Supplementary Information for

**Multivalent Grafting of Hyperbranched Oligo- and Polyglycerols Shielding Rough Membranes to Mediate Hemocompatibility**

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1. Characterization of hyperbranched oligoglycerols, hyperbranched polyglycerols, and linear oligoglycerol.

$^1$H and $^{13}$C NMR spectra were recorded on a Jeol ECX spectrometer operating at 400 MHz at concentrations of 100 mg·mL$^{-1}$. The chemical shifts are reported in $\delta$ (ppm) values and were referenced to the indicated solvents.

1.1. hOG-OH:

$^1$H NMR (400 MHz; MeOD): $\delta$ =3.91-3.42 (m, 189 H, PG-backbone); 1.45-1.34 (m, 2 H, CH$_2$CH$_3$ of starter); 0.95-0.85 (m, 3 H, CH$_2$CH$_3$ of starter) ppm. $^{13}$C NMR (400 MHz; MeOD): $\delta$ = 81.4, 79.8, 73.9, 72.8, 70.6, 64.3, 62.6 (PG-backbone); 45.2 (CHNH$_2$); 44.6 (CH$_2$NH$_2$); 23.6 (CH$_2$CH$_3$ of starter); 7.7 (CH$_2$CH$_3$ of starter) ppm. IR $\nu_{\text{max/cm}^{-1}}$: 3362, 2871, 1640, 1575, 1461, 1329, 1259, 1078, 847.

1.2 hOG-OMe:

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ =3.67-3.39 (m, 181 H, PG-backbone, -CH$_2$- and –CH-); 3.33-3.31 (s, 39 H, primary -OMe); 3.31 (s, 14 H, secondary -OMe); 1.37-1.33 (m, 2 H, CH$_2$CH$_3$ of starter); 0.80 (t, 3 H, CH$_2$CH$_3$ of starter) ppm. $^{13}$C NMR (700 MHz; CDCl$_3$): $\delta$ = 79.4, 78.7, 72.5, 71.5, 70.6, 69.8 (PG-backbone); 59.3 (primary -OMe); 58.0 (secondary -OMe); 43.2 (CHNH$_2$); 42.8 (CH$_2$NH$_2$) 22.9 (CH$_2$CH$_3$ of starter); 7.7 (CH$_2$CH$_3$ of starter) ppm. IR $\nu_{\text{max/cm}^{-1}}$: 3362; 2875; 1643; 1461; 1356; 1296; 1196; 1085; 960; 832.

1.3 hPG-OH:

$^1$H NMR (400 MHz; D$_2$O): $\delta$ =4.00-3.51 (m, 304 H, PG-backbone); 1.39-1.32 (m, 2 H, CH$_2$CH$_3$ of starter); 0.87-0.83 (m, 3 H, CH$_2$CH$_3$ of starter) ppm. $^{13}$C NMR (700 MHz; D$_2$O): $\delta$ = 79.4, 77.9, 72.2, 70.4, 68.9, 60.8 (PG-backbone); 43.1 (CHNH$_2$); 41.3 (CH$_2$NH$_2$); 21.9(CH$_2$CH$_3$ of starter); 6.9 (CH$_2$CH$_3$ of starter). IR $\nu_{\text{max/cm}^{-1}}$: 3320, 2909, 1663, 1455, 1082, 1028.
1.4 hPG-OMe:

$^1$H NMR (700 MHz; DMSO): $\delta = 3.70-3.31$ (m, 393 H, PG-backbone –CH$_2$- and –CH-); 3.25-3.24 (m, 74 H, primary -OMe); 1.39-1.32 (m, 2 H, CH$_2$CH$_3$ of starter); 0.87-0.83 (m, 3 H, CH$_2$CH$_3$ of starter) ppm.$^{13}$C NMR (700 MHz; DMSO): $\delta =$78.9, 77.9, 71.8, 70.5, 69.1, 63.1, 60.5 (PG-backbone); 58.4 (primary -OMe); 57.1 (secondary -OMe); 42.9 (CHNH$_2$); 42.3 (CH$_2$NH$_2$); 22.7 (CH$_2$CH$_3$ of starter); 7.5 (CH$_2$CH$_3$ of starter) ppm. IR $\nu_{\text{max}}$/cm$^{-1}$: 3318, 2903, 1663, 1457, 1199, 1083, 1028.

1.5 OG-OMe:

$^1$H-NMR (DMSO-d6, 500 MHz): $\delta = 1.65$ (m, 2 H, H$_2$N-CH$_2$), 2.58 (s, 2H, H$_2$N-CH$_2$-CH$_2$), 3.23 (s, 74H,OMe), 3.40-3.74 (134 H, other CH and CH$_2$) ppm. MALDI-TOF: $M_n = 2270$ g·mol$^{-1}$, PDI = 1.03 (calculated from main series). GPC: $M_n = 1850$ g·mol$^{-1}$, PDI = 1.3.

1.6 SAXS investigations

The obtained SAXS curves are most appropriately described by the single-polymer form factor published recently by Hammouda based on scaling arguments.$^1$ Therein the scattering of a branched polymer chain in different solution conditions is given by

$$I(q) = \frac{k}{Norm} \left[ \frac{1}{\nu_m U_B^{\gamma/2}} \gamma \left( \frac{c}{2\nu_m}, U_B \right) - \frac{1}{\nu_m U_B^{\gamma/2}} \gamma \left( \frac{c+1}{2\nu_m}, U_B \right) \right]$$

with the scaling factor, $k$, a normalization factor $\text{Norm} = \frac{2}{c(c+1)}$, the alternative incomplete gamma function $\gamma(d,U_B) = \int_0^{U_B} dt \exp(-t)t^{d-1}$ and $U_B = \frac{q^2 R_g^2 (2\nu_m + c)(2\nu_m + c + 1)}{6}$. The model parameters are the radius of gyration, $R_g$, the excluded volume parameter, $\nu_m$, and the scaling exponent, $c$. Values of $c$ range from 1 to 2, where a value of 1 corresponds to a linear polymer and 2
for a highly branched polymer. The excluded volume parameter increased in the line 1/3 for a collapsed polymer, 1/2 for a polymer in theta solvent, 3/5 for a polymer in a good solvent and 1 for a polymer in stretched conformation. It is linked to the minimum fractal dimension, $d_m$, by $\nu_m = \frac{1}{d_m}$. 
Figure S1. SAXS curves of A) hOG-OH, B) hPG-OH, and C) hPG-OMe in aqueous solutions and curve fits with single polymer form factors (solid and dashed lines, respectively). The solvent quality was decreased by increasing the amount of acetone. Water to acetone ratios are 1:0.1:1 and 1:3. Curves with solvent ratios of 1:1 and 1:3 are multiplied by a factor of 10^3 and 10^6, respectively, for better visibility.
2. Membrane characterization

**Figure S2.** SEM images of the surface (left) and cross-section (right) of alkaline treated PEI-pH11 (A) and the OG-OMe (B) and hPG-OMe (C) functionalized PEI membranes (free of non-woven support).

**Residual solvent** of the unfunctionalized membrane PEI-0 was determined by gas chromatography (GC) (DB-Wax, 15 m column, 0.53 mm inner diameter, 1 μm layer thickness, HP 5890 Series II GC, GMI Inc., Ramsey, Minnesota, USA). Two membrane sheets of 175 cm² each (total area by BET 19.5 m²) were cut into small pieces, placed into glass vials, filled with 10 mL Millipore water and sealed with a PTFE septum. For each sample, three replicates of the supernatant, incubated at 37 °C while shaking for 2, 3, and 5 days, were taken and analyzed.
Table S1: Surface roughness of pure PEI-0, alkaline treated PEI-pH11, hOG, and hPG surface functionalized PEI membranes, determined by AFM measurements. An OG-OMe surface functionalized PEI membrane was included for comparison. Samples were analyzed at 1, 10, and 50 µm² scan size in wet state in PBS at pH 7.4 (tapping mode, n = 3 with 3 measurement areas each).

<table>
<thead>
<tr>
<th>Sample ID*</th>
<th>Arithmetic mean roughness $R_a$ and root-mean-squared roughness $R_q$ ($±$ standard deviation) [nm]</th>
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</thead>
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<tr>
<td></td>
<td>Scan Size [µm] 1 10 50</td>
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<tr>
<td>PEI-0</td>
<td>$R_a$ 2.8 ± 2.8 $R_q$ 3.6 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>9.4 ± 12.3 12.6 ± 11.9</td>
</tr>
<tr>
<td>PEI-pH11</td>
<td>$R_a$ 2.1 ± 1.8 $R_q$ 2.7 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>7.4 ± 8 10.5 ± 11.4 17.7 ± 16.5</td>
</tr>
<tr>
<td>hOG-OH</td>
<td>$R_a$ 2.4 ± 0.7 $R_q$ 3.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>3.7 ± 1.1 5.5 ± 1.9 7.9 ± 3.2</td>
</tr>
<tr>
<td>hPG-OH</td>
<td>$R_a$ 2.2 ± 0.6 $R_q$ 2.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>5.4 ± 3.5 9.5 ± 6.2 9.6 ± 4.1</td>
</tr>
<tr>
<td>hOG-OMe</td>
<td>$R_a$ 3.5 ± 3.6 $R_q$ 4.4 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>14.3 ± 18.6 18.0 ± 22.7 25.1 ± 28.3</td>
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<tr>
<td>hPG-OMe</td>
<td>$R_a$ 3.7 ± 3.9 $R_q$ 5.0 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>7.0 ± 5.9 11.2 ± 10.0 17.5 ± 16.2</td>
</tr>
<tr>
<td>OG-OMe</td>
<td>$R_a$ 3.0 ± 1.5                                     $R_q$ 4.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>7.5 ± 6.6 13.4 ± 10.2 13.0 ± 8.6</td>
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</tbody>
</table>

* PEI-0: untreated PEI membrane; PEI-pH 11: pH 11 treated PEI membrane; hOG-OX: hyperbranched oligoglycerol functionalized PEI membranes, with hydroxyl (X=H) or methoxy (X=Me) end groups. hPG-OX: hyperbranched polyglycerol functionalized PEI membranes, with hydroxyl (X=H) or methoxy (X=Me) end groups.
Table S2: Elemental composition of untreated PEI-0, alkaline treated PEI-pH11, as well as hOG, hPG or OG-OMe surface functionalized PEI membranes determined from the survey scans in the angle dependent XPS.

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<th>C1s</th>
<th>O1s</th>
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<td>[°]</td>
<td>[at.%]</td>
<td>[at.%]</td>
<td>[at.%]</td>
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<tr>
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<td>13.4</td>
<td>4.4</td>
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<td>13.4</td>
<td>4.0</td>
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<td>45</td>
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<td>82.4</td>
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<td>75.3</td>
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<td>38.4</td>
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<td>45</td>
<td>75.5</td>
<td>20.7</td>
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<td>74.6</td>
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<td>3.1</td>
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<td>25.6</td>
<td>3.2</td>
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<td>29.2</td>
<td>2.4</td>
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<td>60</td>
<td>71.0</td>
<td>27.0</td>
<td>2.0</td>
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<tr>
<td>hPG-OMe calc.</td>
<td>71.3</td>
<td>27.2</td>
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<td>25.4</td>
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<td>72.3</td>
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<td>60</td>
<td>72.6</td>
<td>24.7</td>
<td>2.8</td>
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<tr>
<td>OG-OMe calc.</td>
<td>66.5</td>
<td>32.9</td>
<td>0.6</td>
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<td></td>
<td>60</td>
<td>76.7</td>
<td>20.5</td>
<td>2.8</td>
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* PEI-0: untreated PEI membrane; PEI-pH 11: pH 11 treated PEI membrane; hOG-OX: hyperbranched oligoglycerol functionalized PEI membranes, with hydroxyl (X= H) or methoxy (X=Me) end groups. hPG-OX: hyperbranched polyglycerol functionalized PEI membranes, with hydroxyl (X= H) or methoxy (X=Me) end groups.

3.1 Endotoxin content and activation of cells

Materials and Methods

LAL-tests were performed for all PEI membranes to determine the content of soluble lipopolysaccharides (LPS). The material eluates were prepared according to ISO10993–12. The eluates were analyzed for LPS contamination using the LAL-Test according to manufacturer’s instructions (Lonza, Cologne, Germany).

The level of C5a in human plasma after incubation with the sample membranes was determined using 300 µL of commercially available pooled normal human plasma (Precision BioLogic Inc. Dartmouth, Nova Scotia, Canada), which was incubated (30 minutes, 37 °C) in a 24-well flat-bottom plate (Costar, Albany, NY, USA). As positive control, 100 µg·mL⁻¹ Zymosan (Sigma-Aldrich, St Lois, MO, USA) was used. A commercial enzyme-linked immunosorbent assay (ELISA) (R&D, Minneapolis, MI, USA) kit was used to determine the amount of generated C5a fragments in 1:100 diluted plasma.

To detect reactive oxygen species (ROS), whole human blood was incubated for 10 min in the presence of the different control and functionalized PEI membranes. Opsonized *E. Coli* provided with the Phagoburst® assay kit (ORPEGENPharma, Heidelberg, Germany) were used as positive control. After incubation the Phagoburst® assay was performed according to manufacturer’s instructions. The evaluation of oxidative burst activity was performed by flow cytometry using the MACSQuant® analyzer. Flow cytometry data were analyzed using the FlowJo software (Tree Star, Ashland, USA).

Results

Before studying protein adsorption, thrombocyte adhesion, and thrombocyte activation, it was of paramount importance to exclude the influence of any potential biologically active contaminations and processes competing with or accompanying the thrombocyte adhesion and activation. Thrombocytes express toll-like receptors and can therefore directly be activated by microbial products such as endotoxins. In order to investigate the endotoxin burden of the different PEI membranes, a LAL-test, which detects endotoxins in material eluates, was performed. The endotoxin values of all examined
hOG-functionalized PEI membranes were far below 0.5 EU·mL$^{-1}$, which is the limit set by the U.S. Food and Drug Administration, indicating a germ free manufacturing process of the investigated membranes (Figure S3A).

The complement system acts as immune surveillance system, which can rapidly react towards foreign bodies including biomaterials such as cardiovascular implants or hemodialysis devices.$^2, 5, 6$ The release of the anaphylatoxin C5a is a key event during complement activation. To examine whether the functionalized and non-functionalized PEI membranes induce C5a release, the membranes were incubated with pooled human plasma and the levels of C5a were determined. Since the C5a levels in untreated plasma were not significantly different compared to plasma incubated in the presence of the different hOG- or hPG-functionalized membranes, it can be concluded that all examined PEI-membranes were incapable of inducing complement activation (Figure S3B). Reactive oxygen species (ROS) produced by distinct immune cells can facilitate inflammation and tissue damage, but could also accelerate the degradation of biomaterials, and especially polyethers,$^7, 8$ which would be unfavorable in view of potential applications. When whole human blood was incubated together with the different hOG/hPG-functionalized PEI membranes the ROS production of neutrophils and monocytes was not induced when compared to the untreated blood (see supporting Figure S4).
Figure S3. Endotoxin levels and complement activation of the hOG/hPG-functionalized PEI membranes. (A) Endotoxins were detected by the LAL-test in material eluates prepared according to the ISO 10993-12. Two material samples were investigated in two independent experiments. Error bars indicate the range of data points. (B) Complement activation in the presence of the different hOG/hPG functionalized PEI membranes. Pooled human plasma was incubated for 30 min on the different membranes. C5a release was determined by ELISA. Data shown are pooled from two independent experiments each performed with two individual material samples. Error bars indicate mean ± SD.

Figure S4. Production of reactive oxygen species (ROS) by neutrophils and monocytes in whole human blood after incubation on hOG/hPG-functionalized PEI membranes and the references PEI-0, PEI pH11, and OG-OMe. Whole human blood was incubated for 10 min on the different PEI-membranes. The percentage of ROS-producing cells was determined by conversion of DHR123 into R123 and analyzed by flow cytometry. Neutrophils (A) and monocytes (B) were discriminated by flow cytometry scatter parameters as described previously. The average values of two healthy human donors analyzed in two independent experiments are shown (median ± range).
4. Statistical evaluation of the thrombocyte adhesion tests

**Table S3.** Statistical evaluation of the numbers of adherent thrombocytes, determined in the static thrombocyte adhesion experiment (6 donors, n = 3 samples each). Repeated measures one-way ANOVA (Tukey adjusted), considering p-values less than 0.05 as significant (marked with hash).

<table>
<thead>
<tr>
<th>PEI-0</th>
<th>PEI-pH11</th>
<th>hOG-OH</th>
<th>hPG-OH</th>
<th>hOG-OMe</th>
<th>hPG-OMe</th>
<th>OG-OMe</th>
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<td>hPG-OH</td>
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<td>hOG-OMe</td>
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<td>hPG-OMe</td>
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**Table S4.** Statistical evaluation of the thrombocyte covered material surface areas, determined in the static thrombocyte adhesion experiment (6 donors, n = 3 samples each). Repeated measures one-way ANOVA (Tukey adjusted), considering p-values less than 0.05 as significant (marked with hash).

<table>
<thead>
<tr>
<th>PEI-0</th>
<th>PEI-pH11</th>
<th>hOG-OH</th>
<th>hPG-OH</th>
<th>hOG-OMe</th>
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5. XPS analyses.

Table S 5. Data of the angle dependent X-ray induced photoelectron spectroscopy for the determined chemical bonds in the C1s, O1s and N1s region scan spectra of untreated PEI-0, alkaline treated PEI-pH11, hOG and OGMe functionalized PEI membranes (non-woven free)

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<th>C-H; C=C</th>
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<th>Imide</th>
<th>Carboxylic Acid</th>
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<th>O=C</th>
<th>O=C shake up</th>
<th>π-→π* shake up</th>
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<td>--</td>
<td>13.3</td>
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1 Calculated according to the chemical structure of PEI and literature references. * PEI-0: untreated PEI membrane; PEI-pH11: pH 11 treated PEI membrane; hOG-OX: hyperbranched oligoglycerol functionalized PEI membranes, with hydroxyl (X= H) or methoxy (X=Me) end groups. hPG-OX: hyperbranched polyglycerol functionalized PEI membranes, with hydroxyl (X= H) or methoxy (X=Me) end groups.
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