDMA was synthesised by RAFT polymerization.[2] The monomer DMA (17 gm) was dissolved in DMF (15 mL). Next, the S-1-Dodecyl-S´-(α,α´-dimethyl-α´´-acetic acid)

trithiocarbonate (0.6 g, 16.4 $x10^{-4}$ mol) chain transfer agent was added into the monomer solution. The admixture was then purged with nitrogen for about 15 min. The radical initiator AIBN (0.016 g, 9.6 $x10^{-5}$ mol) was introduced into the reaction mixture under the stream of nitrogen. After that, the RAFT polymerization was conducted for 12 h at 70 $^{o}$C. After cooling to room temperature, it was then precipitated in excess hexane. The mass was dissolved in methanol and re-precipitated in hexane. The polymer was then dried in vacuum oven at 60 $^{o}$C for 48 h. The monomer conversion was found to be 84%. The molecular weight and PDI was found to be 11000 g/mol and PDI 1.3 respectively as determined by GPC. Quaternization of the PDMA was also performed with butyl bromide, decyl bromide and octadecyl bromide respectively as described above. The extent of alkylation was found to 18-20% mom/mol to the total DMA groups. The prepolymers obtained by the alkylation of PDMA homopolymer with butyl bromide, decyl bromide, and octadecyl bromide are abbreviated as PDMA-QPDMA(C-4), PDMA-QPDMA(C-10) and PDMA-QPDMA(C-18) respectively. These prepolymer (without Dex) were employed for the preparation of PDMA(C-4)-HG, PDMA(C-10)-HG and PDMA(C-18)-HG hydrogels. A PDMA-HG hydrogel of PDMA (without alkylation) and PEG was also prepared.

**GPC Measurements of the Graft Copolymers and Homopolymer**

GPC of the graft copolymers and homopolymers were performed using a Waters model 2695 pump system coupled with Waters 2414 refractive index detector and Waters Styragel columns (HR 0.5 DMF, HR 4E DMF and HR 5 DMF). HPLC grade DMF (Spectrochem, India) containing 0.1% (w/v) LiBr was used as eluent at a flow rate of 0.8 mL/min at 40 $^{o}$C. Calibration was performed with poly(styrene) standards. Only PDI values of the graft copolymers were evaluated since, the effective solvated radius of the graft copolymer is different than the standards. The $M_n$ and PDI of the PDMA homopolymer were calculated by the GPC.

**Calculation of Degree of Initiator Substitution, Composition and Molecular Weight of the Graft Copolymers**

The degree of initiator substitution was calculated by the $^{1}$H NMR spectroscopy using the following equation:

$$\text{Degree of initiator substitution} = (I_{1.92} / I_{3.4-4}) \qquad (1)$$

Where $I_{1.92}$ is the integral area of the six protons of (C−Br)−(C$\underline{H}_3$)$_2$ initiator moiety at δ value, 1.92 and $I_{3.4\text{-}4}$ is the integral area of six protons of glucose unit of Dex (Figure S1).The molecular weight of total grafted PDMA was determined by the following equation:

$$M_n(\text{PDMA}) = [(6 \times 55/I_{3.4-4})I_{4.2}] \times \frac{157}{2} \qquad (2)$$

Where $I_{4.2}$ is the integral area of two protons of –COOC$\underline{H}_2$- of DMA units, 55 x 6 is the total number of protons of each Dex chain, and 157 is the molecular weight of DMA unit. The $M_n$ of per chain of PDMA was calculated by dividing the number of initiator moiety per Dex chain in the graft copolymer. The alkylation (mol %) was calculated by the following equation:

$$\text{Alkylation} = \left(\frac{I_{3.17}/6}{I_{3.17}/6 + I_{4.2}/2}\right) \times 100 \qquad (3)$$

where $I_{3.17}$ is the integral area of the signal of six protons of $N^{+}$(C$\underline{H}_3$)$_2$ and $I_{4.2}$ are the integral area of two protons of (methyl of ester) PDMA. Table 1 (main text) shows the characteristics data of the graft copolymers.

## Characterizations

### Scanning Electron Microscopy (SEM) Analysis

SEM samples were prepared by freeze drying of the completely swollen hydrogel or bandage to avoid shrinkage of pores.[2] The fully swollen hydrogel was broken under liquid nitrogen and then freeze-dried to prepare the sample for the Cross-sectional SEM image. The dried samples were coated in a sputter coater (LEICA EM ACE 200 gold coater) and then SEM images were taken using scanning electron microscope (FESEM, JSM-7100F).

### FT-IR Spectroscopy and Solid State $^{13}$C NMR Spectroscopy

Solid state $^{13}$C NMR (600 MHz, Bruker) spectra of the lyophilized hydrogel were recorded at temperature 25 $^{o}$C. FTIR spectra of completely dried (lyophilized) hydrogels were recorded using Agilent Cary 600 series at room temperature by preparing KBr pellets.

### Measurement of Degree of Swelling

Prepared hydrogels were extracted with DMF and then with water. The samples were dried completely by lyophilisation. The lyophilized and pre-weighed hydrogels (0.5 g) each were submerged in water of pH 7.4 and pH 5 separately and were incubated in an incubator shaker (INFORS AGCH-4103) at shaking speed of 100 rpm at temperature 37 $^{o}$C. The hydrogels were removed at a specific time interval; excess water was wiped off, and weights were recorded. The swelling (%, w/w) was determined by using the following equation:

$$\text{Swelling (\%)} = \frac{W_{swollen} - W_{dried}}{W_{dried}} \times 100 \qquad (4)$$

where $W_{swollen}$ and $W_{dried}$ are the weights of the swollen and the dry hydrogel respectively.

These experiments were conducted in triplicate and average values were taken.

**Determination of Sol Fraction**

For the determination of sol fraction, the preweighed lyophilised hydrogels (0.5 g) were immersed in DMF for 48 h in the incubator shaker (INFORS AGCH-4103) at shaking speed of 100 rpm and temperature 37 $^{o}$C. After 48 h hydrogels were washed with methanol to remove DMF and then dried at 50 $^{o}$C. The dried hydrogel was weighed, and sol fraction was determined by the following equation:

$$\text{Sol fraction } (\%) = \frac{w_b - w_a}{w_b} \times 100 \qquad (5)$$

where $W_b$ and $W_a$ are the weight of the hydrogels before and after extraction respectively. The experiments were conducted in triplicate and average values were considered. The total soluble fraction (DMF and acetone) was collected after evaporation of solvent. The $^{1}$H NMR of the soluble mass was recorded using $CDCl_3$. The soluble mass of representative Dex-HG-1 hydrogel contains signals of PEG, Dex and PDMA.

**Powder X-ray diffraction measurements**

Powder X-ray Diffraction analysis was performed using PANalytical EMPYREAN Goniometer theta/theta diffractometer with Cu Kα radiation (30 mA, 40 kV, 1.54 A$^{o}$ wavelength). The copolymers and hydrogels were lyophilized to a dried and loaded on a quartz plate. The experiment was performed at a step size of 0.013$^{o}$ at the scan rate of 0.02$^{o}$/min. Similarly, the fully water swollen hydrogels were scanned for comparison.

**Bioadhesive Stress of the In Situ Hydrogels**

Bioadhesive stress of the hydrogels was assessed using a universal testing machine (ISO 527 S2 method using Zwick Roell Z2.5). Fresh and separate goat skins were incubated in PBS (0.01 M, pH 7.4) at 37 $^{o}$C for 1 h. The skins were kept in moist condition. The mixed prepolymers solution (16.7%) was then applied on the skin surface (bonding area: 1x1 cm$^{2}$).

The other piece of the fixed-skin specimen was immediately stacked up with the prepolymers-skin layer at room temperature. The specimen was avoided to dryness. Thus the prepolymer solution stayed in between two skin specimens. The specimens sandwich by the prepolymers were then immediately kept inside the incubator of temperature 37 $^{o}$C for 1h in moist condition to provide complete curing time. The thickness of the formed hydrogel layer was determined to be 0.2 mm. Next, the bioadhesive stress was assessed by fixing the two ends of the individual skin layer using specimen holder. The testing was performed at pulling speed of 5 mm/min at room temperature, and the value of the bonded area (1 cm$^2$) was considered for determination of adhesive stress. Each measurement was repeated at least 3 times to obtain average adhesive strength. The bioadhesive stress was the ratio of load (force) at the detachment of the specimens to the bonded (overlap) area.

**Determination of Degradation Behavior of the Hydrogels**

For the determination of the degradation behaviour, the pre-weighed lyophilized hydrogels (0.5 g each) were submerged in PBS of pH 7.4 and 5 separately. Degradation experiments were performed in an incubator shaker (INFORS AGCH-4103) at shaking speed of 100 rpm at 37 $^{o}$C. The hydrogels were then submerged in water to remove buffer salt. The weight of the lyophilized hydrogels was then recorded. The degradation (% w/w) was calculated by the following equation: [2,3]

$$\text{Degradation } (\%) = \frac{W_i - W_t}{W_i} \times 100 \qquad (6)$$

where $W_i$ and $W_t$ are the initial weight and the weight of the hydrogel obtained after the certain time of degradation.

**Loading and Release of Ornidazole**

A representative example of drug loading and release experiment is as follow. The ornidazole was dissolved in phosphate buffer pH 7.4 to maintain final concentration 0.017

g/mL. The Cl-PEG-Cl was dissolved in above drug solution. The final concentration of Cl-PEG-Cl was 0.5 g/mL and the ornidazole concentration, 0.017 g/mL respectively. Similarly, Dex-*g*-PDMA-1 was dissolved in the above drug solution. The concentration of the copolymer was 0.1 g/mL. The hydrogel was prepared by mixing solutions of Cl-PEG-Cl (1 mL) and Dex-*g*-PDMA-1 (5 mL) in the proportion of 1:1 by weight. The total amount of ornidazole was 0.10 g in the admixture where prepolymer amount was 1 g. The loading of the drug was thus 10%, w/w. The drug-containing mixed solution underwent rapid gelation. The gelation time did not change by the presence of the drug. The prepared hydrogel was then put into a dialysis membrane (containing 5 mL PBS) with the molecular cut-off of 1200 g/mol. The dialysis membrane was submerged in phosphate buffer pH 7.4 (0.1 M, 50 mL) and kept in the incubator shaker (INFORS AGCH-4103) with shaking speed of 100 rpm at 37 $^{o}$C. After the certain time, the dissolution media was changed with fresh media and the drug release was measured using UV-Visible spectrophotometer (Shimadzu UV-1800) at $\lambda_{max}$ 319 nm. A standard calibration curve (absorbance vs. concentration of ornidazole) was used for the determination of cumulative release of ornidazole.

**Disc Diffusion Experiment for Evaluation of Antimicrobial Activity of the Ornidazole Loaded Hydrogels**

The drug-loaded bandage was cut into pieces (2 cm$^2$) and submerged in PBS (40 mL). The drug-loaded bandage was incubated in the incubator shaker (100 rpm) for 8 days at 37 $^{o}$C. The media was changed after every 24 h with fresh PBS. After 8 days of incubation, the sample was removed and washed with PBS (pH 7.4). The samples were then cut into the disc (6 mm dia) and subjected to bacterial disc diffusion assay as follows.

The discs were placed on nutrient agar Petridish (90 mm) with the help of sterile forceps. The nutrient agar media was autoclaved at 121°C for 15 min before the experiment. The media (25 mL) was then poured in the Petridish. The media were get solidified at room temperature. Next, *E.coli* or *B. subtilis* bacterial culture (1 mL) was spread on the plate and

dried for 1 min, kept in the incubator at 37 $^{o}$C for 12 h. The inhibition zone was measured for each disc. Similarly, hydrogels samples with and without ornidazole were subjected to the above experiments.

**Coating of Commercial Bandage**

Commercial sterile gauze bandage was taken four folded (4.5 cm x 5 cm) fixed on glass plate and coated with 16.7 % (w/v) homogeneous solution (1:1 v/v) of Cl-PEG-Cl (7.4 pH, 0.5 g/mL, 1 mL) and Dex-*g*-PDMA-QPDMA-1B (7.4 pH, 0.1g/mL, 5 mL) by pouring the solution. The mixed solution contains ornidazole (0.1 g). The prepolymer solution adsorbed bandage was then kept at room temperature for 1h. The coated bandage was then submerged in sterile deionized water for one hour, and used for further characterization. For the preparation of SEM sample, the wet bandage was lyophilized. The hydrogel coated bandage (containing drug) was then rolled in wet condition in packed condition.

**Hemocompatibility of Hydrogel**

Hemocompatibility assay was performed as follows. About 3 mL of Human blood was collected (healthy volunteer) in heparin tube. It was then centrifuged at 1500 rpm for 10 min. The RBC pellet was re-suspended in 0.01 M PBS (pH 7.4) and collected via the centrifugation. This process was repeated trice to remove blood serum if any. The RBC pellets were diluted to 10 mL PBS to prepare RBC stock solution. The premade dry hydrogels (30 mg each) was then saturated with 0.01 M PBS. The samples were then incubated with RBC stock solution (1 mL) for 3 h at temperature 37 $^{o}$C. After incubation, it was centrifuged at 2500 rpm for 5 min and the supernatant was collected. The released haemoglobin in the supernatant was analysed at 540 nm using UV−Visible spectrophotometer (Bio-RAD 680, USA). The experiment was performed in triplicate and percent haemolysis was calculated with respect to haemolysis caused by PBS (negative control) and Triton X-100 (positive control, 1% w/v in PBS) using following equation:

$$\text{Hemolysis (\%)} = \frac{(\text{Sample}_{540\text{ nm}} - \text{negative control}_{540\text{ nm}})}{(\text{Positive control}_{540\text{ nm}} - \text{negative control}_{540\text{ nm}})} \text{ X } 100 \qquad (7)$$

The individual prepolymer solutions of Cl-PEG-Cl and Dex-*g*-PDMA-QPDMA-1B in PBS (pH 7.4) were added in the RBC solution wherein the concentration of the each prepolymer was 10% w/v in the RBC solution. The extent of hemolysis was then performed. A prepolymer solution, i.e. the mixture of Cl-PEG-Cl and Dex-*g*-PDMA-QPDMA-1B with the total concentration of 16.7% w/v in RBC solution was similarly tested. The mixture of prepolymer solution formed hydrogel in the RBC solution.

**DNA Encapsulation and Stability by the DNA-Hydrogel Hybride**

For the DNA adsorption experiment, *Vibrio cholerae* bacterial genomic DNA was used, the DNA was isolated by well-known chloroform phenol method. DNA isolation was done using slightly modified chloroform method.[4] Briefly, 1.5 ml of overnight culture of Vibrio cholerae was taken centrifuged at 7000 rpm for 5 min at 4°C. Followed by addition of 567 µl TE buffer (100 mM pH 8.0). Pellet was resuspended and 30µl of 10% SDS was added with 2 µl protinase K (20mg/ml). Samples were incubated at 37 °C for 1h. After incubation 80 µl lysis buffer (CATB/NaCl) and 100 µl of 5 M NaCl. Samples were mixed vigorously and incubated at 65°C for 10 min. after incubation equal volume of chilled chloroform: isoamyl alcohol (29:1) was added samples were mixed by inverting at least 50 times. The samples were then centrifuged at 13000 rpm for 5 min at 4°C. The supernatant was transferred to new tube equal volume of Phenol: chloroform: isoamyl alcohol was added (25:24:1). The samples were then centrifuged at 13000 rpm for 5 min at 4°C. Supernatant was taken in fresh tube the DNA was precipitated using 0.6 volume of chilled isopropanol. DNA was resuspended in the 30 µl TE buffer (100 mM pH 8.0) Stored until further use. The quality and concentration of freshly isolated genomic DNA were measured by NanoDrop 1000 Spectrophotometer instrument. Next, the dried Dex-HG-1BQ hydrogel (20 mg) was fully swollen in PBS 7.4 pH and

submerged in 0.1 mL (472.12 ng/μL) of DNA solution and incubated for 3 h at 37 $^{o}$C. There after the hydrogel was removed out from DNA solution and again measured the concentration on DNA in solution and found (81.6 ng/μL) remains in solution. The hydrogel was washed thoroughly with PBS and subjected in UV light UV light in Bio-Red doc system the hydrogel DNA hybrid gel shows fluorescence whereas pristine hydrogel could not. The stable immobilization of DNA over the hydrogel verified by elution of immobilized DNA with TE buffer and their PCR amplification of 16S gene with before and after DNA solution samples. The universal 16S gene primers 27F 5′ AGAGTTTGATCMTGGCTCAG 3′ and 1492 R 5′ TACGGYTACCTTGTTACGACTT 3′ were used. The PCR condition was initial denaturation 94˚C for 7 min, denaturation 94 $^{o}$C for 45 seconds, annealing 55˚C for 1 min and extension 72˚C for 1 min for 30 cycles followed by final extension at 72 ˚C for 10 min. after that all PCR product were run on 1% agarose gel with 1 KB ladder at 70 mV for 15 min and gel was observed in Bio-Red Gel Doc system and captured the image. Hemocompatibility of the prepolymers, hydrogels and species formed by the degradation of the hydrogels were tested using red blood cells.

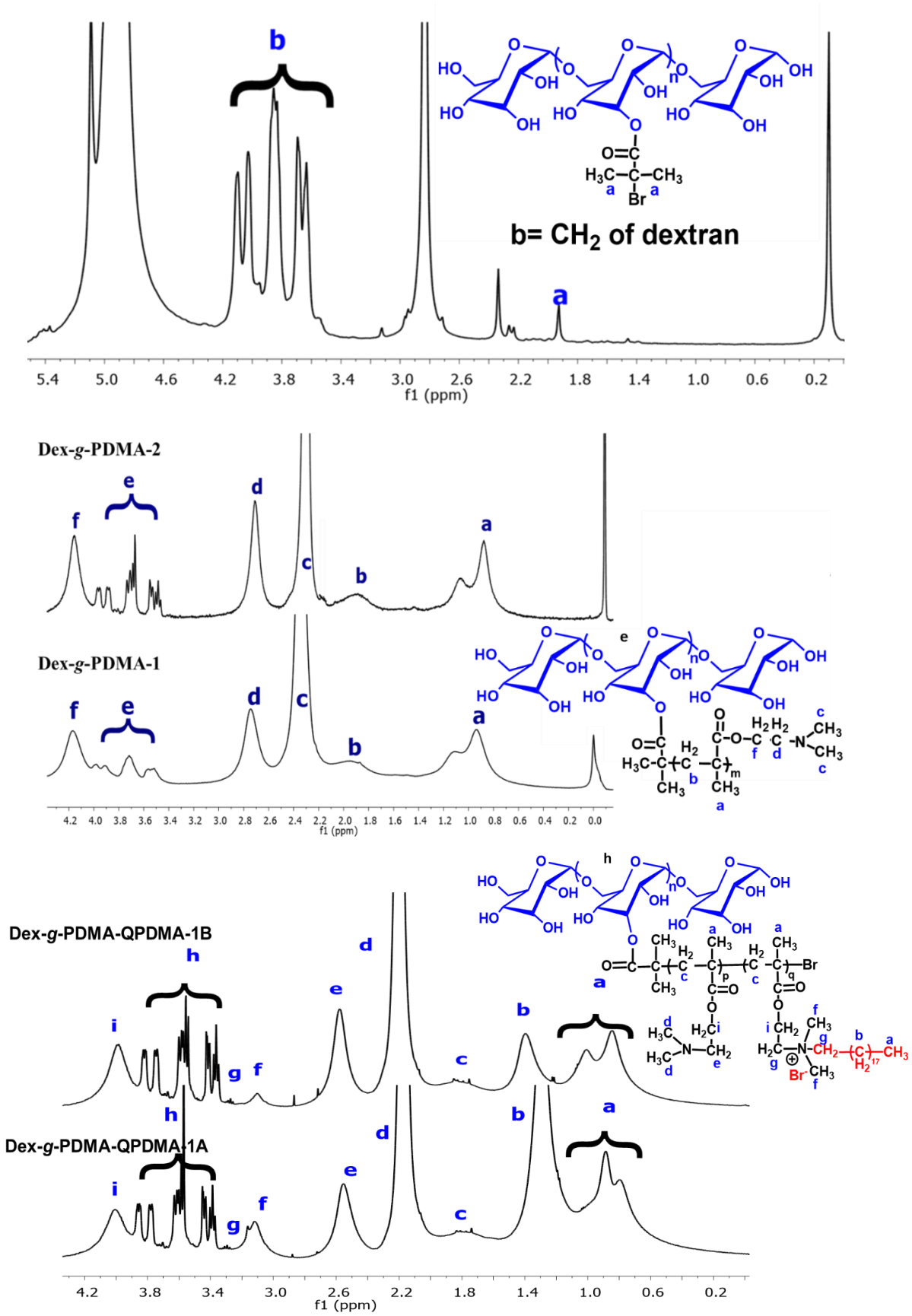



**Figure S1**. $^{1}$H NMR (600 MHz, $D_2O$) spectra of Dex-Br macroinitiator and different graft copolymers.

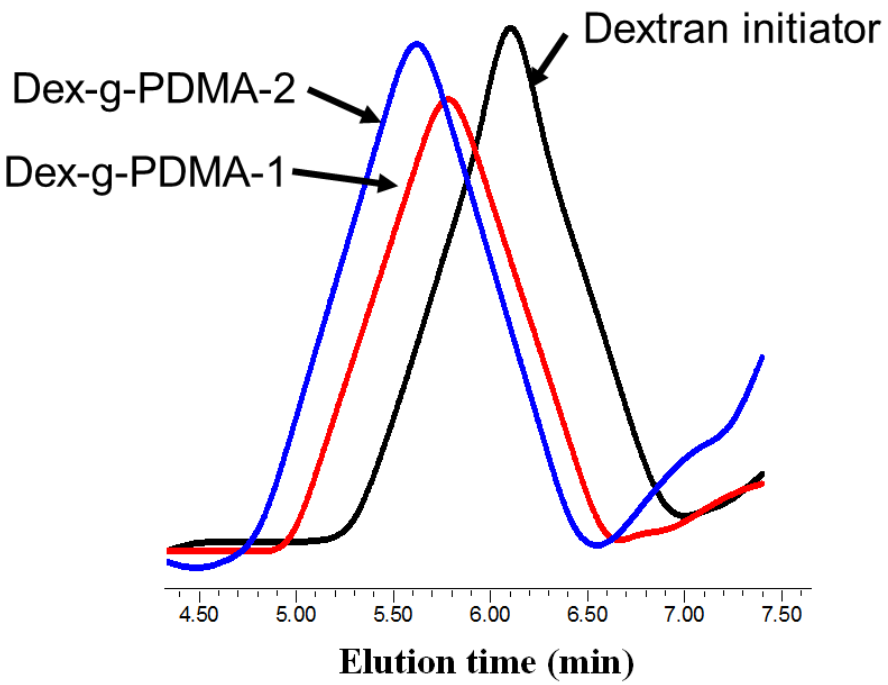



**Figure S2.** GPC traces of Dex-based initiator and synthesized graft copolymers. GPC was performed using a Waters model 2695 separation module coupled with Waters 2414 refractive index detector and Waters Styragel columns (HR 0.5 DMF, HR 4E DMF and HR 5 DMF). HPLC grade DMF containing 0.1% (w/v) LiBr was used as eluent with flow rate of 0.8 mL/min. Poly(styrene) standards were used for calibration.

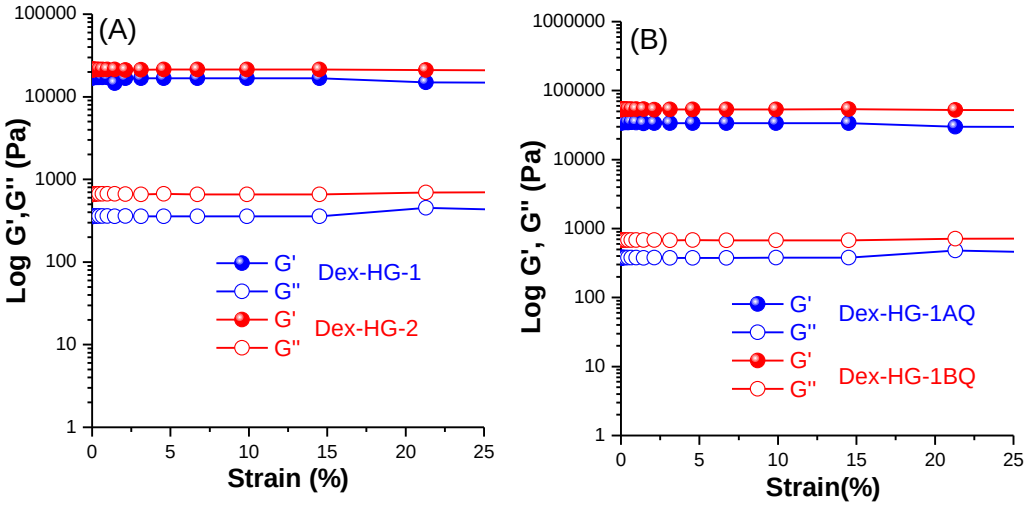



**Figure S3**. Modulus of the hydrogels with increasing applied strain (%) showing viscoelastic region.

The IR band at 1725 $cm^{-1}$ is attributed to the ester carbonyl of DMA moieties of PDMA (-COO-$CH_2$-). IR vibrational bands at 943 $cm^{-1}$ and 1100 $cm^{-1}$ are due to C-O stretch and -$CH_2$- rocking of PEG. A band at 1152 $cm^{-1}$ is present due to the additional presence of C–O–C bond and glycosidic bridge from Dex moiety of hydrogels.[5] The $^{13}C$ NMR signals at δ value 180 ppm is due to the carbonyl carbon of ester moieties (-$^{*}$COO-$CH_2$-). The signals at δ values 70 ppm and 63 ppm are due to the ether carbon of PEG ($^{*}CH_2$-O-), and methylene carbon of PDMA ($^{*}CH_2$-N). The signals at 57 ppm and 54 ppm are due to the methyl carbon of PDMA (N-$^{*}CH_3$). The new signal appeared at δ value 28 ppm for the Dex-HG-1AQ and Dex-HG-1BQ hydrogels which are due to methylene carbons of long chain (C-18) alkyl. This signal is absent in the Dex-HG-1 and Dex-HG-2 hydrogels. The signals at 70-75 ppm are also due to the C-2, -3, -4, and -5 chemical-shifts of Dex moieties in the hydrogels.[6] The weak IR band at 1550 $cm^{-1}$ is probably due to the $^{+}N(CH_3)_2$ group which is formed by the reaction between tertiary amine and halide ends of PEG.

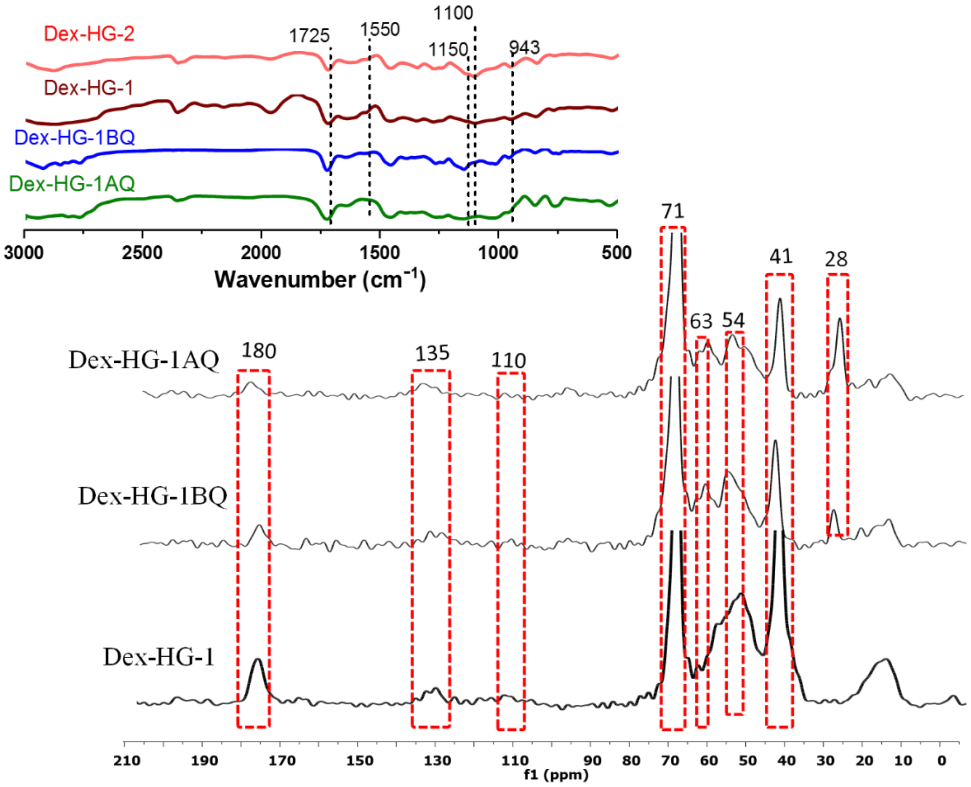



**Figure S4**. FT-IR (top) and solid state $^{13}C$ NMR (600 MHz) (bottom) spectra of the hydrogels.

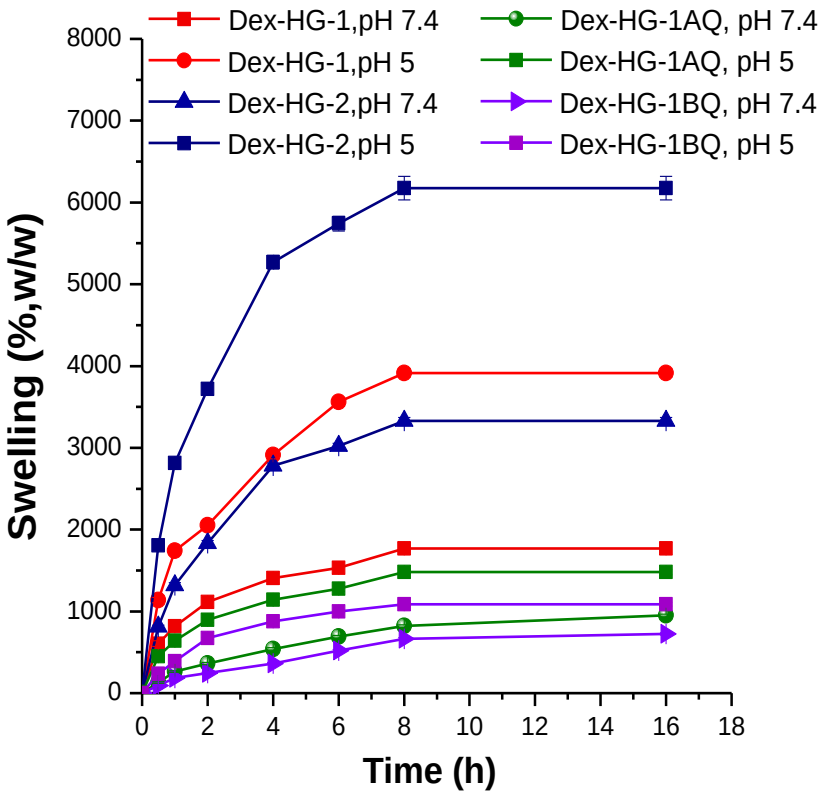



**Figure S5**. Water swelling values of different hydrogels with time at pH 7.4 and 5 respectively.

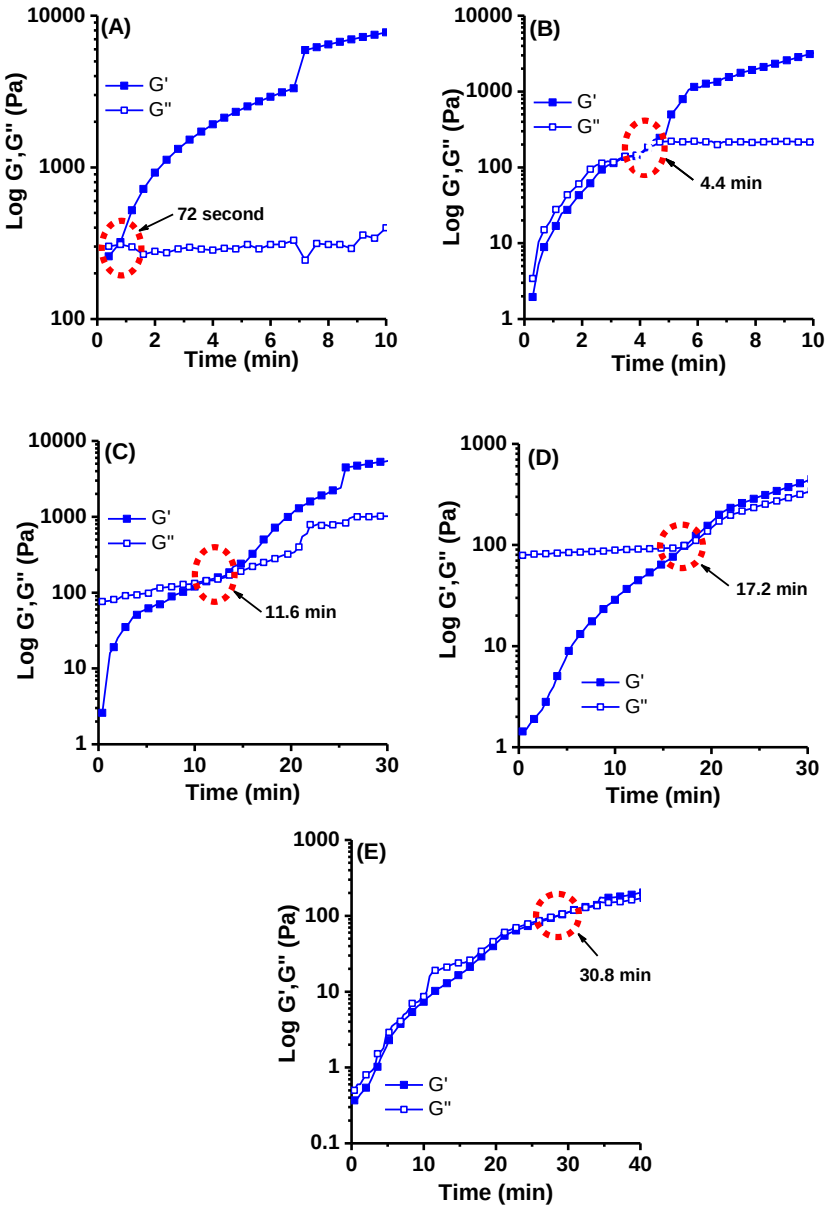



**Figure S6.** Effect of concentration of prepolymer solution (Cl-PEG-Cl:Dex-g-PDMA-QPDMA-1B=1:1 w/w) on the gelation time. The gelation time was determined by the time sweep experiments (expanded scale). Time sweep experiments A-E are for prepolymers solutions of total concentrations 28 % w/v, 16.7% w/w, 12% w/w, 8% w/w and 4% w/w respectively. The experiments were conducted at 37 $^{o}$C. The obtained values of gelation time were plotted against the total concentration of prepolymers in Figure 1A of main text.

**Effect of Composition of Prepolymer Solution on Gelation Time and G' Values of the Hydrogel**

The reaction between benzoyl chloride and amine follows second-order kinetic.[7] The amounts (mol) of Cl-PEG-Cl and DMA groups were 0.000125 mol and 0.00137 mol when Cl-PEG-Cl to graft copolymer ratio (w/w) was 0.25:0.75. The mol amounts were 0.00025 mol and 0.00091 mol when Cl-PEG-Cl to graft copolymer ratio (w/w) was 0.50:0.50. This amount changed to 0.000375 mol and 0.000445 mol when Cl-PEG-Cl to graft copolymer ratio (w/w) was 0.75:0.25. The total mol of reactive DMA was calculated by subtracting the quaternization amount (18% mol) and half of the remaining DMA group from the original amount, since the pKa of the PDMA was ca. 7.4. Considering the second order reaction, the reaction rate is calculated to be highest when Cl-PEG-Cl to graft copolymer ratio is 0.50:0.50 (w/w) and lowest when the ratio is 0.75:0.25 (w/w). Thus, probably the crosslinking rate of prepolymer solution (16.7% w/v) with Cl-PEG-Cl to graft copolymer ratio (w/w) 0.50:0.50 should be higher. Presumably, if the reaction rate lowers to a small extent, it may take relatively longer time to create required crosslinking for initial sol to gel transition. This increases the gelation time of other systems compared to 1:1 w/w prepolymers mixture (Figure S7 and Figure 1B in the main text). When the Cl-PEG-Cl to graft copolymer ratio (w/w) was 0.75:0.25, the concentration of DMA was too low compared to other systems. In this condition the second order reaction rate decreased. Thus the highest crosslinking rate i.e. lowest gelation time was obtained with the prepolymer solution of Cl-PEG-Cl to graft copolymer ratio 0.50:0.50. This is may be the probable reason why the value of G' also increased with the prepolymer solution containing an equal amount (w/w) of the components. It may be noticed that the initial modulus of the resulting hydrogel was also higher with the prepolymer solution containing equal amounts of the components. This is due to the faster

reaction between halide ends of PEG and DMA moieties of the graft copolymer which enhanced the viscosity during mixing the two prepolymer solutions before injection in the rheometer. It is noted that the lag time before injection into the rheometer was kept same for all the systems. The water swelling of hydrogels formed by the prepolymers Cl-PEG-Cl and the graft copolymers with ratio 25:75 (w/w) and 75:25 (w/w) was about two times higher than that of hydrogel formed by the equal amounts of the prepolymers. This strongly indicates lowering of crosslinking density in the other hydrogels compared to that of hydrogels formed by the prepolymers of 1:1 (w/w) ratio.

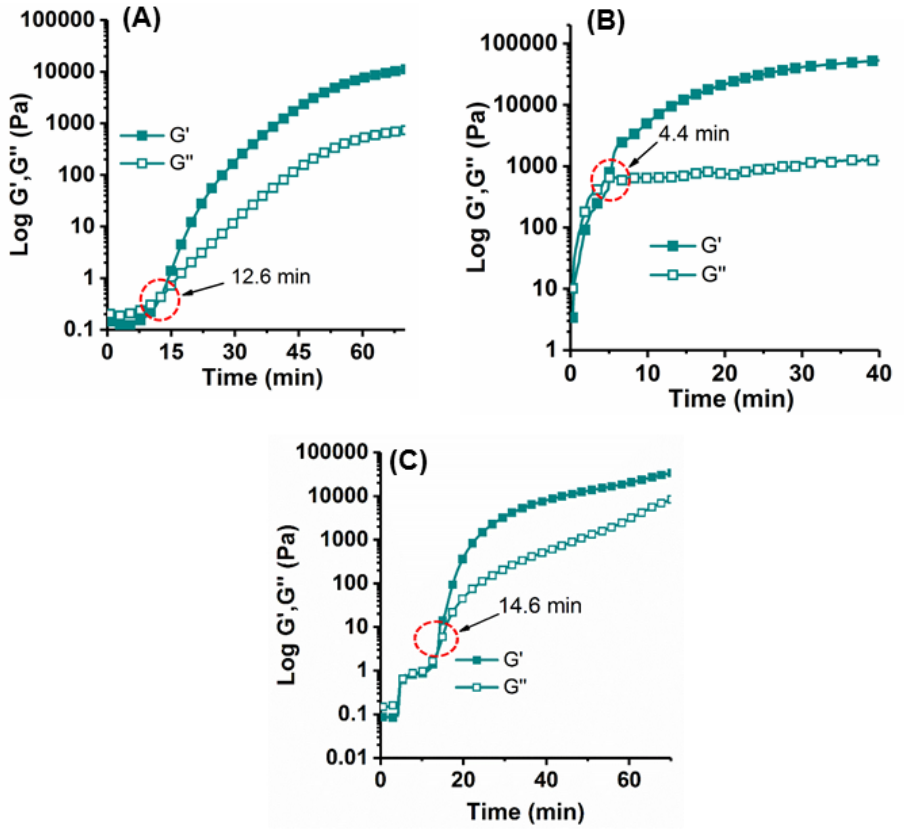



**Figure S7**. Effect of prepolymers composition on the gelation time and modulus of the formed hydrogels. Plots A-C are for the prepolymers solutions (16.7% w/v) with Cl-PEG-Cl to Dex-*g*-PDMA-QPDMA-1B ratios 25:75, 50:50, and 75:25 respectively. The gelation time was determined by the time sweep experiments. The prepolymers mixing time before injection into the rheometer was ca. 20 sec.

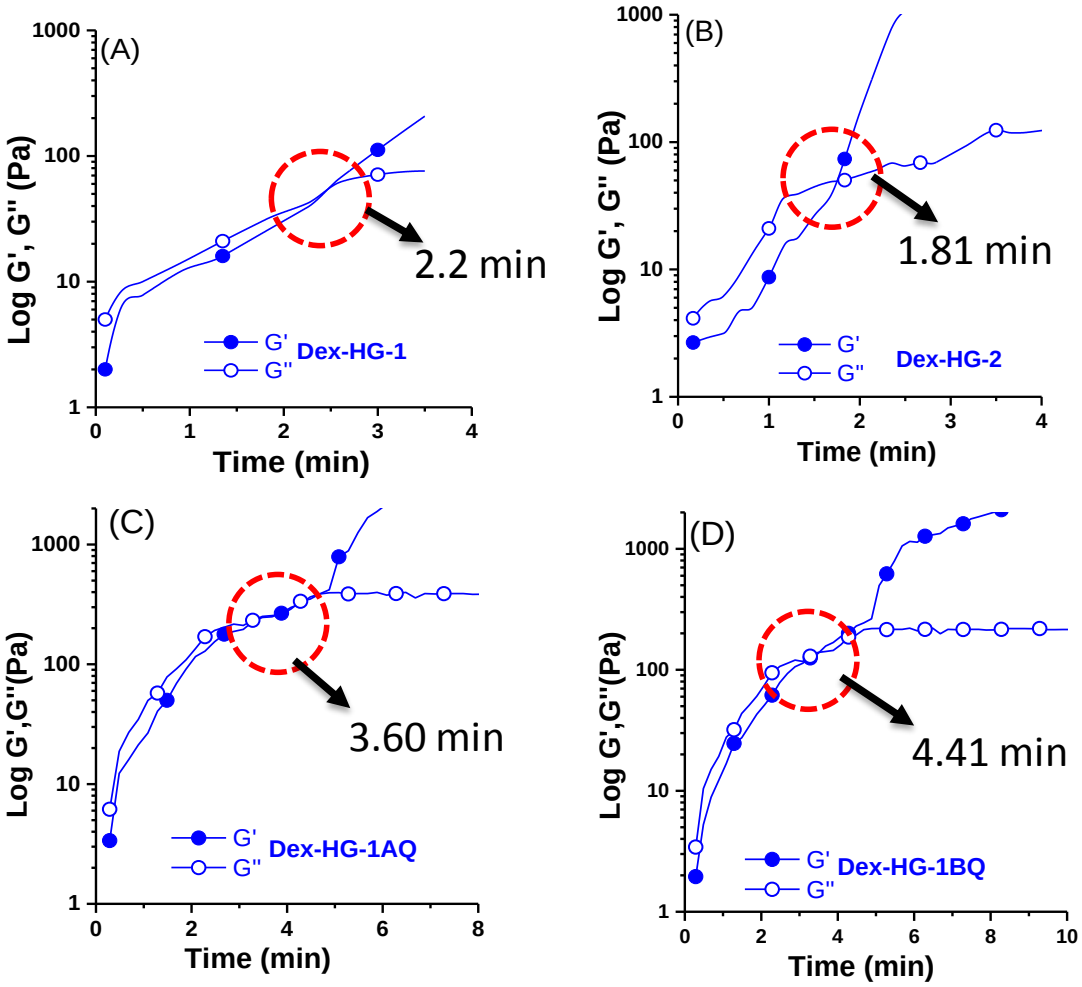



**Figure S8.** Effect of alkylation of Dex-PDMA-1 on the gelation time of the injectable aqueous mixture (total concentration 16.7% w/v). The gelation time was determined by the time sweep experiments (expanded scale of Figure 1 of main text). The prepolymer mixing time before injection into the rheometer was ca. 20 sec.

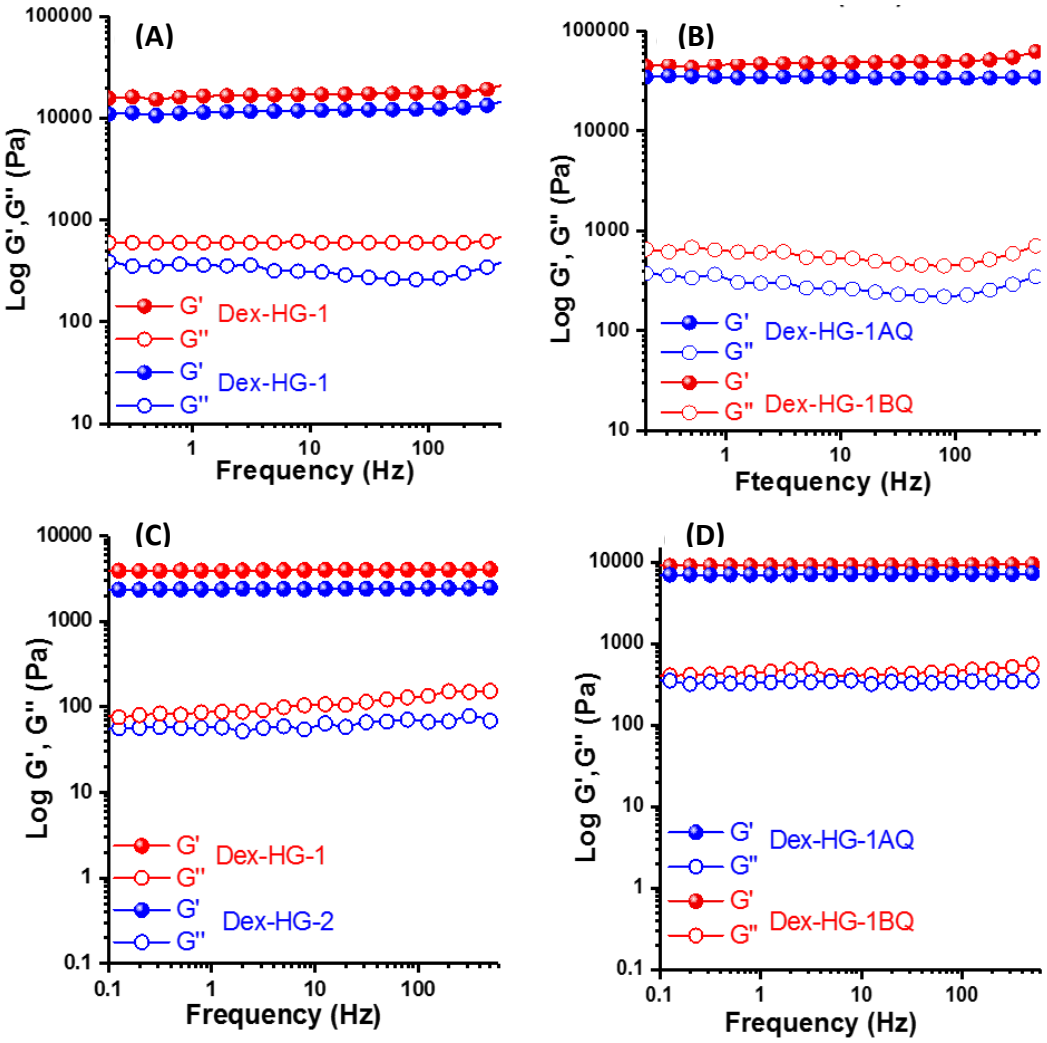



**Figure S9.** Effect of alkylation of the prepolymer (Dex-PDMA) on the rheological properties of the hydrogels. The injections were performed by mixing the aqueous solutions (PBS, pH 7.4) of Dex-*g*-PDMA-1 or alkylated copolymerS (10 %, w/v) and Cl-PEG-Cl (50 %, w/v). The total concentration was 16.7 %, w/v. After 40 min of injection the frequency sweep experiments (profiles A and B) were conducted at 37 $^{o}$C. Profiles C and D: frequency sweep experiments with the fully hydrated premade hydrogels (equilibrium water swollen state).

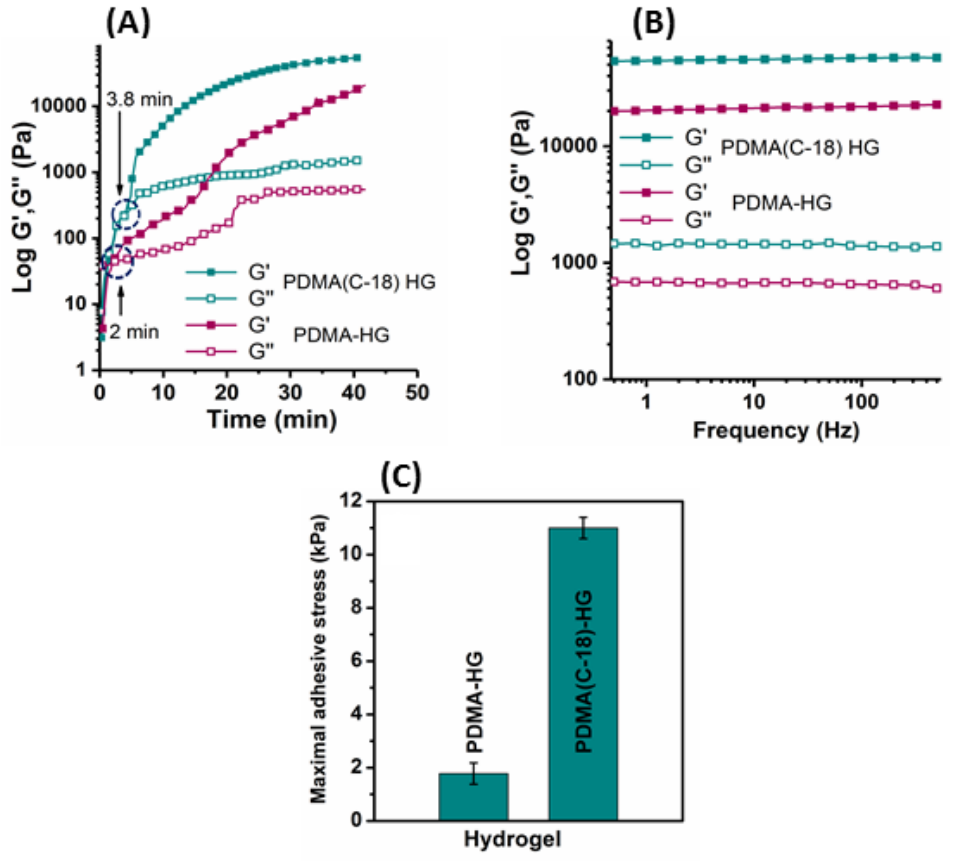



**Figure S10. (A)** Progress of formation of PDMA-HG and PDMA(C-18)-HG hydrogels as a function of time, monitored by oscillatory time sweep experiments. The oscillatory time sweep experiments were undertaken at frequency 1 Hz and at 37 $^{o}$C. (B) Frequency sweep experiments with the formed hydrogels. The injections were performed by mixing the aqueous PBS of Cl-PEG-Cl (50 %, w/v) and corresponding PDMA or PDMA-QPDMA(C-18) solution (10 %, w/v) to obtain each type of hydrogel. The total concentration of the prepolymers was 16.7 %, w/v in the experiments. (C) Bioadhesive stress of PDMA-HG and PDMA(C-18)-HG hydrogels. The prepolymer mixture was applied in between two goat mucous skin.

**Effect of C-18 on the Crystallinity of the Hydrogels**

The C-18 chains induced crystallinity in our hydrogels particularly, in dry state. Intense diffraction peaks at d=4.559 Å and 3.75 Å appeared for the dried Dex-HG-1AQ and Dex-HG-1BQ hydrogels. A weak diffraction at d=3.32 Å was also visible for the above two hydrogels. These peaks were absent in the non- alkylated hydrogel viz. Dex-HG-1 (Figure S11, profile A). On the other hand, the prepolymers Dex-*g*-PDMA-QPDMA-1 and Dex-*g*-PDMA-QPDMA-2 in the dry state showed hump over the amorphous region indicated some

degree of packing of the alkyl chains (Figure S11, profile B). This meant that the packing of alkyl chains was different in the hydrogels than that of the copolymers. The low degree of alkylation (6 % and 18 %) restricts the formation of regular structure in the PDMA-QPDMA-1 and Dex-*g*-PDMA-QPDMA-2 prepolymers. Presumably, the formation of the confined structure through crosslinking restricts the free movement of the dangling alkyl chains. This induced crystallinity in the hydrogels even with the low degree of alkylation.

To confirm the sole effect of alkyl chains, hydrogels PDMA-HG, PDMA(C-18)-HG, PDMA(C-10)-HG, and PDMA(C-4)-HG were prepared by reacting Cl-PEG-Cl with PDMA, PDMA-QPDMA(C-18), PDMA-QPDMA(C-10), and PDMA-QPDMA(C-4) separately. There is no effect of Dex backbone on the packing pattern of the alkyl chains as was evident from the appearance of similar XRD pattern in the dried hydrogels (without Dex) of PDMA-QPDMA and PEG (Figure S11, profile C). The effect was prominent with dodecyl (C-12) and C-18 *N*-alkylation of the DMA groups. Alkylation with butyl alkyl (C-4) showed some indication of the development of regular structure (Figure S11, profile C).

However, the crystallinity induced by the alkyl chains was considerably decreased in the hydrated hydrogels compare to that in the dry samples (compare Figure S11, profile A and profile D). Presumably, the hydrophobic C-18 chains remained as coagulated form in the hydrated hydrogels and reduced the regular structure. The volume change in the swollen state also disturbed the crystallinity. On the other hand, the C-18 chains remained in the extended conformation in the dry state. The considerable reduction in crystallinity of swollen hydrogel of the copolymer containing long chain alkyl was reported earlier.[8] In contrast, Miyazaki reported that some amount of water (3-30 %) induced crystallinity in the C-18 alkyl chains bearing amphiphilic copolymer network.[9] The higher glass transition temperature of the polymer constitutes of the dried hydrogels was the main reasons for such contrasting results.[10] The crystallinity of PEG in our hydrogels was also not observed as evident by the

absence of any sharp peak in the XRD patterns of Dex-HG-1 and PDMA-HG (Non-alkylated) hydrogels in both dry and water swelling states.

The alkylated hydrogels in the water swollen state did not show such sharp diffraction pattern, instead broad signals appeared (Figure S11, profile D). These signals were absent in Dex-HG-1 hydrogel in both dry (Figure A) as well as in the hydrated state (Figure S11, profile D). The comparative XRD profiles of Dex-HG-1 and the alkylated hydrogels (Figure S11, profile D) strongly suggests that the later hydrogels still contain some degree of crystallinity.

The neat Cl-PEG-Cl showed crystalline structure in its XRD profile (Figure S11E). However, the Dex-HG-1 did not show such crystallinity (Figure S11A). This indicated that after crosslinking, the crystalline structure of PEG was disrupted due to the crosslinking of PEG chain. The crosslinking of PEG chain formed Dex-g-PDMA-linked-PEG conetwork hydrogel. Such type of disruption of crystallinity due to crosslinking was reported earlier.[10,11] Hence, long chain alkyl remained in stretch conformation, particularly in dry state and induced crystallinity in the hydrogel.

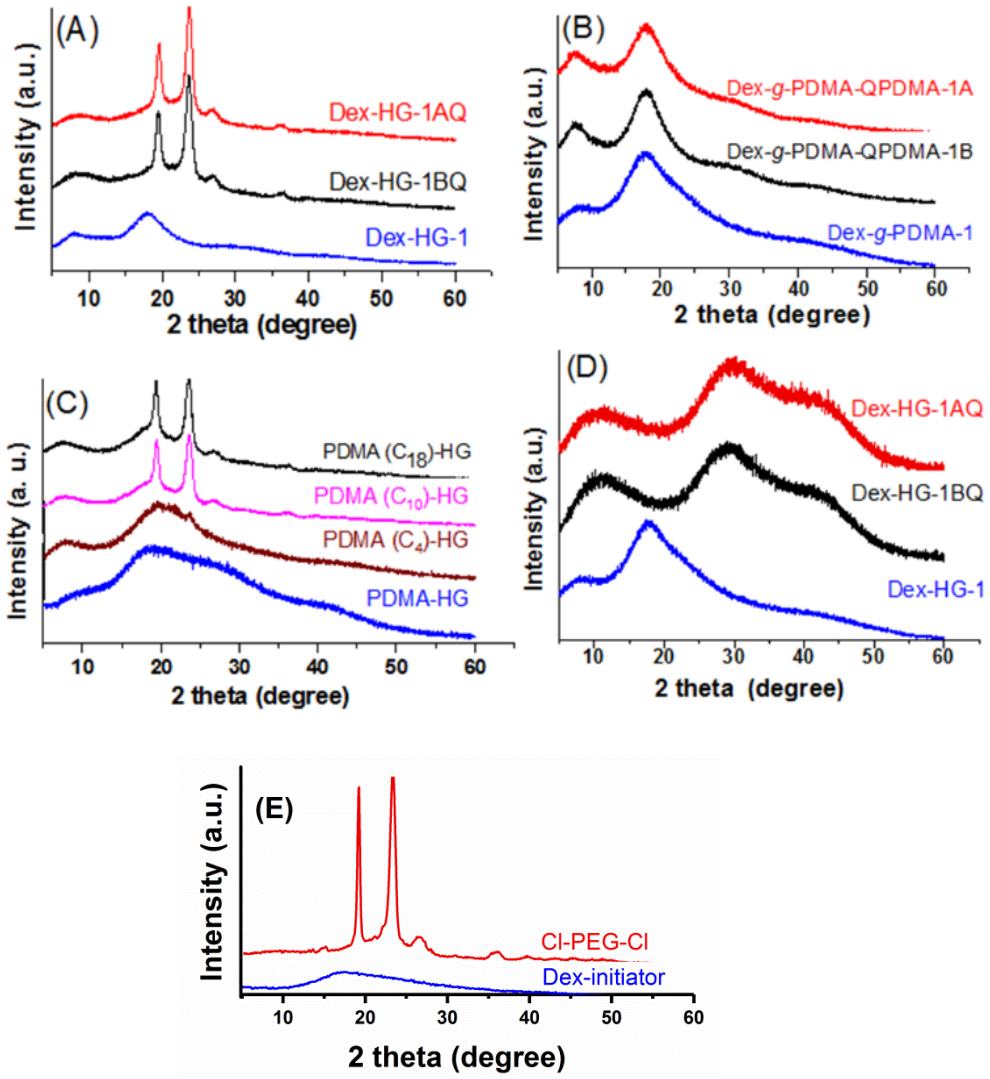



**Figure S11.** X-ray diffraction patterns of (A) dry hydrogels, (B) dry precursors (graft copolymers), (C) hydrogels (dry state) formed by the non-alkylated and alkylated PDMA homopolymer no Dex) with Cl-PEG-Cl, and (D) hydrated hydrogels. (E) XRD profiles of individual Cl-PEG-Cl and Dex-initiator.

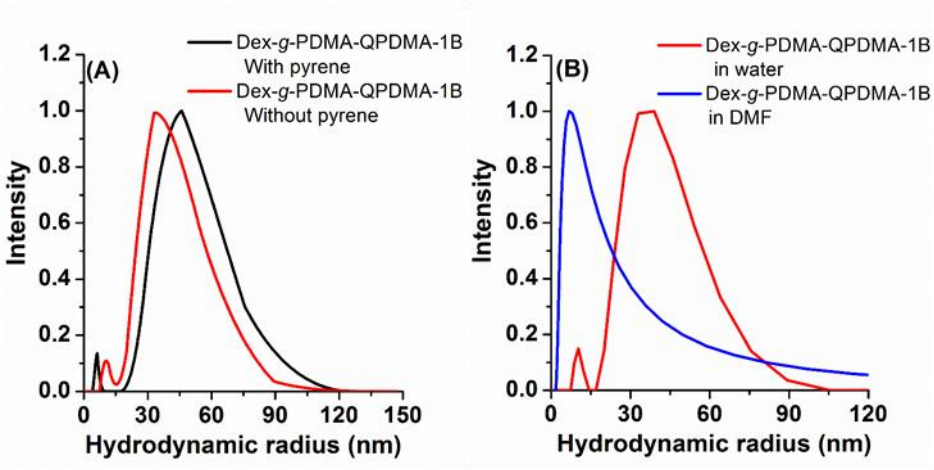



**Figure S12**. (A) DLS profiles of aqueous (1 g/L, pH 7.4) Dex-g-PDMA-QPDMA-1B copolymer micelles and the corresponding pyrene encapsulated micelles. (B) DLS profiles of aqueous (1 g/L, pH 7.4) and DMF solution of Dex-g-PDMA-QPDMA-1B copolymer.

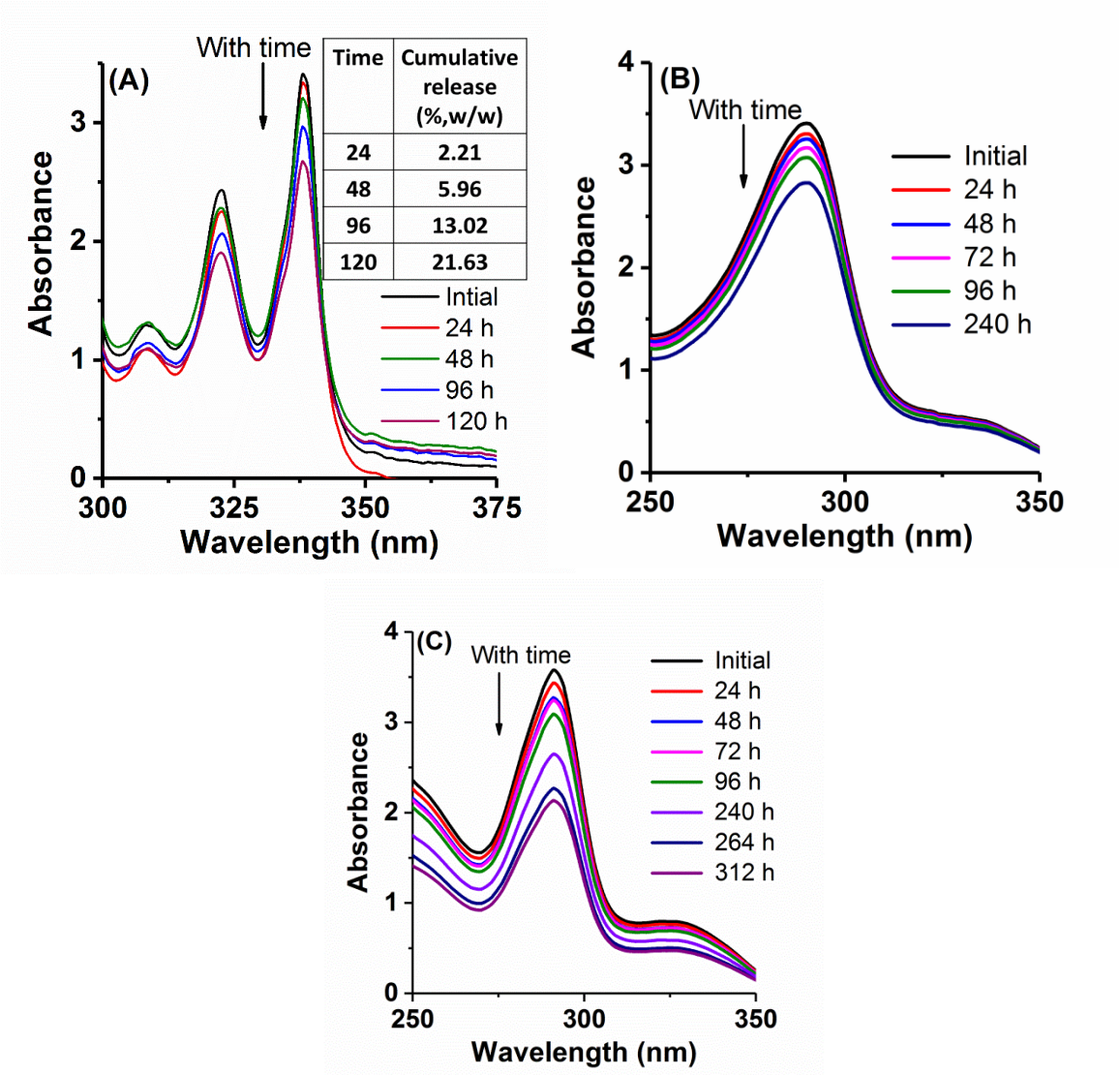
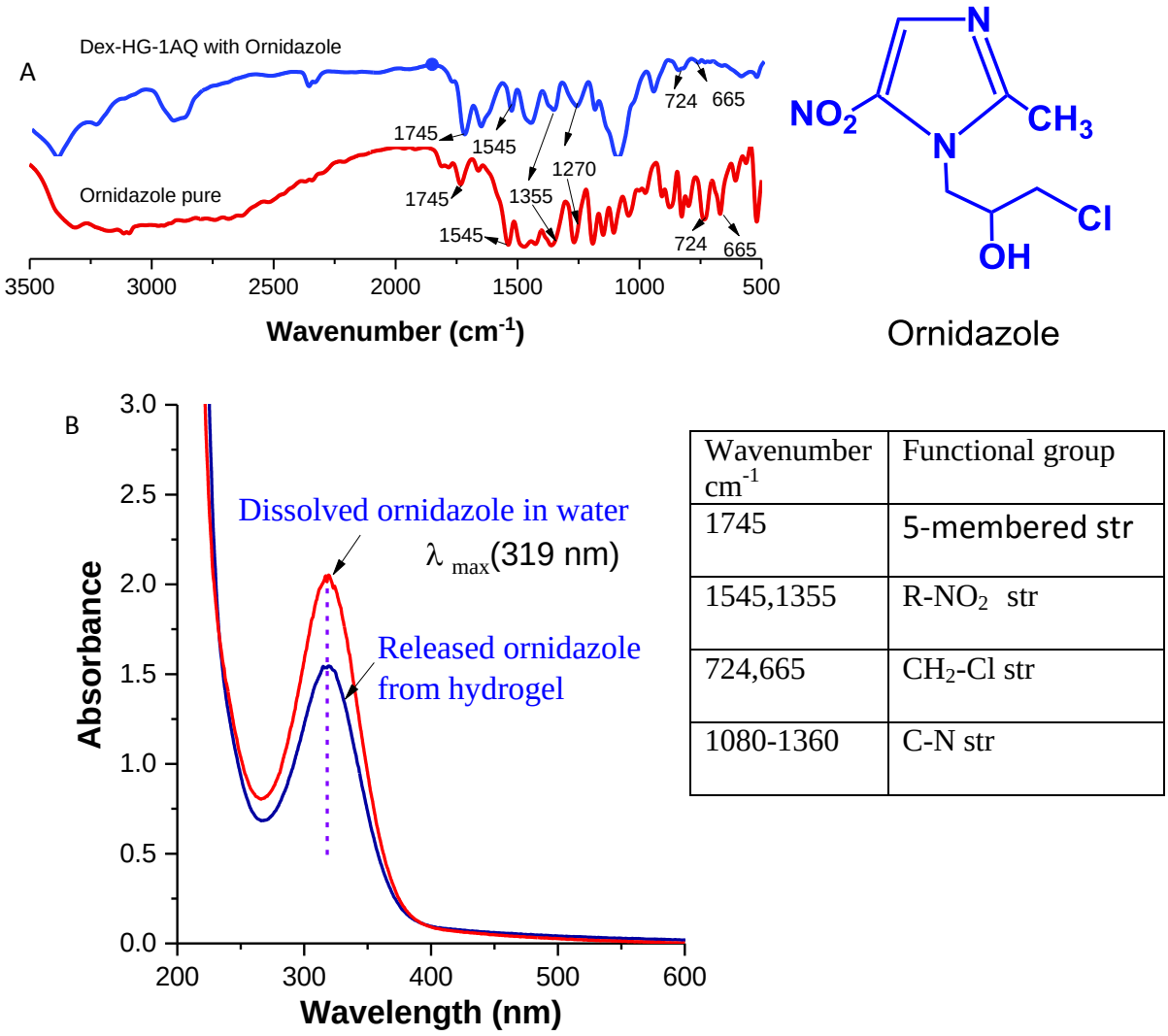



**Figure S13**. Change of UV-Visible spectra of (A) pyrene, encapsulated into the Dex-Hg-1BQ hydrogel with incubation time at pH 5 and at temperature 37 $^{o}$C. Inset: Cumulative release of pyrene with time as computed from the spectral intensity. Change of UV-Visible spectra of griseofulvin encapsulated into the Dex-Hg-1BQ with incubation time at media pH (B) 7.4, and (C) 5 respectively. The drug or pyrene loaded hydrogel formed in the quartz cuvette was incubated in PBS.




| Wavenumber $cm^{-1}$ | Functional group |
|---|---|
| 1745 | 5-membered str |
| 1545,1355 | R-$NO_2$ str |
| 724,665 | $CH_2$-Cl str |
| 1080-1360 | C-N str |

**Figure S14**. (A) FT-IR spectra of a representative Dex-HG-1AQ hydrogel loaded with ornidazole and neat ornidazole. (B) UV-Visible spectra showing absorbance maxima of neat ornidazole dissolved in water and the released ornidazole from the hydrogel.

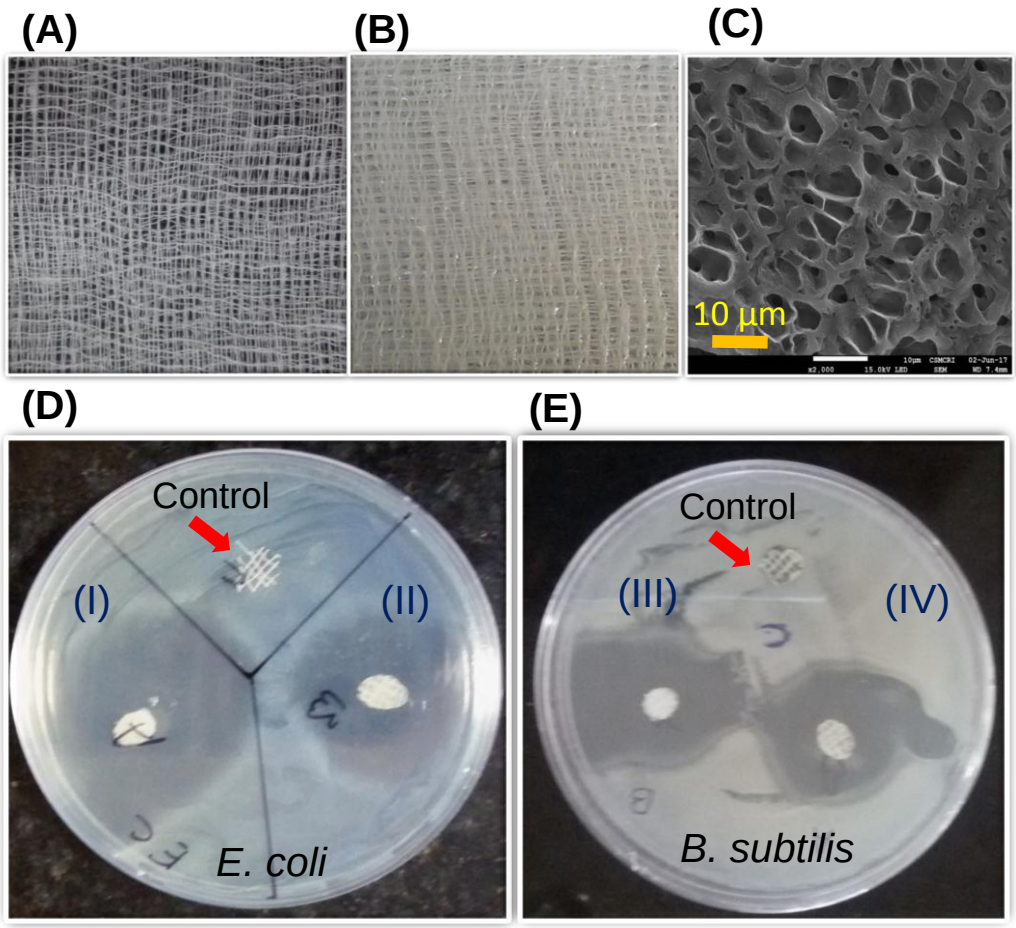



**Figure S15.** Digital photographs of commercial sterilised uncoated bandage (A) and hydrogel coated bandage (B). (C) SEM image of the hydrogel coated bandage obtained after lyophilisation (C). Pictures D and E showing diffusion disc experiments by placing drug loaded freshly prepared bandage.

**Table S1.** Drug Release Kinetics and Mathematical Models Fitted Data of In Vitro Gresiofulvin Release from Various Hydrogel at pH 7.4 and 5.

| Hydrogel | Release condition | *Zero Order | | *First order | | *Higuchi Model | | *Kors Meyar Peppas | | *Hixon Crowell | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope |
| Dex-HG-1BQ | pH 7.4 | 0.99 | 0.076 | 0.98 | 0.0004 | 0.956 | 1.478 | 0.7016 | 0.377 | 0.99 | 0.001 |
| Dex-HG-1BQ | pH 5 | 0.998 | 0.142 | 0.995 | 0.0008 | 0.933 | 2.714 | 0.653 | 0.380 | 0.99 | 0.002 |
| PDMA(C-18) HG | pH 7.4 | 0.99 | 0.0879 | 0.99 | 0.0029 | 0.8963 | 1.3051 | 0.603 | 0.2697 | 0.99 | 0.0014 |
| PDMA(C-18) HG | pH 5 | 0.99 | 0.1564 | 0.99 | 0.002 | 0.8934 | 2.3216 | 0.601 | 0.2646 | 0.99 | 0.0028 |

**Table S2.** Drug Release Kinetics and Mathematical Models Fitted Data of In Vitro Ornidazole Release from Various Hydrogel at pH 7.4 and 5.

| Hydrogel | Release condition | *Zero Order | | *First order | | *Higuchi Model | | *Kors Meyar Peppas | | *Hixon Crowell | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope | $R^2$ | Slope |
| Dex-HG-1 | pH 7.4 | 0.999 | 0.2688 | 0.99 | 0.0035 | 0.90 | 3.101 | 0.687 | 0.316 | 0.99 | 0.0049 |
| Dex-HG-1 | pH 5 | 0..995 | 0.3636 | 0.97 | 0.0054 | 0.90 | 4.186 | 0.675 | 0.312 | 0.99 | 0.0075 |
| Dex-HG-2 | pH 7.4 | 0.988 | 0.2847 | 0.99 | 0.0038 | 0.91 | 3.289 | 0.700 | 0.319 | 0.99 | 0.0052 |
| Dex-HG-2 | pH 5 | 0.991 | 0.4015 | 0.96 | 0.0069 | 0.90 | 4.627 | 0.682 | 0.311 | 0.98 | 0.0087 |
| Dex-HG-1AQ | pH 7.4 | 0.996 | 0.183 | 0.99 | 0.0022 | 0.89 | 2.1067 | 0.661 | 0.303 | 0.99 | 0.0032 |
| Dex-HG-1AQ | pH 5 | 0.991 | 0.2467 | 0.99 | 0.0032 | 0.90 | 2.842 | 0.678 | 0.312 | 0.99 | 0.0044 |
| Dex-HG-1BQ | pH 7.4 | 0.993 | 0.1647 | 0.98 | 0.002 | 0.85 | 1.881 | 0.621 | 0.288 | 0.98 | 0.0029 |
| Dex-HG-1BQ | pH 5 | 0.99 | 0.2286 | 0.98 | 0.0028 | 0.90 | 2.634 | 0.679 | 0.304 | 0.99 | 0.004 |
| PDMA(C-18) HG | pH 7.4 | 0.98 | 0.169 | 0.97 | 0.002 | 0.84 | 1.930 | 0.612 | 0.279 | 0.98 | 0.003 |
| PDMA(C-18) HG | pH 5 | 0.98 | 0.239 | 0.97 | 0.003 | 0.90 | 2.758 | 0.684 | 0.299 | 0.98 | 0.004 |

*The mechanism of drug release was determined by applying zero-order (Eqn. 8), first-order (Eqn. 9), Higuchi model (Eqn. 10), Peppas model (Eqn. 11) and Hixon Crowell model (Eqn. 12).[12-14]

$$M_t = M_0 + K_0 t \qquad (8)$$

$$\ln(M_{t)} = M_0 + K_1 t \qquad (9)$$

$$M_t = K_H t^{1/2} \qquad (10)$$

$$\frac{M_t}{M_\infty} = K t^n \qquad (11)$$

$$M_0^{1/3} - M_t^{1/3} = Kt \qquad (12)$$

where $M_0$ and $M_t$ are the initial drugs in the solution and amount of drug released at time t. K indicates rate constants for the models.

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