Functionalization of electrospun PLA fibers using amphiphilic block copolymers for use in carboxy-methyl-cellulose hydrogel composites

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**ABSTRACT**

Carboxy-methyl-cellulose (CMC) hydrogels, prepared in the presence of a crosslinker and photoinitiator, were reinforced with 3.7 wt% electrospun PLA fibers to create CMC hydrogel composites. To improve fiber-matrix adhesion, electrospun fiber mats based on hybrids of PLA and amphiphilic block copolymer (BCP) poly(D,L-lactide)-block-poly(2-(dimethylamino)ethyl methacrylate) (PLA-b-PDMAEMA) were produced. The presence of PDMAEMA at the fiber surface induced hydrophilic surface properties, which could be controlled by varying the PDMAEMA chain length. PDMAEMA was quaternized and co-electrospun with PLA fibers, which further enhanced the interaction between fibers and hydrogel matrix via ionic interactions. Physicochemical properties of the electrospun fiber mats and their CMC hydrogel based composites were assessed and revealed a nearly two orders of magnitude increase in modulus. Continuous electrospun fiber mats were chopped into discontinuous fibers to create short fiber reinforced CMC hydrogels. Rheological properties of these reinforced hydrogels incorporating 0.5 wt% discontinuous fibers were evaluated and showed potential as injectable composite systems for biomedical applications.

**GRAPHICAL ABSTRACT**

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**Introduction**

Cellulose is the most abundant renewable resource on the planet and cellulose-based hydrogels are considered to have great potential in the fields of tissue engineering, drug delivery, food, agriculture, etc. \cite{1} Cellulose hydrogels can be prepared by physical crosslinking through ionic, hydrogen bonding or other molecular interactions \cite{2–4}. They are also produced via chemical crosslinking or radiation \cite{5,6}. The 3D network of carboxy-methyl-cellulose (CMC) hydrogels contains large amounts of water, which provides an attractive environment for cell growth. However, most hydrogel materials (especially single-phase gels) have poor mechanical properties whereas many biological gel tissues, such as cartilages, are usually strong and shock-absorbing \cite{7}. Hence their scope for use in biomedical applications is often limited. As a result, considerable efforts have been devoted to the improvement of the mechanical performance of hydrogels. For instance, it was found by repeated freeze-thawing of hydrogels, that polymer molecules tend to aggregate under the expelling of water crystals, forming a crystallite; a two-phase gel structure contributing to increased hydrogel strength \cite{8,9}. Alternatively, a double network gel can be produced which combines two independent gel networks to improve the gel strength and toughness \cite{10,11}. Instead of physical or covalent crosslinks as present in other gels, hydrogels made from supramolecules with topological interlocking structures exhibited good ductility as well as water adsorption \cite{12}.
The use of reinforcing fillers is a common way to improve the mechanical properties of polymer matrices in general and hydrogels in particular without chemical modification or specific synthesis. Nanoscale fillers such as cellulose nanocrystals [13–15], carbon nanotubes [16,17], graphene oxide [2,18,19], halloysites [20] and silica or polymeric nanoparticles [21,22] have all been used in hydrogels due to their large surface area and mechanical or functional properties (drug carriers, sensors, actuators, etc.). Nevertheless, the reinforcing effect of these nanofillers was often rather limited, while the use of high filler loadings could even lead to a reduction in mechanical performance due to filler aggregation and a disturbance of the hydrogel network.

Electrospinning is an easy and rapid technique for the production of continuous or high aspect-ratio polymer nanofibres [23–25]. Electrospun fibers have great potential as reinforcements for soft materials and hydrogels for biomedical applications [26]. Moreover, they have the potential of introducing functional properties such as shape memory behavior [27] or drug delivery [28,29]. Their fibrous structure, high porosity and large surface area make them suitable as reinforcing or functional fibers; while their small diameter, infinite length or high aspect ratio allows for effective stress transfer. Moreover, fiber reinforced hydrogels highly resemble biological tissues which often consist of hierarchical micro- and nano-fibrous structures. Randomly oriented electrospun fiber mats as well as highly organized 3D printed electrospun fiber scaffolds have been used as reinforcements for hydrogels for use in the biomedical field [30–32]. In addition to the use of continuous electrospun fiber mats, short electrospun fibers have also been used for hydrogel reinforcement while maintaining good flowability, processability and injectability [33,34]. Poly(lactic acid) (PLLA) is well-established nontoxic and biocompatible polymer material used for electrospinning. However, the hydrophobicity of PLLA fibers does not allow an easy integration into hydrogels or fiber wetting. Plasma treatment of PLLA fibers has been used to facilitate improved fiber-hydrogel integration [35], however at the same time this technique also introduced defects in these fibers, hence reducing their strength.

In this study, electrospun PLA fiber reinforced CMC hydrogel composites were prepared. CMC hydrogels were prepared in the presence of a crosslinker and photoinitiator. To improve fiber-matrix compatibility, electrospun fibers based on hybrids of PLA and amphiphilic block copolymer (BCP) poly(D,L-lactide)-block-poly[2-(dimethylamino)ethyl methacrylate] (PLA-b-PDMAEMA) were produced. The presence of PDMAEMA on the fiber surface provided hydrophilic surface properties. By varying the PDMAEMA chain length, the hydrophilicity of the PLA could be controlled. Moreover, PDMAEMA was quaternized and co-electrospun with PLA fibers. The quaternized PDMAEMA further enhanced the interaction between fiber and hydrogel matrix via ionic interactions. The physio-chemical properties of electrospun fibers and CMC composite hydrogels were assessed. Finally, continuous electrospun fiber mats were chopped into short electrospun fibers and incorporated into CMC hydrogels. The rheological properties of these composite hydrogels were evaluated. It is proposed that these short fibers are potentially suitable for the reinforcement of injectable composite hydrogel systems.

### Experimental

#### Materials

All chemicals were obtained from Sigma-Aldrich (UK) unless specified. D,L-lactide was recrystallized in toluene; 4-(dimethylamino) pyridine (DMAP) (≥99%) was recrystallized in petroleum:ether-toluene 4:1 mixture; triethylamine (≥99.9%) was distilled over calcium hydride; 2-(dimethylamino) ethyl methacrylate (DMAEMA) (98%) was passed through aluminum oxide to remove inhibitor. Phosphate buffer saline (PBS) was prepared by dissolving one PBS tablet in 200 ml deionized water. Benzyl alcohol (anhydrous, 99.8%), dichloromethane (DCM) (anhydrous, ≥99.9%), tetrahydrofuran (THF) (anhydrous, ≥99.9%), 2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropio phenone (Irgacure 2959, 98%), allyl bromide (98%), N,N,N’,N”-pentamethyldiethylenetriamine (PMDETA) (99%), 5-bromo-1- pentene (95%), copper (I) bromide (98%), aluminum oxide (≥98%), poly(ethylene glycol) dithiol (PEG-dithiol, average Mₙ = 1,000 g/mol), 2-hydroxy-4’-methoxyacetophenone (Irgacure 184, 98%), hexane (≥95%), tetrahydrofuran (THF) (anhydrous, ≥99.9%), methanol (≥99.9%), sulfuric acid (99.99%), hydrogen peroxide solution (30 wt% in water) were used as received. Silicon wafer was purchased from PI-KEM Ltd. CMC power (Aqualon® Sodium CMC, DS≤0.7) and Ingeo PLA 2002D (Mₙ = 200,000 g/mol) were obtained from Hercules Inc. (USA) and NatureWorks (USA), respectively.

#### Synthesis of PLA-b-PDMAEMA and quaternization

PLA-b-PDMAEMA was synthesized by a combination of ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). First, bromide-ended PLA (PLA-Br) was synthesized via ROP of lactide followed by end group functionalization. 3.0 g of D,L-lactide (2.1×10⁻² mol) and 37.1 mg DMAP (3.0×10⁻⁴ mol) were placed in a round bottom flask equipped with a magnetic stirrer. The flask was dried under vacuum and purged with argon (Ar) prior to
adding any reactants. The reactor was then sealed and three cycles of vacuum-argon purge were conducted. 43.0 μl benzyl alcohol (4.2 × 10⁻⁴ mol) was injected into the reactor under Ar protection. The reaction was carried out in an oil bath at 135 °C for 4 h. At the end of the reaction, 10.0 ml anhydrous DCM and 290.0 μl triethylamine (2.1 × 10⁻³ mol) were directly injected to dissolve the products. The reactor was transferred to a cold water bath followed by slowly adding 276.0 μl α-bromoisobutyryl bromide (2.1 × 10⁻³ mol) under Ar atmosphere. The functionalization was continued overnight. PLA-Br was achieved by precipitating in cold methanol twice and dried in a vacuum desiccator.

PLA-b-PDMAEMA was synthesized via ATRP. In flask A, 0.5 g of PLA-Br (≈1.2 × 10⁻⁴ mol) and DMAEMA monomer were dissolved in 2.0 ml anhydrous tetrahydrofuran (THF), which was purged with Ar for 30 min. Meanwhile in flask B, 51 μl PMDETA (2.4 × 10⁻⁴ mol) and 17 μg copper(I) bromide (1.2 × 10⁻⁵ mol) were dissolved in 10.0 ml anhydrous THF and purged with Ar for 30 min. Afterwards, contents in flask B were transferred to flask A. The polymerization was continued in oil bath at 50 °C for 24 h. The reaction was stopped by exposing to air and the contents were diluted with 100 ml DCM and passed through alumina column to remove copper. The product solution was concentrated to 10 ml using a rotavapor (Buchi, Rotavapor RII, UK) and precipitated in cold hexane twice. The product was dried in a vacuum evaporator (Buchi, Rotavapor RII, UK) and stored in a nitrogen box. BCP with low, medium and high PDMAEMA content was synthesized, named as PDMAEMA (L), PDMAEMA (M) and PDMAEMA (H), respectively.

For quaternizing PDMAEMA, PDMAEMA (H) was dissolved in anhydrous dimethylformamide (DMF) at 1.0 g/ml concentration. Then 2.5 eq. (mol) of 5-bromo-1-pentene was added and the reaction was carried out at 70 °C for 24 h. The product (named as PDMAEMA-Q) was recovered by precipitating in excessive cold diethyl ether and dried in a vacuum desiccator.

**Spin-coating and electrospinning of PLA and PLA-b-PDMAEMA**

The impact of PDMAEMA on PLA's hydrophilicity was first studied on spin-coated films. Homo PLA, PLA-b-PDMAEMA and homo PLA/PLA-b-PDMAEMA hybrids were prepared, respectively. To start, PLA-b-PDMAEMA was dissolved in chloroform/DMF mixed solvent at a concentration of 0.1 mg/ml. The mass ratio of chloroform to DMF was 3:1 and was also used for all spin-coating and electrospinning experiments. The solution was stirred until homogeneous. Meanwhile, silicon wafer was cut into 1.0 × 1.0 cm² substrates and functionalized to hydrophilic by treating them in sulfuric acid/hydrogen peroxide solution (3:1 volume ratio) at 100 °C for 1 h. Substrates were then washed with deionized water and purged with nitrogen. The spin coating was performed by first spinning at 400 rpm for 10 s and then 2,000 rpm for 40 s (G3P-8, Specialty Coating System, USA). The spin coated substrates were left in a fume hood overnight to allow solvent evaporation and stored in a nitrogen box for further characterization. PLA (with and without PLA-b-PDMAEMA) spin-coated films were prepared on the same substrates following the same spin-coating routine. Due to a higher viscosity, they were dissolved until a diluted concentration (0.05 mg/ml). 15 wt% of PLA-b-PDMAEMA was added with respect to homo PLA.

Electrospinning was carried out using an in-house built experimental setup. Homo PLA and PLA-b-PDMAEMA (15 wt% with respect to homo PLA) were dissolved in a 3:1 chloroform:DMF mixed solvent at a total polymer concentration of 9 wt%. PLA-b-PDMAEMA on its own is not suitable for electrospinning due to its low molecular weight. The polymer solution was supplied to the spinning tip by a syringe pump (Kent Scientific Genie, UK) at a feeding rate of 1.0 ml/h. This spinning tip was connected to an EQ high voltage power supply (Glassman High Voltage Inc., USA), which generated 20–24 kV. Electrospun fibers were collected as continuous fiber mats on aluminum foil and left in a fume hood to dry. For comparison, PLA without BCP was electrospun using the same conditions as described above. In the end, five different types of fiber mats were prepared: homo PLA fiber, PLA/PDMAEMA (L) fiber, PLA/PDMAEMA (M) fiber, PLA/PDMAEMA (H) fiber and PLA/PDMAEMA-Q fiber.

**Preparation of fiber mat reinforced hydrogel composites**

The CMC hydrogel was prepared by UV crosslinking of CMC and PEG-dithiol (crosslinker) aqueous solution with photoinitiator Irgacure 2959. First, the commercial CMC powder was modified by allyl bromide using an established method, which resulted in 13% molar ratio of alkene groups in CMC. In detail, 1.0 g CMC powder was dissolved in 50 ml water and stirred until fully dissolved. Another 50 ml DMF was added and this solution was mixed until homogeneous. Approximately 0.1 ml allyl bromide was added into CMC solution and the reaction was continued at 70 °C overnight. The functionalized CMC was obtained by precipitating in acetone twice and dried in vacuum desiccator. To make the precursor solution of the hydrogel, functionalized CMC was dissolved in water at 20 mg/ml; PEG-dithiol and Irgacure 2959 were dissolved in PBS and methanol, respectively, at 225 mg/ml (PEG-dithiol) and 45 mg/ml (Irgacure 2959). A mixed precursor solution of
1.0 ml CMC, 10.6 μl PEG-dithiol and 12 μl Irgacure was prepared.

To create the electrospun fiber mat reinforced hydrogel composites, the as-spun random fiber mat was cut into 20 × 30 mm² rectangular specimen (thickness 0.08–0.10 μm) and placed on a flat substrate. CMC precursor solution was cast onto a single layer fiber mat. To facilitate impregnation of the hydrophobic fibers, the mat was degassed in a vacuum desiccator for 15 min until the precursor solution infiltrated into the fiber mat. A glass slide was placed on top of the layup and pressed down to remove excess solution. The whole system was UV crosslinked at 80 mW/cm² for 120 s (OmniCure® RS1500). After UV crosslinking, composite hydrogels with 3.7 wt% fiber loading were obtained and incubated in PBS.

Molecular characterization

All synthesized polymers were characterized by 1H-NMR and GPC. For 1H-NMR spectra, samples were dissolved in deuterated chloroform (CDCl₃) at a concentration of 10 mg/ml and tested by a Bruker AVIII 400 spectrometer (Bruker Corp., USA). Due to poor solubility of PDMAEMA-Q in CDCl₃, it was dissolved in deuterated dimethyl sulfoxide (DMSO) instead. For gel permeation chromatography (GPC), samples were dissolved in THF at 1.0 mg/ml and tests were performed using an Agilent Technologies 1260 Infinity (Agilent Technologies Inc., USA) equipped with a UV detector at 308 nm wavelength. THF (with 2.0 vol% triethylamine) was used as the eluent at a flow rate of 1.0 ml/min. Polystyrene (1,000–1,000,000 g/mol) was used as standard for calibration.

Physicochemical characterization

Atomic force microscopy (AFM, NT-MDT, NTEGRA, Russia) was used to acquire topography and phase images of BCP-functionalized PLA spin-coated films in tapping mode. AFM was performed using a Bruker RTESP-300 probe (resonant frequency 300 kHz, spring constant 40 N/m) at set-point of 50–70% of free oscillation amplitude and an image scan rate of 1.0 Hz. The hydrophilicity of the spin-coated films and electrospun fiber mats was evaluated by contact angle measurements using drop shape analysis (DSA, Kruss GmbH, DSA100, Germany). 5.0 μl liquid was carefully placed onto film samples and the contact angle was measured. To observe the morphology of the electrospun fibers and hydrogels, samples were sputter coated with a thin gold layer and characterized using scanning electron microscopy (SEM, FEI Inspect F, Netherlands) at a voltage of 10 kV. To investigate hydrogel-fiber interactions and wetting, composites were cold-fractured in liquid nitrogen and lyophilized and then characterized by SEM. Thermal properties of electrospun fibers were measured by differential scanning calorimetry (DSC, Perkin-Elmer DSC 4000, USA) and dynamic mechanical analysis (DMA, TA Instrument Q800, USA). For DSC tests, samples were first equilibrated at 25 °C and then ramped to 180 °C at 10 °C/min. Glass transition temperature (Tg) was determined by the mid-point of the glass transition. The crystallinity of PLA was calculated by,

$$X_c = \frac{\Delta H_m}{\Delta H_{ref}} \times 100\%$$  

where X_c is the crystallinity, ΔH_m is the experimental heat of fusion at melting point determined by DSC, ΔH_{ref} is the theoretical heat of fusion of fully crystalline PLA (93 J/g) [36–38]. Tensile tests were performed on both the electrospun fiber mats and the fiber mat reinforced hydrogel composites using a dynamic mechanical analyzer (DMA, TA Instrument Q800, USA) in controlled force mode. Fiber mats and composite hydrogel films were cut into 20 × 5 mm² rectangular strips before being mounted into the clamps. A preload of 0.05 N was applied and specimens were stretched at a rate of 0.1 N/min until failure at room temperature (RT). Three tests were performed for each sample. The Young’s modulus was derived from the slope of the stress-strain curve at low strain (2%).

Preparation and characterization of discontinuous fiber reinforced hydrogel composites

To create short or discontinuous fibers, electrospun fiber mats were cut into small pieces (10 × 10 mm²) and added to 50 ml deionized water while a homogenizer (Heidolph™ Silent Crusher M, Germany) was used to homogenize the fiber-water mixture at 14,000 rpm for 20 min. A fiber suspension was obtained after homogenization and chopped electrospun fibers were recovered by filtration. The recovered cut fibers, with lengths as high as several millimeters, were dried using a freeze dryer (Labconco FreeZone 2.5, USA) and their morphology was characterized using SEM. Three different types of chopped fibers were prepared from homo PLA fiber, PLA/PDMAEMA (H) fiber and PLA/PDMAEMA-Q fiber, respectively. To prepare the hydrogel composites, 0.5 wt% of chopped fibers were added into the CMC precursor solution and stirred overnight (named as CMC-PLA fiber, CMC-PDMAEMA (H) fiber and CMC-PDMAEMA-Q fiber, respectively). Fiber loading was limited to 0.5 wt% as higher loadings resulted in a too high viscosity for effective mixing. Rheological tests on these discontinuous fiber reinforced hydrogels were carried out using a rheometer (TA
Instrument DHR-3, USA), with stainless steel parallel plate geometry (20 mm). During the tests (at RT), around 0.2 ml fiber-loaded precursor solution was transferred between the plates and a 0.5 mm plate gap was applied. First, a time sweep was performed for 180 s, with a displacement of $10^{-4}$ rad, frequency of 1.0 Hz and UV exposure at 80 mW/cm$^2$. Then a frequency sweep was performed from 0.1 Hz to 100 Hz at a displacement of $10^{-4}$ rad to assess hydrogel stability at different frequencies. Finally, a strain sweep was performed from 1 to 1000% at 1.0 Hz frequency to evaluate hydrogel stability at different deformations.

**Results and discussion**

**PLA-b-PDMAEMA synthesis**

PLA was synthesized via ROP catalyzed by an organic catalyst DMAP [39]. By using an organic catalyst, conventional metallic catalysts could be avoided [40,41], which are potentially harmful in biomedical applications. PDMAEMA blocks were added to the PLA block via ATRP. A schematic of the synthesis process is shown in Figure 1. Representative $^1$H-NMR spectra of products are presented in Figure 2. Peaks corresponding to different protons or solvents are identified and the molecular weight ($M_n$) is calculated by end group analysis. For PLA blocks, the $M_n$ was calculated by comparing the peak integration of proton a (7.34 ppm, multiple peaks) and c (5.16 ppm, multiple peaks overlapping with proton b); for PDMAEMA blocks, the $M_n$ was calculated by comparing the peak integration of proton a (7.34 ppm, multiple peaks) and proton h (4.05 ppm). The $^1$H-NMR spectra of BCP before and after quaternization are shown in Figure 2b. The occurrence of peak n (5.88 ppm) and n’ (5.07 ppm), with the complete shifting of peak h (from 3.99 to 4.43 ppm), i (from 2.50 to 3.59 ppm), j (from 2.20 to 3.29 ppm) indicate the successful quaternization of the PDMAEMA blocks. The reaction yield is calculated by comparing reactant mass and product mass. The yield for ROP, ATRP and quaternization is near 50.5%, 51.7% and 90.0%, respectively. For more details regarding the $^1$H-NMR characterization of PLA-b-PDMAEMA, see also Colak et al. [42]. In addition, GPC was used to measure $M_n$ and $M_w/M_n$. A detailed molecular characterization is listed in Table 1.

**The influence of PDMAEMA content on PLA hydrophilicity**

Due to the thermodynamic incompatibility between different blocks in block copolymers, the same blocks tend to maximize their interactions while minimizing the interactions between different blocks, resulting in self-assembled domains. We expect phase separation to affect the distribution of the hydrophilic PDMAEMA phase, eventually determining the PLA surface hydrophilicity. It is therefore important to investigate the phase separation behavior between PLA and PDMAEMA. Phase separation of PLA-b-PDMAEMA was investigated in spin-coated films (thickness ~1.0 μm) using AFM. In Figure 3a1–b1, randomly distributed phases are observed in PDMAEMA (L) and PDMAEMA (M). With increasing PDMAEMA content, a homogeneous surface was obtained (Figure 3c1,d1). To enhance the mobility of molecules and to drive phase separation, films were annealed in THF.

![Figure 1. Schematic showing synthesis and quaternization of PLA-b-PDMAEMA.](image-url)
atmosphere (50 °C, 12 h). Phase images after THF-annealing are shown in Figure 3a2–d2. No clear phase separation was seen for PDMAEMA (L), PDMAEMA (M) and PDMAEMA (H) (a2–c2). This is due to partial miscibility and insufficient incompatibility between PLA and PDMAEMA blocks, especially at low molecular weight [43]. However, clear phase separation was observed in PDMAEMA-Q, which can be explained by the poor compatibility between PLA and quaternized PDMAEMA. After spin-coating with homo PLA, the hybrid surface properties were investigated using AFM and DSA. Sn-coating produced rough and porous surfaces due to rapid solvent evaporation and homo PLA crystallization, while no self-assembly was observed (images not shown). The hybrid films were again solvent annealed to allow molecular migration and reduce roughness. After annealing, films became smoother with crystalline PLA domains observed in both topographic and phase images (Figure 4). However, PLA/PDMAEMA phase separation did still not occur due to the formation of PLA stereocomplexes which disrupt BCP self-assembly [43]. Therefore, the surface of PDMAEMA-functionalized PLA films is considered to have no phase separation.

The influence of PDMAEMA on PLA surface hydrophilicity was investigated by water droplet contact angle measurements under both neutral and acidic conditions (Table 2). With increased PDMAEMA content, the water contact angle under neutral conditions decreased from 82.9° to 65.6°, implying an increase in PLA’s hydrophilicity. Under acidic conditions, the water contact angle was further decreased, from 80.6° for homopolymer to 43.3° for PLA with PDMAEMA (H). The quaternized PDMAEMA only modified the

![Figure 2. (a) Representative 1H-NMR of PLA-Br and PLA-b-PDMAEMA in deuterated chloroform (CDCl3), (b) 1H-NMR of PDMAEMA (H) and PDMAEMA-Q in deuterated DMSO.]

| Sample      | $M_n$ PLA $^a$ | $M_n$ PDMAEMA $^a$ | $M_n$ $^b$ | $M_w/M_n$ $^b$ |
|-------------|----------------|-------------------|------------|---------------|
| PLA         | 3.2            | N/A               | 7.5        | 1.7           |
| PDMAEMA (L) | 3.9            | 1.1               | 3.3        | 1.9           |
| PDMAEMA (M) | 3.1            | 6.5               | 5.5        | 1.5           |
| PDMAEMA (H) | 3.8            | 20                | 18         | 1.4           |

$^a$Molecular weight calculated from 1H-NMR end group analysis (kg/mol). $^b$Obtained from GPC test (PS standard, kg/mol).
hydrophilicity of PLA slightly compared with PDMAEMA. As PDMAEMA is pH-responsive, the pH-dependent water contact angle results suggested the presence of PDMAEMA blocks on the hybrids’ surface.

Physicochemical properties of functionalized PLA fiber mats and hydrogel composites

Homo PLA and hybrids with PLA-b-PDMAEMA were electrospun into fiber mats and their morphologies are characterized using SEM. Average fiber diameters were determined using Image J software and listed in Table 3. SEM images of electrospun fibers together with their diameter distribution are presented in Figure 5. Both homo and functionalized PLA were electrospun into continuous fibers without beading. The addition of PLA-b-PDMAEMA results in a decrease in fiber diameter from 1.2 μm to 400–600 nm. The low molecular weight BCP lowered the viscosity of the spinning solution, which allowed for easier deformation and stretching of the solution jet during electrospinning and smaller diameter fibers. The incorporation of PLA-b-PDMAEMA also narrows the fiber diameter distribution. Fiber diameters become more uniform compared to homo PLA fibers. Thermal properties of the electrospun fibers are characterized by DSC. Their thermograms and data are presented in Figure 5 and Table 3. All samples have a melting peak (Tm)
around 154 °C and a glass transition temperature ($T_g$) at 60–62 °C. Cold crystallization is seen in all fiber samples; its peak becomes narrower and the cold-crystallization temperature ($T_{cc}$) is shifted toward lower temperature. The crystallinity of PLA is increased by the addition of PDMAEMA. The highest crystallinity is found when using PDMAEMA-Q, indicating that PDMAEMA-Q facilitated PLA crystallization during electrospinning.

Water contact angles were measured on the electrospun mats. Homo PLA fibers exhibited a higher hydrophobicity than PLA films due to the air entrapped within the porous fibrous structure, with a water contact angle measured at 131 ± 6°. Addition of PDMAEMA (L) increased the contact angle to 152 ± 6° due to increased porosity (increased air entrapment) as a result of decreasing fiber diameter from 1.20 μm to 0.58 μm [44]. Water contact angle was not measured for fibers containing PDMAEMA (M), PDMAEMA (H) and PDMAEMA-Q as water droplets infiltrated quickly into the mat. This rapid wetting of these electrospun mats suggested greatly improved PLA fiber hydrophilicity after functionalization, which also allows for an easy integration and wetting of the fibers by the hydrogel.

### Table 3. Average diameter of electrospun fibers and their thermal properties.

| Composition          | Fiber diameter (μm) | $T_g$ (°C) | $T_m$ (°C) | $T_{cc}$ (°C) | Crystallinity (%) |
|----------------------|---------------------|------------|------------|---------------|------------------|
| PLA                  | 1.23 ± 0.47         | 60.0       | 154.0      | 95.4          | 3.4              |
| PLA/PDMAEMA (L)      | 0.58 ± 0.17         | 60.6       | 153.0      | 85.9          | 8.8              |
| PLA/PDMAEMA (M)      | 0.58 ± 0.21         | 61.9       | 154.3      | 85.9          | 8.7              |
| PLA/PDMAEMA (H)      | 0.42 ± 0.10         | 60.0       | 154.0      | 87.9          | 10.4             |
| PLA/PDMAEMA-Q        | 0.55 ± 0.10         | 61.1       | 153.7      | 86.2          | 14.4             |

![Figure 5](image-url)
Fiber-hydrogel interaction in the PLA and functionalized PLA fiber mat reinforced CMC hydrogel composites were characterized by SEM. By comparing the mass of fibers and the mass of composite hydrogels, the fiber weight fraction in the hydrogel composites is calculated at 3.7 ± 0.1 wt% (≈3.0 vol%). A hydrogel-rich layer was seen at the composites’ surface. The cryo-fracture surfaces of lyophilized hydrogel composites are shown in Figure 6. Lyophilized CMC exhibits a porous structure (Figure 6a) while all hydrogel composites exhibit a fibrous structures (Figure 6b–f). However, it can be seen that as PDMAEMA content increases, fiber pull-out length and fiber protrusions become shorter, indicating improved fiber-matrix adhesion. CMC is adhering well to the fibers (Figure 6d–f) due to increased fiber hydrophilicity and fiber-hydrogel interaction. Conversely, hydrophobic fibers (homo PLA and PLA fibers with low PDMAEMA contents) show debonding and detachment from the CMC matrix; while for hydrophilic or quaternized fibers, a better wetting and stronger interaction between fibers and CMC is observed, leading to improved stress transfer and reinforcing efficiency of the PLA fibers in the hydrogel composite.

The mechanical properties of the electrospun fiber mat reinforced CMC hydrogels were measured by tensile tests in a DMA. For comparison, tensile tests were also performed on dry electrospun fiber mats. Detailed test results are listed in Table 4. All electrospun mats have a much lower modulus and strength than bulk PLA (3.5 GPa and 53 MPa, respectively, as provided by supplier). The weak mechanical performance of the random fiber mats is caused by the high porosity, poor fiber alignment and poor fiber-fiber interactions resulting in sliding of fibers during stretching. The observed decrease in Young’s modulus and tensile strength of the fiber mats with increasing PDMAEMA content is caused by increased fiber sliding. For homo PLA fibers, some level of fiber-fiber fusion is observed, leading to a physical network (see Figure 5). Functionalization using PDMAEMA produces ‘drier’ fibers that show less fiber fusion, leading to a more loosely connected fibrous network structure. The mechanical properties of the CMC composite hydrogels are highly dependent on the mechanical properties of the electrospun fibers once fully integrated, and stronger fiber mats are expected to produce stronger composite hydrogels. In contrast, hydrogel composites incorporating PDMAEMA-Q fibers exhibit the highest Young’s moduli and tensile strengths despite the fact that these fiber mats have the lowest intrinsic mechanical properties. The high Young’s modulus and tensile strength of these composites is the result of the excellent fiber-matrix adhesion and effective stress-transfer between the PDMAEMA-Q fibers and CMC hydrogel matrix as already identified by SEM. Here, the CMC matrix acts as an effective bridge between the fibers, limiting fiber sliding when the composite is stretched. Hence, as a result of this improved reinforcement efficiency in the case of PDMAEAM-Q fibers, the mechanical properties of the resulting composite are improved.

In order to get a better insight into the reinforcing effect of electrospun PLA fibers in CMC hydrogel matrix...
we used the generalized rule of mixtures (RoM) to estimate the reinforcing efficiency of the fibers \([26,45,46]\).

\[
E_r = k \eta_L \eta_0 E_f V_f + E_m (1 - V_f)
\]

Where \(k\) is the reinforcing efficiency. \(E_f\) is taken as the modulus of bulk PLA (3.5 GPa). As a result of chain alignment, electrospinning can lead in the case of thin nanofibres (<200 nm) to slightly higher moduli as compared to bulk polymer. However, in case of relatively thick electrospun fibers as used here we expect properties similar to those of the bulk polymer [24]. \(V_f\) is the fiber volume fraction (3 vol.%), and \(E_m\) is the modulus of the pure CMC hydrogel (0.1 MPa) [47]. \(\eta_0\) is the fiber orientation distribution factor (3/8 for 2D in-plane randomly-oriented fiber) [45]. The length efficiency factor \(\eta_L\) in case of continuous fiber is 1. Using Equation 2, the reinforcing efficiency parameter \(k\) can now be calculated at around 0.25 (25%). This rather low reinforcing efficiency for the CMC/PLA system can be partly explained by variations in fiber and matrix properties, out-of-plane fiber orientation as well as imperfect adhesion, wetting or fiber aggregation, all inhibiting effective stress transfer between fibers and matrix, emphasizing once more the importance of fiber surface modification through PDMAEMA.

### Rheological behavior of discontinuous fiber CMC hydrogel composites

The morphology of the chopped fibers after homogenization and their hydrogel composites was characterized by SEM (Figure 7). The discontinuous fiber ends are

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**Table 4.** Tensile properties of electrospun fiber mats and fiber mat reinforced CMC hydrogel composites (3 vol.% fiber).

| Composition       | Young’s modulus (MPa)* | Failure strain (%) | Tensile strength (MPa) |
|-------------------|-------------------------|--------------------|------------------------|
| Fiber mat         |                         |                    |                        |
| PLA               | 119 ± 32                | 79 ± 26            | 4.53 ± 1.06            |
| PDMAEMA (L)       | 136 ± 14                | 91 ± 4             | 5.31 ± 0.65            |
| PDMAEMA (M)       | 133 ± 8                 | 70 ± 6             | 4.65 ± 0.32            |
| PDMAEMA (H)       | 85 ± 14                 | 52 ± 3             | 3.77 ± 0.27            |
| PDMAEMA-Q         | 21 ± 11                 | 61 ± 3             | 2.17 ± 0.30            |
| Composite         |                         |                    |                        |
| CMC/PLA           | 9.4 ± 1.2               | 78 ± 4             | 0.68 ± 0.08            |
| CMC/PDMAEMA (L)   | 8.0 ± 0.2               | 94 ± 8             | 0.57 ± 0.04            |
| CMC/PDMAEMA (M)   | 6.4 ± 0.8               | 54 ± 25            | 0.47 ± 0.07            |
| CMC/PDMAEMA (H)   | 3.9 ± 0.7               | 28 ± 5             | 0.24 ± 0.04            |
| CMC/PDMAEMA-Q     | 9.4 ± 3.4               | 57 ± 11            | 0.77 ± 0.08            |

*Young’s modulus is calculated at 2.0% strain from stress-strain curve.
Figure 8. Rheological tests of CMC hydrogel matrix and discontinuous fiber reinforced hydrogel composites: (a) time sweep for 180 s with UV exposure from 30 to 150 s, at $10^{-4}$ rad displacement and 1 Hz frequency, (b) frequency sweep from 0.1 to 100 Hz at $10^{-4}$ rad displacement, (c) strain sweep from 1 to 1000% at 1 Hz frequency.

Figure 9. Photographs demonstrating the injectable composite hydrogel system based on chopped electrospun fibers: (a) dispensing CMC precursor solution, (b) dispensing CMC/0.5 wt% chopped PDMAEMA-Q fiber precursor solution, (c) two solutions injected in PDMS mold, (d) free-standing chopped fiber reinforced CMC hydrogel composite after UV curing.
indicated by yellow arrows in a1-c1. Fiber diameter hardly changed compared to as-spun fibers. Massive entanglement in case of PLA fibers was observed due to their poor hydrophilicity, while fiber aggregation occurred during homogenization. After functionalization, dispersion of fibers in water was greatly improved during homogenization. The chopped fibers were then integrated into the CMC hydrogels. PLA fiber bundles were found in lyophilized hydrogels, indicating poor fiber distribution. In contrast, PDMAEMA (H) and PDMAEMA-Q fibers were more homogeneously distributed, leading to a more controllable and stable composite hydrogel system.

Rheological tests of CMC precursor solution (without chopped fibers) showed viscous fluid behavior before UV exposure (loss modulus G'' > storage modulus G') (Figure 8). Upon UV exposure, quick gelation occurs (cross-over of G' and G'' after only 2.5 s UV exposure) and G' reaches around 275 Pa after complete UV exposure. With the addition of 0.5 wt% chopped fibers (≈0.4 vol%), the CMC solution becomes more viscous and behaves like a gel even prior to UV curing (G' > G''). Chopped PLA fibers showed little or no reinforcing effect, which can be explained by the weak interaction between the PLA fibers and the CMC hydrogel as well as the poor fiber distribution. The addition of PLA/PDMAEMA (H) fibers improved G' from 275 Pa to 390 Pa, indicating that functionalized PLA fibers provide better reinforcement of the CMC hydrogel. PLA/PDMAEMA-Q fibers showed the greatest increase in G' (960 Pa), implying further enhanced fiber-hydrogel interaction. Compared with previously reported short fiber reinforced hydrogels [33,35], the composite hydrogels in our current work demonstrated significant reinforcement at very low fiber loading. The frequency sweep presented a plateau in the range of 0.1 to 10 Hz, indicating that the hydrogels are stable under these oscillatory conditions. At higher frequencies (10 to 100 Hz), G' starts to decrease, which is due to a loss of inertia. The failure strain of the hydrogels was reduced with the addition of chopped electrospun fibers, possibly due to the low failure strain of the fibers or the presence of defects and fiber aggregates. Rheology tests indicated that the PLA/PDMAEMA-Q fibers exhibited the best reinforcement effect, which can be explained by the ionic interactions between fibers and CMC hydrogel.

Photographs demonstrating the injectability of the chopped fiber reinforced hydrogel systems are presented in Figure 9. For comparison, CMC precursor solutions with and without chopped fibers were both dispensed using a syringe (needle diameter 0.5 mm) into PDMS molds. After UV curing, the free-standing fiber reinforced composite hydrogels could be removed easily from the molds, while hydrogels without fibers were difficult to remove from the mold or to handle without damaging them.

Conclusions

Functionalized electrospun PLA fibers were fabricated by electrospinning hybrids of homo PLA and amphiphilic block copolymer PLA-b-PDMAEMA. Block copolymers with different PDMAEMA block chain lengths were synthesized by a combination of ROP and ATRP. The introduction of PLA-b-PDMAEMA successfully increased the hydrophilicity of homo PLA due to the presence of PDMAEMA blocks on the fiber surface. By varying the PDMAEMA chain length, the hydrophilicity of the electrospun PLA fibers was modulated. Compared with previous reports, the hydrophilicity of these functionalized PLA fibers was better controlled and more easily modulated without damaging the fiber structure and their integrity. Functionalized PLA fiber mats were easier to incorporate into CMC hydrogels and showed improved fiber-matrix bonding with increased PDMAEMA content or PDMAEMA quaternization. Finally, chopped electrospun PLA fibers were produced and integrated into the CMC hydrogel. The addition of a very small amount (0.5 wt%) of functionalized PLA fibers resulted already in a three-fold increase in storage modulus of the CMC hydrogel. Their easy integration, strong interfacial interaction and high reinforcing efficiency make PLA-b-PDMAEMA-functionalized PLA fibers a promising candidate for the reinforcement and design of novel injectable CMC based hydrogel composite systems.

Disclosure statement

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