Supplementary Data

Enzymatically cross-linked hydrogels based on a linear poly(ethylene glycol) analogue for controlled protein release and 3D cell culture

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Fig. S1. $^1$H NMR spectrum of the PEGDA-DTT-HPA copolymer in DMSO-$d_6$ after $D_2O$ exchange.

Fig. S2. $dn/dc$ results of the indicated samples determined by a refractive index detector in DMF.

Table S1. Effect of molar ratio on the properties of PEGDA-DTT-HPA.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sample</th>
<th>Molar ratio$^a$</th>
<th>$DS_{HPA}^b$</th>
<th>Solubility in water</th>
<th>Gel formation by HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PEGDA-DTT-HPA-1</td>
<td>0.04</td>
<td>0.035</td>
<td>soluble</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>PEGDA-DTT-HPA-2</td>
<td>0.14</td>
<td>0.13</td>
<td>soluble</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>PEGDA-DTT-HPA-3</td>
<td>0.80</td>
<td>0.425</td>
<td>insoluble</td>
<td>−</td>
</tr>
</tbody>
</table>

$^a$ Molar ratio of 3-(4-hydroxyphenyl) propionic acid (HPA) to the hydroxyl groups of the PEGDA-DTT copolymer; $^b$ Determined by $^1$H NMR.
Fig. S3. Phase angle ($\phi$) of PEGDA-DTT-HPA hydrogels with varying concentrations of H$_2$O$_2$ as a function of time. The measurement was taken with constant strain of 1.0% (selected according to the LVER test) and the oscillation frequency was set at 1 Hz. The $DS_{HPA}$ of PEGDA-DTT-HPA was 0.13. The final concentrations of the PEGDA-DTT-HPA copolymer and HRP were 8.0 wt% and 0.0125 mg mL$^{-1}$, respectively for all the tested samples.

Fig. S4. Storage modulus $G'$ and loss modulus $G''$ of PEGDA-DTT-HPA hydrogels with indicated concentrations of the PEGDA-DTT-HPA copolymer as a function of time. The measurement was taken with constant oscillation strain of 1.0% (selected according to the LVER test) and the oscillation frequency was set at 1 Hz. The $DS_{HPA}$ of PEGDA-DTT-HPA was 0.13. The final concentrations of H$_2$O$_2$ and HRP were 0.0175 wt% and 0.0125 mg mL$^{-1}$, respectively for all the tested samples.
Fig. S5. Storage modulus $G'$ and loss modulus $G''$ of PEGDA-DTT-HPA hydrogels with different $D_{S_{HPA}}$ of PEGDA-DTT-HPA as a function of time. The measurement was taken with constant oscillation strain of 1.0% (selected according to the LVER test) and the oscillation frequency was set at 1 Hz. The final concentrations of PEGDA-DTT-HPA ($D_{S_{HPA}} = 0.035$, $D_{S_{HPA}} = 0.13$), $H_2O_2$ and HRP were 8.0 wt%, 0.0175 wt% and 0.0125 mg mL$^{-1}$, respectively for all the tested samples.

Fig. S6. (A) Frequency sweep and (B) amplitude sweep of PEGDA-DTT-HPA hydrogels with varying concentrations of $H_2O_2$. The $D_{S_{HPA}}$ of PEGDA-DTT-HPA was 0.13. The final concentrations of the PEGDA-DTT-HPA copolymer and HRP were 8.0 wt% and 0.0125 mg mL$^{-1}$, respectively for all the tested samples.
**Fig. S7.** Relative fluorescent intensity of 3D confocal microscopy images of hMSCs-LifeAct-EGFP in Gel-2 after culture for 24, 48, and 72 h determined by confocal laser scanning microscope.