Tough fibrous mats prepared by electrospinning mixtures of methacrylated poly(trimethylene carbonate) and methacrylated gelatin

Jia Liang\textsuperscript{a}, Honglin Chen\textsuperscript{b,*}, Zhengchao Guo\textsuperscript{a}, Piet Dijkstra\textsuperscript{a}, Dirk Grijpma\textsuperscript{a}, André Poot\textsuperscript{a,\dagger}

\textsuperscript{a} Department of Biomaterials Science and Technology, University of Twente, Enschede 7522NB, the Netherlands
\textsuperscript{b} Institute for Life Sciences, School of Medicine, South China University of Technology, Guangzhou 510640, China

\textbf{A R T I C L E  I N F O}

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Synthetic-natural hybrid material

\textbf{A B S T R A C T}

Electrospun scaffolds prepared from combinations of synthetic and natural macromers (macromolecular monomers) are interesting materials for tissue engineering applications. In this study, hybrid fibrous mats composed of 10/0, 8/2, 6/4, 3/7, 1/9 and 0/10 (wt/wt) three-armed methacrylated poly(trimethylene carbonate) (PTMC-tMA) and methacrylated gelatin (GelMA) were prepared by electrospinning and photo-crosslinking. The elastomeric PTMC fibers only maintained a stable structure when electrospun in combination with GelMA. The hybrid materials showed two glass transition temperatures, which indicated the occurrence of phase separation. By varying the ratios of PTMC-tMA/GelMA from 8/2 to 1/9 (wt/wt), electrospun mats with a fiber diameter ranging from 1037 to 419 nm, water uptake from 106 to 315%, porosity from 88.3 to 94.3%, tensile modulus from 0.643 to 0.216 MPa, elongation at break from 445 to 96%, maximum strength from 4.127 to 0.381 MPa and toughness from 1023 to 15 N/mm\textsuperscript{2} were prepared. Culturing of human smooth muscle cells on hybrid fibrous mats made from 6/4, 3/7 and 1/9 PTMC-tMA/GelMA showed similar cell attachment and proliferation as compared to 100% GelMA fibrous mats. The toughness of the latter hybrid fibrous mats (539, 33 and 20 N/mm\textsuperscript{2}) was significantly higher than that of the 100% GelMA fibrous mats (3 N/mm\textsuperscript{2}). In conclusion, tough hybrid fibrous mats with good cell adhesive properties were prepared by electrospinning and photo-crosslinking of mixtures of PTMC-tMA and GelMA.

\section{1. Introduction}

Electrospinning is a reliable and mature technology to fabricate continuous fibers with diameters in the nanometer to micrometer scale, and is a popular method to make tissue engineering scaffolds [1–4]. To fabricate such scaffolds, a potential (high voltage) difference is applied between a polymeric liquid and a collecting target [5]. The fibrous structure of the resulting scaffold has generally a high porosity and can mimic the hierarchically organized fibrous structure of the native extracellular matrix (ECM) [6]. The materials that can be used for electrospinning are diverse. Various polymers of both synthetic and natural origin have been used to prepare electrospun fibers using solvent or melt spinning. During the process, the fibers will solidify by solvent evaporation or temperature change, facilitating the formation of a stable fibrous structure [7]. Hence, most of the applied materials for electrospinning are thermoplastic polymers, whereas the use of elastomeric polymers for electrospinning can be challenging due to the viscous behavior of the material at room temperature (RT) [8].

Poly(trimethylene carbonate) (PTMC) is a non-crystallizing flexible polymer, that is suitable for tissue engineering applications because of its biocompatibility and degradation by surface erosion without the formation of acidic compounds. Crosslinked PTMC networks are usually flexible, elastic and tough [9–11]. A drawback of the use of PTMC for electrospinning is its low glass transition temperature \(T_g\), approximately \(-20^\circ\text{C}\), because of which it is in a viscous state at RT [12,13]. The collected PTMC fibers will irreversibly deform, resulting in loss of porosity. This can be overcome by electrospinning of PTMC together with other polymers which are stable at RT, such as PCL [14] and PLA [15]. Gelatin, which has a \(T_g\) around 50 \(^\circ\text{C}\), is another candidate for this purpose [16]. As a natural polymer, gelatin is a widely used tissue engineering material and fibrous structures can be easily manufactured by electrospinning [17]. Moreover, scaffolds made from gelatin and gelatin/PCL blends were shown to support the growth of various kinds of cells [18]. Recently, Joy \textit{et al.} reported on the electrospinning of a tubular scaffold for blood vessel regeneration using a gelatin/PTMC blend [19]. The scaffold showed minimal \textit{in vitro} toxicity and good

\textsuperscript{*} Corresponding authors.
E-mail addresses: chenhl@scut.edu.cn (H. Chen), s.a.poot@utwente.nl (A. Poot).

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biocompatibility upon subcutaneous implantation in the rat.

In the present study, three-armed methacrylated PTMC (PTMC-tMA) and methacrylated gelatin (GelMA) were synthesized. Single and hybrid macromer mixtures in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)/acetic acid (AcOH) were prepared for electrospinning. During and after electrospinning, UV irradiation was applied to photo-crosslink the fibers. We used PTMC of relatively low molecular weight around 20,000 g/mol, as this would increase the amount of methacrylate groups in the macromer mixtures, and thus increase the crosslink density of the fibers. For the same reason, three-armed instead of linear PTMC was used. GelMA was used, as we hypothesized this would increase the cell adhesive properties of the electrospun meshes. The resulting photo-crosslinked electrospun mats were characterized with respect to morphology, fiber diameter, gel content, water uptake, porosity, thermal and mechanical properties, in vitro degradation and adhesion and proliferation of human smooth muscle cells (hSMCs).

2. Materials and methods

2.1. Materials

Trimethylene carbonate (TMC) was provided by Huizhou Foryou Medical Devices, China. Gelatin from porcine skin (Type A, gel strength 90–110 g Bloom), tin(II)-2-ethyl-hexanoate (Sn(Oct)$_2$), 1,1,1-trimethylopropane, triethylamine, methacrylic anhydride, hydroquinone, Irgacure 2959, deuterated chloroform (CDCl$_3$), deuterium oxide (D$_2$O), HFIP, AcOH, phosphate-buffered saline (PBS), cholesterol esterase (260 U/mg), collagenase (1000 U/mg) and sodium azide (Na$_3$N) were purchased from Sigma Aldrich, The Netherlands. Ethanol (99.8%) and dichloromethane (DCM) were bought from VWR Chemicals, The Netherlands. Ethanol, deuterium oxide (D$_2$O), phosphate-buffered saline (PBS), cholesterol esterase (260 U/mg), collagenase (1000 U/mg) and sodium azide (Na$_3$N) were purchased from Sigma Aldrich, The Netherlands. Ethanol (99.8%) and dichloromethane (DCM) were bought from VWR Chemicals, The Netherlands. Dialysis membrane (MWCO = 12–14 kDa) was purchased from Spectra/Por®, VWR. Dulbecco’s PBS (DPBS), advanced Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), glutamax, trypsin/EDTA and penicillin/streptomycin were obtained from Gibco, Thermo Fisher, The Netherlands. CyQuant assay reagent and Live/Dead cell viability kit were purchased from Thermo Fisher. Gelatin solution (Type B, 2% (wt/v) in H$_2$O) for coating of tissue culture flasks and plates was purchased from Sigma Aldrich.

2.2. Synthesis of PTMC-tMA

Three-armed PTMC macromer with an intended molecular weight of 20,000 g/mol was synthesized by ring-opening polymerization of TMC. In a three-neck round bottom flask with a magnetic stirring bar, TMC (51 g, 0.5 mol) was heated to 80 °C in an argon atmosphere. After the TMC monomer was melted, 1,1,1-trimethylopropane (0.34 g, 2.55 mmol) and 5 drops of Sn(Oct)$_2$ were added. Next, the temperature was increased to 130 °C and after 2 d the reaction was stopped by cooling to RT. Subsequently, the PTMC hydroxy end groups were converted into methacrylate groups by reaction with methacrylic anhydride in DCM in the presence of triethylamine and hydroquinone. First, the PTMC (51 g, 7.65 mmol OH end groups) was dissolved in 150 mL of DCM, followed by addition of 0.05 g hydroquinone, triethylamine (6.5 mL, 46 mmol) and methacrylic anhydride (7 mL, 46 mmol). The reaction was conducted for 5 d at RT under continuous stirring in the dark. The PTMC-tMA macromer was purified by precipitation in cold ethanol and dried in a vacuum oven at RT in the dark. The molecular weight (Mn) and degree of functionalization of the macromer were calculated from $^1$H NMR spectral data, using CDCl$_3$ as a solvent (Bruker Ascend 400/Avance III 400 MHz NMR spectrometer).

2.3. Synthesis of GelMA

To 10 g of gelatin, 100 mL of Millipore water was added at RT. The gelatin was swollen for 1 h and then heated to 50 °C with magnetic stirring. After the gelatin was fully dissolved, methacrylic anhydride (20 mL, 0.135 mol) was slowly added to the solution under vigorous stirring. The resulting emulsion was stirred at 3 °C. Subsequently, the mixture was transferred to a centrifuge tube and centrifuged at 4,000 g for 5 min while still warm. The supernatant was collected and diluted with an equal volume of Millipore water of 40 °C to decompose the remaining methacrylic anhydride. The resulting solution was transferred to a 12–14 kDa MWCO dialysis tube and dialyzed against water for 5 d at 40 °C. The water was refreshed 4 times per day. Finally, the GelMA was freeze-dried and stored at –25 °C. The degree of functionalization was analyzed by $^1$H NMR spectral data, using D$_2$O as a solvent.

2.4. Electrospinning

PTMC-tMA and GelMA were dissolved in HFIP/AcOH (20/1, v/v) at different ratios (10/0, 8/2, 6/4, 3/7, 1/9 and 0/10, wt/wt). The mixtures (12.5%, wt/v) were stirred overnight at a speed of 300 rpm at RT. Then, Irgacure 2959 (5 wt% relative to macromer) was added to the mixture and stirring was continued for 30 min before electrospinning. The mixture (2.5 mL) was transferred to a 5 mL syringe equipped with a blunted 27G stainless-steel needle. Electrospinning was performed using a syringe pump (Multi-Phaser, Model NE-1000) at a rate of 1.0 mL/h and an applied voltage of 16 kV. The working distance from the tip of the spinneret to an aluminum collector plate was set at 15 cm. During electrospinning the fibers were irradiated with a UV lamp (365 nm, 0.32 mW/cm$^2$). A low intensity lamp was used to avoid blocking of the needle by fast crosslinking of the macromers. The collected fibers on the plate were exposed to irradiation from a identical UV lamp. The two light bulbs were positioned at a distance of 10 cm from the fibers, which were continuously irradiated during spinning (2.5 h). The collected mesh was subsequently immersed in ethanol containing Irgacure 2959 (3%, wt/v) for 1.5 h and then post-cured in a UV box (365 nm, 7.0 mW/cm$^2$, 5 cm distance to the lamp, Ultra Lum, San Diego, USA) for 40 min. Preparation is shown in Scheme 1.
2.5. Differential scanning calorimetry (DSC)

DSC measurements were carried out using a DSC25 (TA Instruments, New Castle, USA). An extracted and dried sample (see 2.7) of 10 mg was sealed in an aluminum pan. Samples were equilibrated for 1.0 min in the DSC at −75 °C, after which the temperature was increased to 150 °C at a heating rate of 10 °C/min. After 1.0 min, the sample was cooled to −75 °C at the same rate. After 1.0 min, a second heating and cooling scan were recorded for all samples. The Tg was determined as the midpoint value of the heat capacity change of the second heating scan.

2.6. Scanning electron microscopy (SEM) and determination of fiber diameters

The morphology of the electrospun scaffolds was examined by secondary electron SEM (JSM-T100, JEOL, Japan). Extracted samples washed with water (see 2.7) were freeze-dried and subsequently gold-sputtered with a Cressington Sputter Coater 108 Auto set at 30 mA for 60 s. The average fiber diameter and fiber diameter distribution were determined using Adobe Photoshop CS4 software by analyzing at least 100 segments per SEM image of which 5 were made for each scaffold. For analysis of fiber cross-sections, samples were frozen in liquid nitrogen, broken and examined by high resolution SEM (Zeiss MERLIN, Germany) without coating at a voltage of 0.9 kV.

2.7. Gel content, water uptake and porosity of scaffolds

To determine the gel content, water uptake and porosity, circular specimens with a diameter of 1 cm and an approximate thickness of 300 μm were cut from the electrospun fibrous mats and weighed (m0). For all measurements 3 samples were used.

Samples were extracted in HFIP for 2 d and dried in a fume hood until constant weight (m1). The gel content is defined by Eq. (1):

\[ \text{Gel content} = \frac{m_1 - m_0}{m_0} \times 100\% \]  

(1)

Dried samples (m2) were immersed in distilled water for 1 d. The excess water was removed by blotting with tissue paper and the samples were weighed (m3). The water uptake was calculated according to Eq. (2):

\[ \text{Water uptake} = \frac{m_3 - m_2}{m_2} \times 100\% \]  

(2)

The porosity of the specimens was calculated using Eq. (3) in which h is the thickness, V is the volume, m3 is the dry mass, and ρ is the density of the polymer mixture. The latter was calculated using the densities of PTMC (1.31 g cm\(^{-3}\)) and gelatin (1.27 g cm\(^{-3}\)) and the fraction (a) of PTMC present.

\[ \text{Porosity} = \left[1 - \frac{(m_3/V \times \rho)}{\rho}\right] \times 100\% \]  

(3)

\[ V = \pi d^2/4 \times h \quad \rho = 1.31a + 1.27(1-a) \]

2.8. Mechanical properties

The electrospun fibrous mats were extracted in HFIP and dried as described above. After swelling in water for 24 h, the mechanical properties of dumbbell-shaped specimens (50 × 9 mm) were assessed using a Zwick Z020 tensile tester equipped with a 500 N load cell according to ASTM D6822. A measurement was started at a grip-to-grip separation of 30 mm and the specimen was elongated at a speed of 10 mm/min at RT. From the stress–strain curve the elastic modulus (Eelastic at 10% strain), elongation at break (εb) and maximum strength (Fmax) were determined. The toughness was calculated as the area under the stress–strain curve.

2.9. In vitro degradation

In vitro degradation of the meshes was evaluated under different conditions using extracted circular specimens with a diameter of 1 cm, an approximate thickness of 300 μm, and dry mass m1. Specimens were placed in well plates containing 1 mL of either PBS (pH 7.4), 20 U/ml cholesterol esterase in PBS or 0.02 U/ml collagenase in PBS. All buffers contained 0.02 wt% NaN3 as a bactericide and the buffer was refreshed two times per week. After 1, 7, 14 and 21 d of incubation at 37 °C, specimens were rinsed with water and freeze-dried to constant weight (m2). The percentage of mass remaining (M%t) was calculated from Eq. (4). In vitro degradation experiments were performed in triplicate (3 specimens of each mesh, per time point, per incubation solution).

\[ \text{M}_t = \frac{m_0 - m_2}{m_0} \times 100\% \]  

(4)

2.10. Cell culturing, proliferation and viability

hSMCs (passage 4) were cultured at 37 °C in humidified air containing 5% CO2, in 175 cm\(^2\) culture flasks containing culture medium (advanced DMEM with 1% (v/v) glutamax, 1% (v/v) penicillin/streptomycin and 10% (v/v) FBS). The culture flasks were coated with 0.1% (wt/v) gelatin solution before seeding the cells. The culture medium was refreshed 3 times per week until the cells reached confluence. Upon confluence, the cells were trypsinized and counted using a Neubauer cell counting chamber.

Circular specimens with a diameter of 11 mm were cut from extracted electrospun mats and sterilized in 70% ethanol (n = 4). After evaporating the ethanol in a sterile environment, the films were placed in 48-well cell culture plates. Cells were seeded on the films at a density of 10,000 cells/cm\(^2\) and cultured in a cell culture incubator for 7 d. Culture media were changed every 2–3 d. The proliferation and viability of hSMCs on the scaffolds were determined using the following methods:

2.10.1. CyQuant assay

On day 1, 3 and 7, the specimens were rinsed with DPBS to remove culture medium and non-adhering cells. Next, the adhering cells were lysed by 30 min incubation in CyQuant lysing buffer after which the culture plate was stored at −25 °C. After thawing, the number of adhering cells was quantified by adding 20 μl lysate to 180 μl CyQuant GR dye solution diluted 1:400 in lysing buffer, and measuring the fluorescence at an excitation wavelength of 480 nm and an emission wavelength of 520 nm using a Tecan Saphire fluorometer.

2.10.2. Live/Dead staining

Live/Dead staining was performed 7 days after cell seeding. The specimens were rinsed with warm DPBS (37 °C), and incubated with 2 μM calcein-AM/4 μM ethidium homodimer-1 solution for 1 h at 37 °C. After rinsing with warm DMEM, pictures were taken using an EVOS FL Cell Imaging System. Calcein-AM is retained within living cells, producing an intense uniform green fluorescence upon hydrolysis by esterase. Ethidium homodimer-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells.

2.11. Statistical analysis

All analyses were conducted with at least 3 specimens and presented as mean ± standard deviation. Statistical differences between the experimental data of groups were analyzed using one-way Analysis of Variance (ANOVA) with GraphPad 8.0. Statistical significance was set to a p value < 0.05.
3. Results and discussion

3.1. Characterization of PTMC-tMA and GelMA

The Mn of the synthesized PTMC-tMA was 18,500 g/mol. Successful reaction of the oligomer with methacrylic anhydride was confirmed by the presence of $-\text{C}=$CH$_2$ and $-\text{CH}_2$ peaks of the methacrylate group around $\delta = 6.1, 5.5$ and at $\delta = 1.87$ ppm, respectively. The degree of functionalization of the PTMC-tMA macromer was determined by comparing the integral values of the $-\text{CH}_3$ of the initiator at $\delta = 1.32$ ppm and the $-\text{C}=$CH$_2$ peaks around $\delta = 6.1$ and 5.5 ppm (shown in Fig. 1). A degree of substitution (DS) of 98% was calculated.

The $^1$H NMR spectra of gelatin and GelMA are shown in Fig. 2. The $-\text{C}=$CH$_2$ peaks of the methacrylate group are shown at $\delta = 5.58$ and 5.34 ppm. The DS of GelMA was determined by comparing the integral value of $-\text{CH}_2\text{NH}_2$ at 2.91 ppm for GelMA to that of the unmodified gelatin. A DS of 90% was calculated.

<table>
<thead>
<tr>
<th>PTMC-tMA/GelMA Ratio</th>
<th>$T_g$ 1 (°C)</th>
<th>$T_g$ 2 (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/0</td>
<td>-15.9</td>
<td>-</td>
</tr>
<tr>
<td>8/2</td>
<td>-15.5</td>
<td>61.1</td>
</tr>
<tr>
<td>6/4</td>
<td>-14.9</td>
<td>57.4</td>
</tr>
<tr>
<td>3/7</td>
<td>-14.5</td>
<td>56.9</td>
</tr>
<tr>
<td>1/9</td>
<td>-13.3</td>
<td>52.7</td>
</tr>
<tr>
<td>0/10</td>
<td>-</td>
<td>44.8</td>
</tr>
</tbody>
</table>

3.2. Thermal analysis of PTMC-tMA/GelMA electrospun fibers

$T_g$ values of the electrospun fibers are shown in Table 1. The $T_g$ of the 100% PTMC-tMA specimens ($T_g^{PTMC-tMA}$) was $-15.9$ °C, which is close to the values for PTMC networks reported by Schüller et al. [20]. The $T_g$...
of the 100% GelMA fibers ($T_g$-GelMA) was 44.8 °C, in agreement with values for crosslinked gelatin reported in the literature [16]. Unlike the fibers made from a single macromer, the hybrid fibers made from PTMC-tMA and GelMA mixtures showed two $T_g$ values, $T_g^1$ and $T_g^2$, which are related to phases rich in PTMC-tMA and GelMA, respectively. The presence of a single $T_g$ is commonly used as an indicator to reflect blend miscibility. For immiscible polymers multiple $T_g$s will be observed [21]. Therefore, we inferred that the PTMC-tMA/GelMA hybrid fibers had a two-phase structure. Although the initial macromer mixtures were homogeneous in solution, the evaporation of solvent during electrospinning likely decreased the miscibility of the macromers, resulting in phase separation. In addition, with increasing amount of GelMA in the fibers the value of $T_g^1$ (-15.5, -14.9, -14.5 and -13.3 °C) slightly increased compared to the value of $T_g$-PTMC-tMA (-15.9 °C). Moreover, with increasing amount of PTMC-tMA in the fibers the value of $T_g^2$ (52.7, 56.9, 57.4 and 61.1 °C) increased compared to the value of $T_g$-GelMA (44.8 °C). The latter increase may be due to a lower amount of structured water bound to the gelatin with increasing PTMC content of the fibers.

3.3. Morphology of PTMC-tMA/GelMA electrospun fibers

The morphology of the fibers one week after electrospinning and photo-crosslinking is shown in Fig. 3. Although it was possible to spin
fibers of 100% PTMC-tMA, the fibrous structure was not stable as shown in Fig. 3a and b. Because of the low T\text{g} of PTMC-tMA and low molecular weight of the PTMC-tMA, the electrospun fibers were in a viscous state, resulting in loss of the fibrous structure before post-curing. Fiber spinnability during electrospinning is promoted by application of linear polymers with high molecular weight, as this increases entanglement and alignment of the polymer chains. However, as we aimed to prepare crosslinked networks, we used three-armed PTMC of relatively low molecular weight to increase the amount of photo-crosslinkable methacrylate groups in the electrospun fibers. The T\text{g} of the hybrid fibers in this work was around 44.8 °C, indicating that the electrospun GelMA fibers were in the solid state at RT. The GelMA electrospun meshes maintained their structure as shown in Fig. 3p and q. The incorporation of GelMA in PTMC-tMA electrospun fibers improved fiber stability, as shown in Fig. 3d and n. This can be explained by the presence of a second glass transition in the hybrid fibers (T\text{g,2}) originating from the gelatin component. The diameter of the hybrid fibers increased with increasing GelMA content, see Fig. 3f and o. The mean fiber diameters of the 8/2, 6/4, 3/7 and 1/9 PTMC-tMA/GelMA fibers were 419, 501, 821 and 1037 nm, respectively. This is likely due to changing properties of the macromer mixtures during electrospinning, such as viscosity, surface tension and conductivity, with varying ratios of PTMC-tMA and GelMA [22,23]. The cross-section of the fibers was investigated with high-resolution SEM, see Fig. 3b–q. The hybrid fibers had a relatively rough cross-section and surface compared to the GelMA fibers, indicating microphase separation of the macromers in accordance with the DSC results. It should be noted, however, that the hybrid fibers had a macroscopically homogeneous structure. Compared to electrospun hybrid fibers made from unfunctionalized PTMC and gelatin [19], the photo-crosslinked PTMC-tMA/GelMA fibers prepared in this work show a superior structure in terms of continuity and uniformity.

Fig. 4. Stress–strain curves of GelMA and PTMC-tMA/GelMA electrospun fibrous mats in the hydrated state.

Table 2. Gel content, water uptake and porosity of the electrospun mats.

<table>
<thead>
<tr>
<th>PTMC-tMA/GelMA Ratio</th>
<th>Gel content (%)</th>
<th>Water Uptake (%)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/0</td>
<td>92 (2)</td>
<td>3 (2)</td>
<td>–</td>
</tr>
<tr>
<td>8/2</td>
<td>92 (0)</td>
<td>106 (9)</td>
<td>88.3 (1.6)</td>
</tr>
<tr>
<td>6/4</td>
<td>95 (1)</td>
<td>179 (9)</td>
<td>89.6 (2.0)</td>
</tr>
<tr>
<td>3/7</td>
<td>91 (2)</td>
<td>241 (9)</td>
<td>94.2 (0.9)</td>
</tr>
<tr>
<td>1/9</td>
<td>93 (1)</td>
<td>315 (9)</td>
<td>94.3 (2.0)</td>
</tr>
<tr>
<td>0/10</td>
<td>92 (2)</td>
<td>445 (23)</td>
<td>94.4 (0.9)</td>
</tr>
</tbody>
</table>

Table 3. Elastic modulus, strain at break, maximum strength and toughness of GelMA and PTMC-tMA/GelMA electrospun fibrous mats in the hydrated state.

<table>
<thead>
<tr>
<th>PTMC-tMA/GelMA Ratio</th>
<th>E\text{mod} MPa</th>
<th>εb %</th>
<th>F\text{max} MPa</th>
<th>Toughness N/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/2</td>
<td>0.643</td>
<td>445</td>
<td>4.127</td>
<td>1023(59)***</td>
</tr>
<tr>
<td></td>
<td>(0.021)</td>
<td>(20)</td>
<td>(0.248)</td>
<td></td>
</tr>
<tr>
<td>6/4</td>
<td>0.450</td>
<td>375(5)</td>
<td>2.373</td>
<td>539(43)**</td>
</tr>
<tr>
<td></td>
<td>(0.015)</td>
<td></td>
<td>(0.015)</td>
<td></td>
</tr>
<tr>
<td>3/7</td>
<td>0.299</td>
<td>110</td>
<td>0.512</td>
<td>33(6)*</td>
</tr>
<tr>
<td></td>
<td>(0.021)</td>
<td>(10)</td>
<td>(0.017)</td>
<td></td>
</tr>
<tr>
<td>1/9</td>
<td>0.216</td>
<td>96(2)</td>
<td>0.381</td>
<td>15(4)*</td>
</tr>
<tr>
<td></td>
<td>(0.013)</td>
<td></td>
<td>(0.020)</td>
<td></td>
</tr>
<tr>
<td>0/10</td>
<td>0.121</td>
<td>66(4)</td>
<td>0.111</td>
<td>3(1)</td>
</tr>
<tr>
<td></td>
<td>(0.010)</td>
<td></td>
<td>(0.005)</td>
<td></td>
</tr>
</tbody>
</table>

Significant difference compared to the 100% GelMA fibrous mat: *p < 0.01; ***p < 0.0001; **p < 0.00001.

3.4. Physical and mechanical characterisation of the PTMC-tMA/GelMA fibrous mats

Extraction of the electrospun mats in HFIP revealed that the gel contents of all meshes were at least 91%, as shown in Table 2, indicating that both macromers were efficiently crosslinked. As PTMC is hydrophobic and gelatin hydrophilic, the mats made from PTMC-tMA and GelMA had a water uptake of 3 ± 2% and 445 ± 23%, respectively. The water uptake of the hybrid meshes increased with increasing GelMA content of the fibers. As shown in Table 2, the water uptake of the 8/2, 6/4, 3/7 and 1/9 PTMC-tMA/GelMA hybrid meshes amounted to 106 ± 9%, 179 ± 9%, 241 ± 9% and 315 ± 9%, respectively. All hybrid mats, as well as the 100% GelMA fibrous mats, had a high porosity of at least 88%.

In addition to affecting hydrophilicity, incorporation of GelMA in the electrospun fibers influenced the mechanical properties of the meshes. All fibrous mats except those made from 100% PTMC-tMA, were mechanically tested to evaluate their tensile behavior in the hydrated state. The 100% PTMC-tMA samples were not tested because fibrous mats could not be formed. As shown in Fig. 4 and Table 3, the E\text{mod}, εb, F\text{max} and toughness decreased with increasing proportion of GelMA in the fibrous mats, due to the relatively weak mechanical properties of gelatin. Despite this effect on the mechanical properties of incorporation of GelMA in the meshes, the hybrid fibrous mats showed several times higher toughness values than reported for electrospun hydrogel fibrous mats [24–26]. In particular, fibrous mats made from 8/2 and 6/4 PTMC-tMA/GelMA mixtures showed outstanding mechanical performance, with E\text{mod} of 0.643 ± 0.021 MPa and 0.450 ± 0.015 MPa, εb of 445 ± 20% and 375 ± 5% and F\text{max} of 4.127 ± 0.248 and 2.373 ± 0.015 MPa, respectively. These fibrous mats had a toughness of 1023 ± 59 and 539 ± 43 N/mm², respectively. Compared to a non-crosslinked electrospun fibrous scaffold made from 60% PTMC and 40% gelatin (toughness 133 N/mm²) [19], the electrospun fibrous mesh made from 6/4 PTMC-tMA/GelMA in the present study had a 4 times higher toughness (539 N/mm²). We envisage that our electrospun hybrid fibrous meshes can be useful for vascular tissue engineering. Upon collecting the fibers on a rotating mandrel, a porous tubular scaffold is formed. A high toughness of the mesh is one of the prerequisites for successful vascular tissue engineering.

3.5. In vitro degradation of the electrospun mats

As PTMC is a surface eroding polymer, the mechanical properties of PTMC scaffolds are preserved during a relatively long time in a biological environment [27]. For tissue engineering applications, scaffolds must possess suitable degradation profiles to allow successful tissue regeneration [28]. Scaffolds prepared from GelMA show a rapid enzymatic degradation in an in vivo environment [29]. Incorporation of gelatin in a synthetic polymer fiber can reduce the degradation time and...
the degradation rate can be adjusted with the gelatin proportion [28,30]. The remaining mass of the PTMC-tMA/GelMA fibrous scaffolds during incubation in PBS, cholesterol esterase and collagenase solutions is shown in Fig. 5. Generally, the degradation rate of the mats increased with increasing GelMA content. After incubation for 21 days, specimens prepared from 100% PTMC-tMA did not show significant mass loss in any of the media. GelMA meshes showed the fastest degradation. They were completely degraded after incubation for 21 days in both cholesterol esterase and collagenase solutions. Even in PBS, the GelMA scaffolds showed around 30% mass loss after 21 days of incubation. Thus, at the enzymatic concentrations used in this work, degradation of the hybrid fibers was dominated by the degradation of GelMA in the macromer networks. It is noteworthy that after 21 days of degradation in collagenase solution, the remaining mass of the 8/2, 6/4 and 3/7 PTMC-tMA/GelMA fibrous mats was approximately 79, 66 and 32%, see Fig. 5c. This is similar to the PTMC amount in the hybrid fibers, and may indicate that the gelatin was mainly located at the outside of the fibers. As we used enzyme solutions, the shown degradation profiles are not indicative of degradation upon cell culturing or in vivo application, which needs further investigation.

3.6. Cell culturing on the electrospun mats

Except for the contribution to stable fiber formation, another important purpose for incorporating GelMA in the hybrid fibers was to enhance the biological properties of the scaffolds. In our previous study [9], we showed that although PTMC is a biocompatible polymer, the incorporation of gelatin in the polymer network further enhanced the proliferation of cells on the hybrid networks. As shown in Fig. 6, hSMCs adhered and proliferated on the electrospun scaffolds. After 7 days of

![Fig. 5. In vitro degradation of the electrospun mats in a. PBS, b. 10 U/ml cholesterol esterase and c. 0.02 U/ml collagenase.](image)

![Fig. 6. Proliferation of hSMCs on PTMC-tMA, GelMA and PTMC-tMA/GelMA electrospun mats as assessed by CyQuant assay, *p < 0.05.](image)
culturing, cell numbers on the 6/4, 3/7 and 1/9 PTMC-tMA/GelMA fibrous mats were similar as compared to the 100% GelMA meshes, and significantly higher as compared to the 100% PTMC-tMA specimens. This is also reflected by the Live/Dead staining images shown in Fig. 7. These results are in agreement with reports demonstrating that incorporation of gelatin in various electrospun fibers enhanced the attachment and proliferation of different cell types [31,32]. It is well-known that cell morphology and orientation are dependent on the architecture of scaffold materials [33,34]. On electrospun fiber substrates, adherent cells will be elongated and oriented in parallel with the fiber direction [35]. In the present study, the cells were indeed more elongated than on the flat PTMC/gelatin films used in our previous work [9]. As the fibers in the electrospun mats had a random orientation, the cells were also randomly oriented. In addition to the high toughness, the good adhesion and proliferation of hSMCs on our hybrid fibrous mats indicate that these meshes are potentially useful in tubular form for vascular tissue engineering.

4. Conclusions

The preparation of tough electrospun fibrous mats from homogeneous PTMC-tMA and GelMA solutions with a wide range of compositions was demonstrated and discussed. Electrospinning solutions of only PTMC-tMA did not yield a fibrous structure upon photo-crosslinking. This could be overcome by the addition of GelMA, which conferred form-stability to the electrospun fibers, yielding stable photo-crosslinked hybrid meshes. The fiber diameter, water uptake, mechanical properties and degradation rate could be controlled by adjusting the ratio of GelMA to PTMC-tMA. The incorporation of GelMA in the fibers enhanced the proliferation of hSMCs on the fibrous mats. Hybrid electrospun fibrous mats made from 6/4 PTMC-tMA/GelMA showed both good mechanical and cell adhesive properties.

5. Author statement

All authors have approved the revisions.

CRediT authorship contribution statement

Jia Liang: Conceptualization, Methodology, Investigation. Zhengchao Guo: Conceptualization, Methodology, Investigation. Piet Dijkstra: Conceptualization, Methodology, Validation, Writing - review & editing. Dirk Grijpma: Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. André Poot: Conceptualization, Methodology, Validation, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

References


Fig. 7. Live/Dead staining of hSMCs cultured for 7 days on PTMC-tMA, GelMA and PTMC-tMA/GelMA electrospun mats (scale bars 400 μm).
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