Supporting Information

Bioactive Hydrogel Coatings of Complex Substrates using Diffusion-Mediated Redox Initiation

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Experimental Section

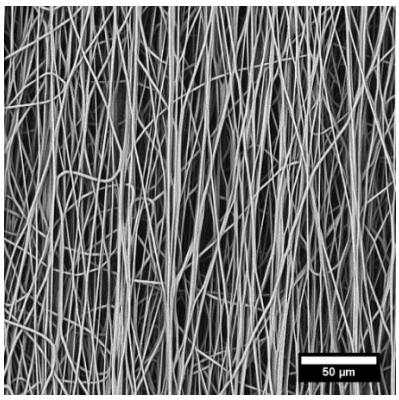
Characterization of Fe(II) by FerroZine™ assay

A protocol for characterizing the Fe(II) content in iron(II) gluconate dihydrate (IG) using the FerroZine™ iron reagent (Thermo Fisher Scientific) was adapted from Stookey et al. and others.[1-6] A 1.0 mM (0.025 wt%) solution of IG was prepared in sodium acetate buffer (2.5 M sodium acetate, 1.24 M acetic acid, pH = 5.2). A standard curve was prepared by diluting the IG stock with sodium acetate buffer to a final concentration ranging from 17-48 μM (0.000833 – 0.0023 wt%) with ascorbic acid (10.0 mM) and the Fe(II) detecting reagent FerroZine™ (0.2 mM). Molar excess of ascorbic acid reduces all Fe(III) in the standard solutions to Fe(II). Samples of IG were diluted within standard's range and 0.2 mM FerroZine™ was added. Samples and standards were placed in a 96 well plate and mixed by orbital shaking (1 mm amplitude, 2 minutes). Absorbance was read at 562 nm using a plate reader (BioTek Synergy 2). The amount of Fe(II) in samples was determined using the reduced calibration curve.

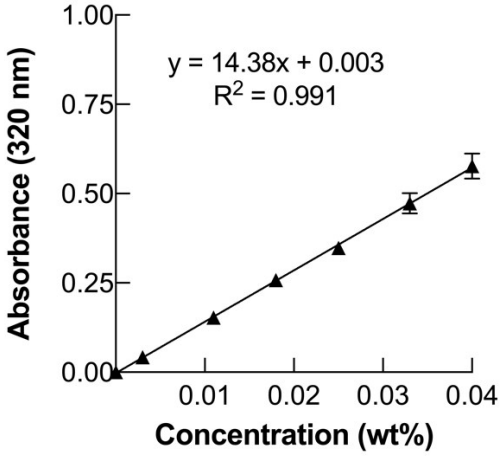
Effect of leachables on HUVEC viability

A standard LIVE/DEAD assay kit (Molecular Probes) was used to determine the effect of uncoated and hydrogel coated mesh samples on Human umbilical vein endothelial cell (HUVEC) viability. Briefly, confluent HUVECs were cultured in 24-well tissue-culture plates and exposed via transwell to uncoated and hydrogel coated samples. To prepare hydrogel samples, meshes were coated in 5 wt% IG, then immersed in a solution of PEGDA 3.4 kDa 10 wt% and APS 0.137 wt% for 30 seconds in a 96 well plate. Both uncoated and coated samples were immersed in sterile 10 mM phosphate buffered saline (PBS) supplemented with 1% penicillin/streptomycin after preparation. Solutions were exchanged 3x overnight, and samples were exposed to UV sterilization for 30 min during this time. Circular specimens (diameter = 6 mm) were punched from the samples and placed into transwell inserts of the confluent HUVECs wells. Culture proceeded for 24 hours. For LIVE/DEAD analysis, specimens were removed, and a dead control was prepared by soaking cells in 70% ethanol for 15 minutes. Cells were rinsed with PBS and stained for imaging with 4 μM calcein AM (live) and 4 μM ethidium homodimer-1 (dead) for 30 minutes at 37°C. Cells were rinsed with PBS prior to imaging. Imaging was conducted with a fluorescence microscope (Nikon Eclipse TE2000-S) and characterized using ImageJ software (n = 8).

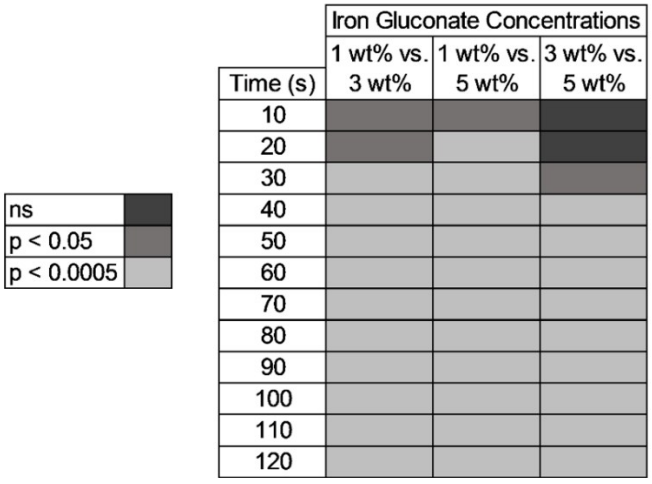
Additional Results and Discussion



Supplemental Figure S1: Scanning electron microscopy image of Bionate® polyurethane electrospun mesh.



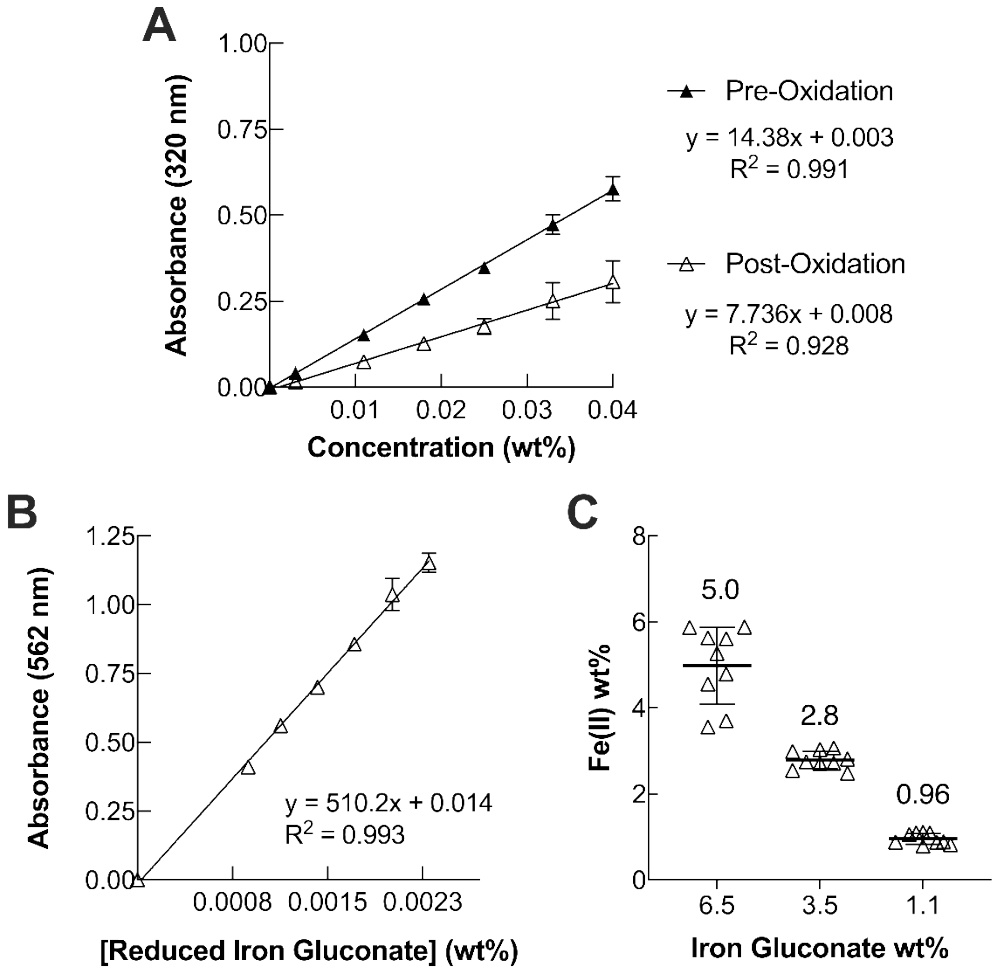
Supplemental Figure S2: Iron (II) gluconate calibration curve, absorbance measured at 320 nm.



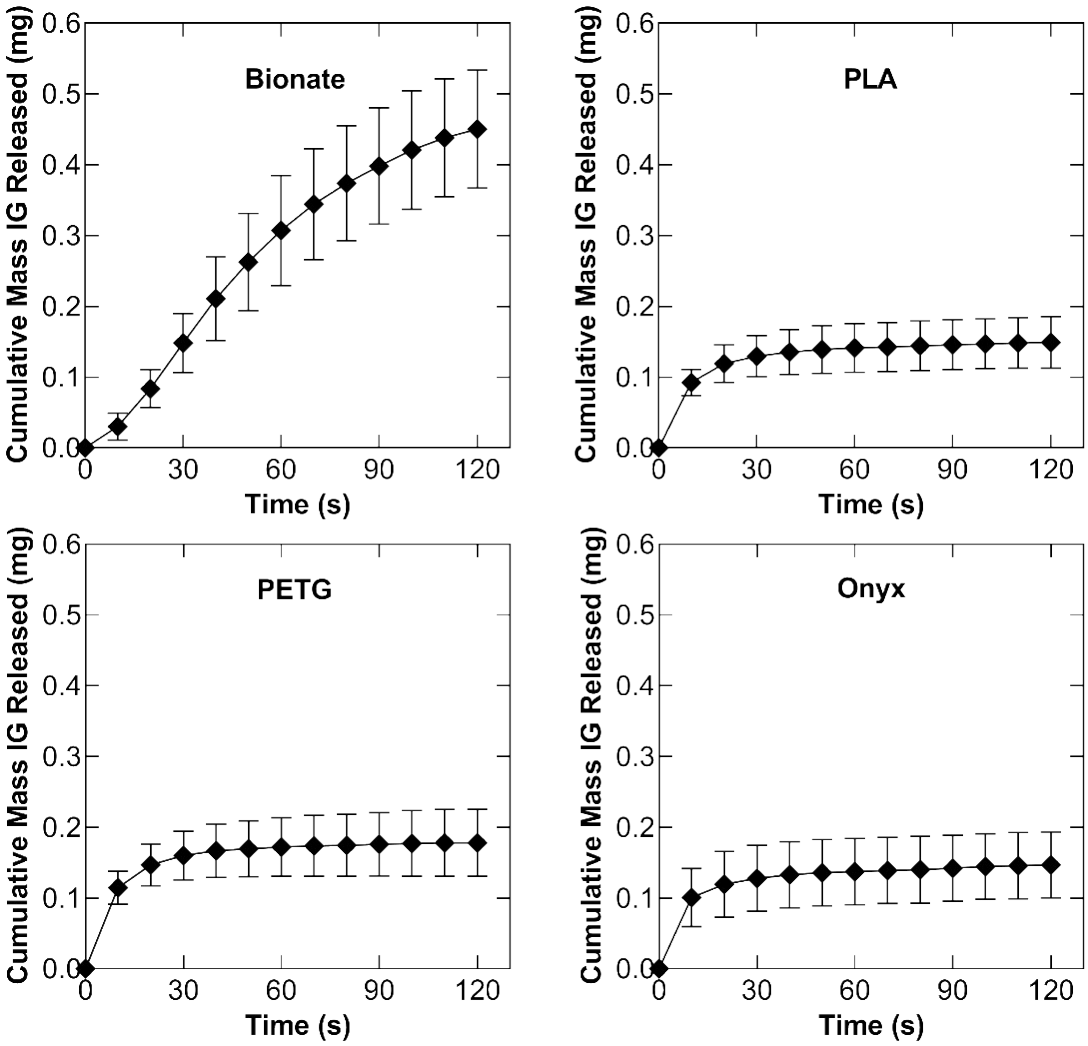
Supplemental Figure S3: Statistical analysis of iron (II) gluconate desorption over time between varying initial concentrations of iron (II) gluconate. Statistical significance determined as $p < 0.05$ or $p < 0.0005$ for $n = 18$ in ANOVA with Tukey's multiple comparison test.

Effect of oxidation on iron (II) gluconate

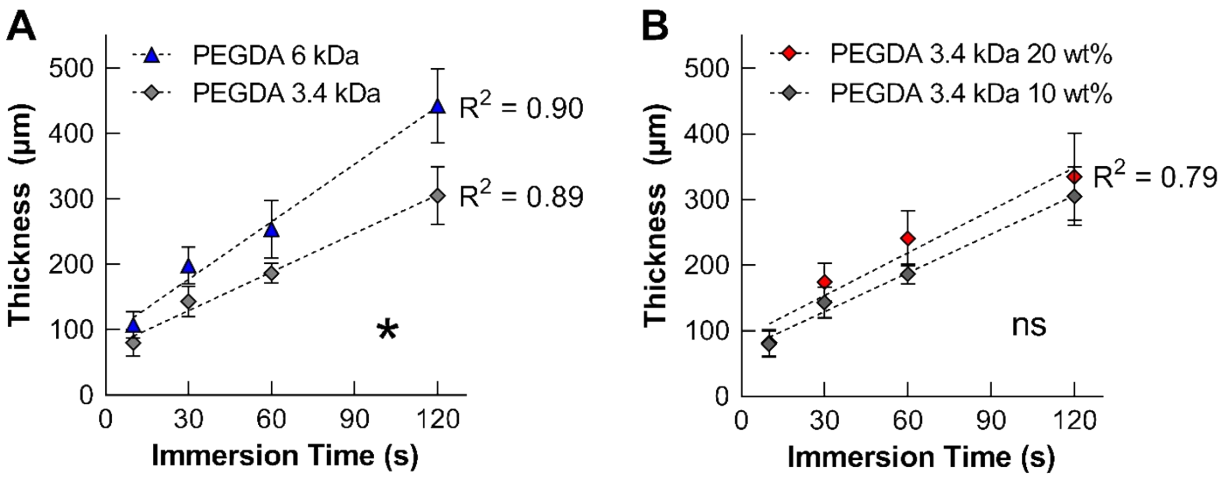
Over time (~6 months), a significant drop in the slope of the calibration curve of IG in water was observed (**Supplemental Figure 4A**). Additionally, particulates were observed in stock solutions of IG. It was hypothesized that these particulates were water insoluble ferric oxides. Fe(II) gluconate is susceptible to oxidation in the presence of moisture in air.[7, 8] To determine if the amount of redox-active ferrous (Fe(II)) iron in solution was changing over time as oxidation occurred, the FerroZine™ assay was used to characterize the amount of Fe(II) (**Supplemental Figure 4B**). Solutions of 1, 3, and 5 wt% IG used in initial studies showed Fe(II) concentrations lower than expected (data not shown). The slope of original IG calibration curves in water was used to back calculate effective concentrations of IG in oxidized samples. To match the original concentration of IG, solution concentrations were increased until slopes matched pre-oxidation values (data not shown). The FerroZine™ assay was run on solutions with the increased IG concentrations (1 to 1.1 wt%, 3 to 3.5 wt%, and 5 to 6.5 wt%). On average, the Fe(II) concentrations in these samples matched approximately original 1, 3, and 5 wt% Fe(II), respectively.



Supplemental Figure S4: (A) Calibration curves of iron gluconate in water before and after reagent oxidation. (B) Ferrozine reduced calibration curve of Fe(II) content in iron gluconate. (C) Fe(II) content in various stock solutions of iron gluconate. Inset numbers represent averages.

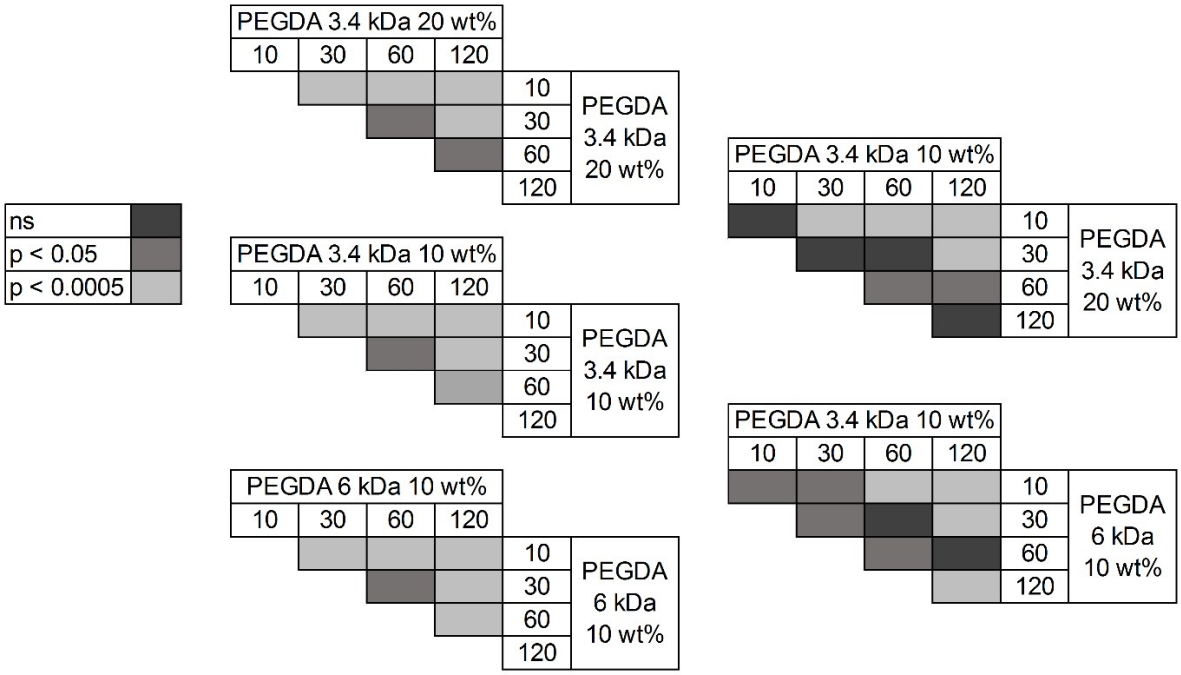


Supplementary Figure S5: Iron gluconate desorption from PCL, PETG, and Onyx 3D printed scaffolds as compared to release from electrospun Bionate meshes. All data represents averages ± standard deviation (n = 18).

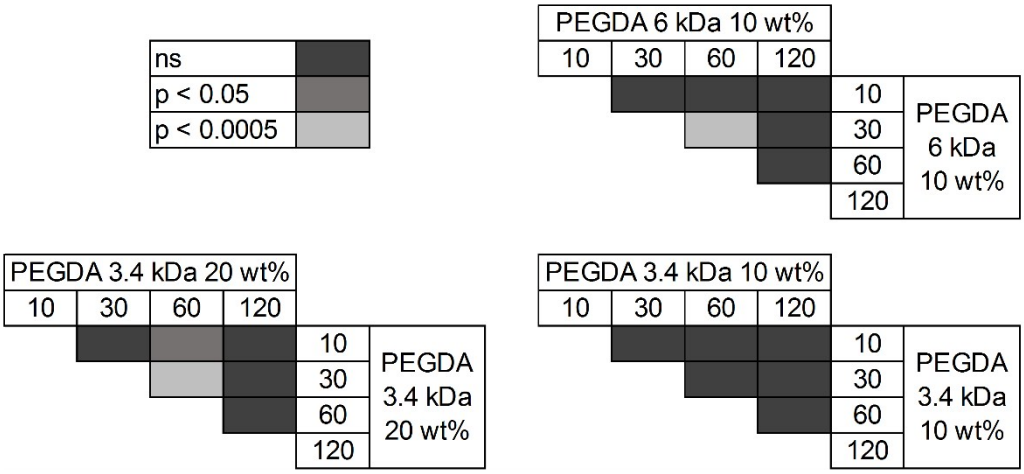


Supplementary Figure S6: Linear regression of hydrogel coating thickness growth kinetics (A) across polymer molecular weight between PEGDA 3.4 kDa and PEGDA 6 kDa at 10 wt% and (B) across polymer concentrations for PEGDA 3.4 kDa. The * represents a significant difference between slopes ($p < 0.0001$).

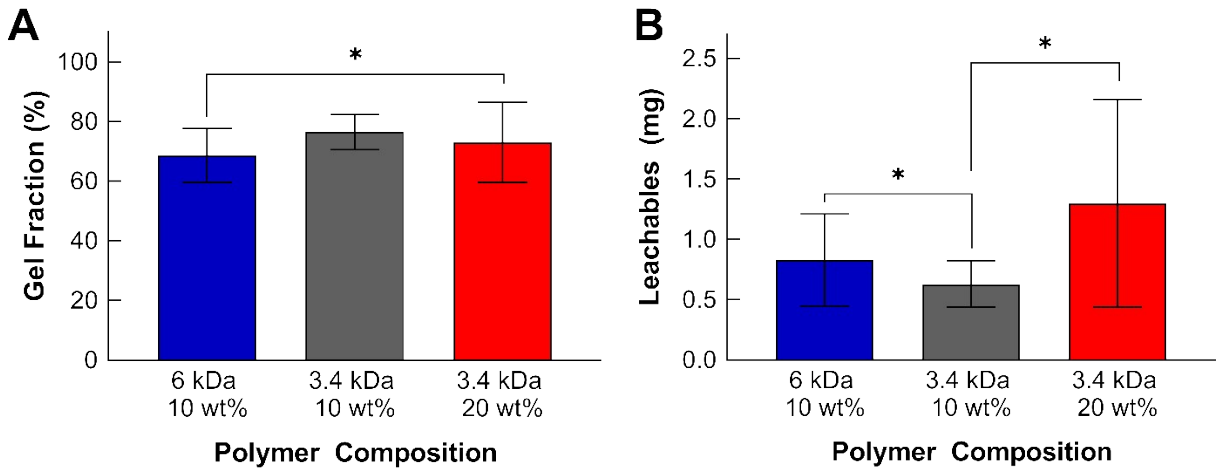
A) Statistical Analysis: Hydrogel coating thickness



B) Statistical Analysis: Hydrogel coating swelling ratio



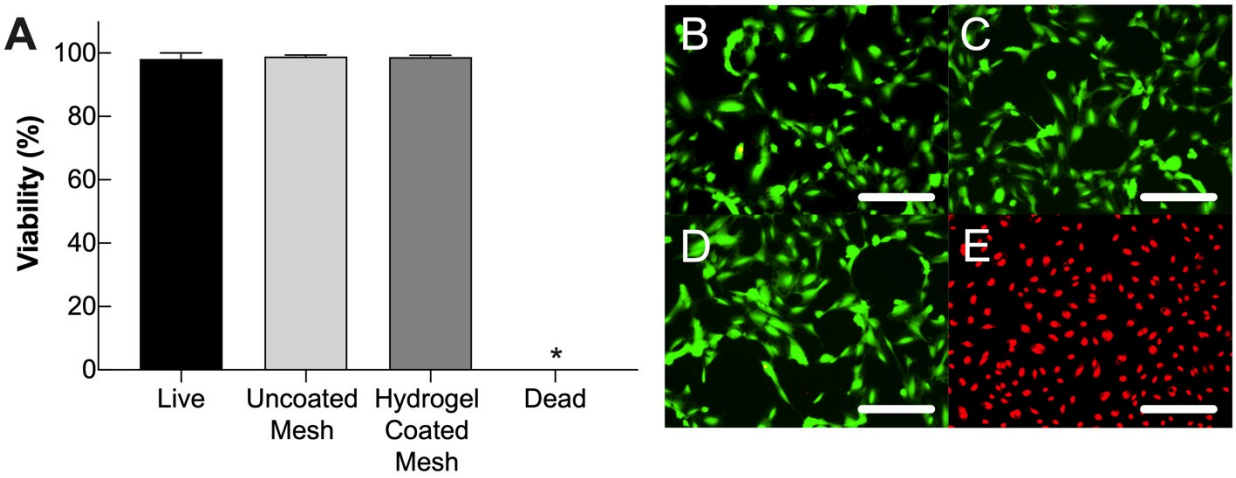
Supplemental Figure S7: Statistical analysis for hydrogel coating characterization. (A) Hydrogel coating thickness within and between varying polymer compositions and immersion times (n = 12). (B) Hydrogel coating swelling ratio across times within a constant composition (n = 6). Statistical significance determined as $p < 0.05$ or $p < 0.0005$ in ANOVA with Tukey's multiple comparison test.



Supplemental Figure S8: (A) Hydrogel gel fraction for all immersion times combined. (B) Hydrogel leachables for all immersion times combined. All measurements represent averages ± standard deviation (n = 24). The * represents a difference from all others ($p < 0.05$) in ANOVA with Tukey's multiple comparison test.

Supplemental Table S1: Swelling ratio, gel fraction, and leachable content of hydrogel compositions (n = 6 per condition, n = 24 per condition average). All measurements represent averages ± standard deviation.

Hydrogel Formulation	Immersion Time (s)	Swelling Ratio (Q)	Gel Fraction (%)	Leachables (mg)
PEGDA 3.4 kDa 10 wt%	10	15 ± 4.2	75.4 ± 7.00	0.4 ± 0.08
PEGDA 3.4 kDa 10 wt%	30	14 ± 0.5	69.9 ± 1.78	0.7 ± 0.05
PEGDA 3.4 kDa 10 wt%	60	12 ± 1.0	79.7 ± 2.78	0.6 ± 0.09
PEGDA 3.4 kDa 10 wt%	120	12 ± 1.5	81.2 ± 3.22	0.8 ± 0.10
PEGDA 3.4 kDa 10 wt%	Average	13 ± 2.5	76.5 ± 5.96	0.6 ± 0.19
PEGDA 3.4 kDa 20 wt%	10	9 ± 0.3	83.7 ± 3.04	0.4 ± 0.08
PEGDA 3.4 kDa 20 wt%	30	9 ± 0.5	57.0 ± 9.29	1.6 ± 0.38
PEGDA 3.4 kDa 20 wt%	60	8 ± 0.5	86.0 ± 1.94	0.7 ± 0.08
PEGDA 3.4 kDa 20 wt%	120	9 ± 0.4	65.4 ± 4.40	2.5 ± 0.42
PEGDA 3.4 kDa 20 wt%	Average	9 ± 1.1	73.0 ± 13.50	1.3 ± 0.86
PEGDA 6 kDa 10 wt%	10	15 ± 0.9	62.5 ± 4.86	0.6 ± 0.06
PEGDA 6 kDa 10 wt%	30	14 ± 1.2	67.6 ± 4.46	0.8 ± 0.14
PEGDA 6 kDa 10 wt%	60	17 ± 1.6	69 ± 14.92	1.0 ± 0.69
PEGDA 6 kDa 10 wt%	120	16 ± 0.9	75.61 ± 1.86	1.0 ± 0.05
PEGDA 6 kDa 10 wt%	Average	16 ± 1.5	68.7 ± 9.04	0.8 ± 0.38



Supplemental Figure S9: (A) Cytocompatibility assay (LIVE/DEAD) of extractables from uncoated and hydrogel coated meshes using a transwell indirect setup in comparison to a positive live control (TCPS) and negative dead control (ethanol fixed TCPS). Images of stained HUVECs after 24 hours of indirect exposure to (B) hydrogel coated meshes and (C) uncoated meshes, (D) TCPS positive control and (E) ethanol-fixed negative control. Scale bar = 250 µm.

References

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