Supporting Information

Photocrosslinkable Dextran Hydrogel Films as Substrates for Osteoblast and Endothelial Cell Growth

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Cryo SEM

To investigate the hydrogel's macroporous structure cryo-SEM measurements were carried out. Therefore, a part of dried bulk hydrogel was photo-crosslinked, swollen in buffer, and frozen in liquid nitrogen. The water was sublimed, then the hydrogel was coated with platinium and observed with the cryo-SEM under high vacuum (Fig S1). The freezing step is expected to influence the structure in the swollen state. The macroporous structure is clearly visible in the micrometer range, which is consistent with the literature for dextran based hydrogel systems.¹⁻⁵ These are expected to be a more flexible network in the swollen state rather than expanded rigid pores. A macroporous hydrogel structure was recently described as well by Chalal et al. observed in the swollen state by two-photon fluorescence microscopy.⁶ They reported macropores up to 75 µm for pure PNIPAAm and up to almost 250 µm for pHEMA-LLA-D (poly(hydroxyethyl methacrylate-L-lactide-dextran) hydrogels.



Figure S1: Cryo SEM images after freeze drying in liquid nitrogen and water evaporation in high vacuum. A-C show cross sections of a crosslinked bulk hydrogel (**3**) (BP:CH=1:25; CM:CH=1:6) in HEPES (10mM). A hydrogel film on a silizium substrate after swelling in water, freeze drying, and coating with platinium is shown in D.

Experimental: To investigate the overstructure of the hydrogel a polymer sample was crosslinked in bulk or as a film on a silicon wafer, swollen in buffer, rapidly frozen in liquid nitrogen, and transferred into the precooled (about -160°C) cryo preparation chamber (PP2000T, Quorum Technologies). The sample was broken and sputtered with a thin platinium layer (~20 nm) after the frozen water was sublimed off and then placed into the precooled chamber of the Focused Ion Beam (Nova, 600 Nanolab (FIB), FEI). The stage was slowly heated up to room temperature while measuring.

UV-VIS

To determine the effect of the UV-irradiation a hydrogel film was prepared on a quartz slide (PGO) covered by a selfassembled monolayer of 4-(3-triethoxysilyl)propoxybenzophenone and the UV-VIS spectra were recorded after increasing crosslinking intervals (Fig S2A). The decrease of the benzophenone $\pi\pi^*$ -absorption at 259 nm is clearly visible. Furthermore, an absorption around 345 nm, the wavelength of the n π^* -transition, occurs during crosslinking. This absorption reaches its maximum value directly after exposing the sample to UV-light for every crosslinking time. It subsequently decreases with time after the irradiation but does not vanish completely (Fig. S2A inset), while the benzophenone absorption at 259 nm increases simultaneously. Therefore, the second absorption at 345 nm might be attributed to longliving radical produced by UV-irradiation. This phenomenon was already observed by Horie and co-workers in 1987 for benzophenone moieties in a poly(vinyl alcohol) matrix⁷ and attributed to some reaction product with an absorption around 340 nm. The different recombination pathways of benzophenone radicals are summarized by Dorman et al.⁸. The small increase of the benzophenone absorption and simultaneously decreasing absorption at 345 nm with time show a relation between both signals (Fig. S2A inset).



Figure S2: UV-VIS spectra for decreasing crosslinking times (0, 1, 3, 6, 10, 15, 25 minutes) and 10 minutes waiting after crosslinking (A). The change of the UV-VIS absorption with increasing time (4, 7, 10 min, 12 h) after crosslinking for 3 minutes is shown in the inset of A. The decrease of the benzophenone absorption (@259nm) with increasing crosslinking time @254nm (A) and the decrease in swelling degree in relation to the crosslinking time for pH 3 (10mM) and pH 8 (10mM) (B and B inset) of a ~390nm thick (after spincoating) dextran hydrogel film on a quartz slide with an adhesion layer of 4-(3-triethoxysilyl)propoxybenzophenone. The degree of benzophenone substitution was 0.04 and carboxymethylation was 0.16.

Regarding the benzophenone absorption after approximately 15-20 minutes of irradiation with 254 nm, the crosslinking seems to be completed. Besides the UV-VIS spectra the influence of varying the crosslinking time on the swelling degree for a 390 nm thick hydrogel film (degree of carboxymethylation 0.16 and degree of benzophenone functionalization 0.04) at pH 3 and pH 8 (c(buffer) = 10mM) is shown in figure S2B. The data are consistent with the UV-VIS data. For crosslinking times up to 13 minutes the variation of swelling degree with crosslinking time is very high, wile for crosslinking times of 30 minutes and more only minor change of the swelling degree was observed.

The exponential relation between the crosslinking and the swelling of the hydrogel film is in agreement with the theory for polyelectrolyte gels with long crosslinked chains.⁹

Experimental: To investigate the crosslinking UV-VIS spectra were recorded with a UV-VIS/NIR (Perkin Elmer, Lambda 900) for different crosslinking times and different periods after the crosslinking was finished.

Reversed Wentzel Kramer Brillouin (rWKB) Approximation

The refractive index-thickness hydrogel film profile is calculated using the rWKB approximation. As an example its implementation is shown for a hydrogel film at pH 4. The angular positions of the four optical waveguide modes (Fig. S3A black curve) are used to calculate the hydrogel film profile (Fig. S3B). The value for the lowest thickness is set to 150 nm assuming a penetration depth of the surface plasmon of 150-200 nm.¹⁰ The refractive index value at the hydrogel gold interface (thickness=0nm) is derived experimentally from the surface plasmon by setting the thickness in a one box model to 1 μ m or higher and simulating the surface plasmon by fitting the refractive index. The angular scan cannot be reproduced by simulating the one-box model, which is consistent with the results of the rWKB approximation and reported hydrogel systems.¹¹ The rWKB profile shows a 3-box structure: one box for the first 150 nm from the gold-hydrogel interface, one box up to approximately 5 μ m and the last box for the hydrogel-buffer interface, showing a gradient towards the solution. If this 3-box structure is simulated the experimentally recorded scan can be reproduced (Fig S3A, red curve). For an angular scan with four optical waveguide modes 2 boxes for the hydrogel film plus the "surface plasmon box" leads to one parameter per

signal which is the maximum of parameters that can be reasonably fitted. The exact values used for the simulation are summarized in the table of figure S3.



(C)			
Layer	Thickness /nm	ε (real)	ε (imaginary)
LaSFN9	0	3.407	0
Chromium	1.9	-4	16.8
Gold	45	-12.4	1.6
SAM	2	2.06	0
Hydrogel-1	150	1.828	0
Hydrogel-2	4800	1.8105	0.006
Hydrogel-3	1600	1.802	0.001
Buffer	0	1.774	0
	1		

Figure S3: Implementation of the rWKB approximation and the procedure of data simulation exemplified for a 30 minutes crosslinked (254 nm) hydrogel film at pH 4. The hydrogel film profile (B) is derived from the angular position of the optical waveguide modes (A black curve) and the refractive index of the surface plasmon. The rWKB profile indicates a 3-box system, which reproduces well the angular scan (A, red curve). The exact values of the simulated curve are shown in table C. The hydrogel had a degree of benzophenone substitution of 0.04 and a degree of carboxymethylation of 0.16.

Usually, the hydrogel refractive index-thickness profile doesn't resemble a one-box structure, but is denser at the gold interface and shows a gradient transition at the hydrogel-solution interface, which is consistent with a PNIPAAm-based system reported in the literature.¹¹ This gradient at the film-solution interface is reduced by compacting the network, either by increasing the crosslinking time or by collapsing the gel. The photocrosslinked dextran-based hydrogel films can be simulated with a two-box layer model for high crosslinking times and a three-box layer model for incomplete crosslinking. A denser layer up to ~150 nm (surface plasmon) is followed by a subsequent box-like layer, and, for low crosslinked films, another box layer, with lower refractive index than the central one, close to the gel-liquid interface. This structure is in agreement with the WKB-based profiles.

Swelling Degrees

The swelling of the hydrogel film increases continuously with decreasing ionic strength of a PBS solution until a concentration of 10 mM. For crosslinking times below 90 minutes and ionic strength below 10mM the swelling of the hydrogel films increases significantly leading to the disappearance of the optical waveguide modes in the spectrum and partly delamination of the hydrogel film. This instability is more distinct if the hydrogel is not swollen under "low swelling conditions" like PBS 150mM or pH 3 first. In these cases the mechanical force upon swelling can obviously not be balanced by the forces of the hydrogel network and the film is destroyed. Therefore, films investigated here are never swollen with a solution of less than 10 mM ionic strength.

Tab. S1 Swelling degreey for stable gels for increasing crosslinking times at pH 3 and pH8 and for 150 mM and 10mM PBS (pH 7,4) derived from the rWKB analysis of a hydrogel film with a dry-state thickness of ~400nm and a degree of benzophenone substitution of 0.04 / degree of carboxymethylation of 0.16.

crosslinking	pH 3, 10 mM	pH 8, 10 mM	PBS 150 mM	PBS 10 mM
@ 254 nm	(WKB)	(WKB)	(WKB)	(WKB)
7min	$26(\pm 3.2)$	$48(\pm 6.0)$	$26(\pm 3.2)$	$42(\pm 5.3)$
10min	$24(\pm 3.0)$	$40(\pm 5.0)$	$25(\pm 3.1)$	$40(\pm 5.0)$
13min	$17(\pm 2.3)$	33(±4.2)	$17(\pm 2.3)$	$33(\pm 4.2)$
30min	$11(\pm 1.5)$	$20(\pm 2.6)$	$13(\pm 1.7)$	$21(\pm 2.7)$
60min	$11(\pm 1.5)$	$19(\pm 2.4)$	$13(\pm 1.7)$	$19(\pm 2.4)$
90min	$11(\pm 1.5)$	$16(\pm 2.0)$	$12(\pm 1.6)$	$17(\pm 2.2)$

AFM measurements

Atomic Force Microscopy (AFM) Measurements: The topography of the hydrogel films before and after swelling / drying cycles was investigated by atomic force microscopy (AFM) in air at room temperature with a commercial AFM (Dimension 3100 CL) in tapping mode. Micro cantilevers (Olympus) 160 μ m long, 50 μ m wide and 4,6 μ m thick with an integrated tip of a nominal spring constant of 42 N/m and a resonance frequency of 300kHz were used. The tip was scanned at rates about 0.7 Hz for scan size regions of ~1 μ m width.

The AFM measurements carried out before and after every pH and ionic strength screening cycle and drying show a very flat surface with a surface roughness of 0.2-0.5 nm (rms) on a scale of $1x1 \mu m$. No change of the surface structure upon swelling and collapsing could be observed. The overall roughness on a scale of cm² is higher and depends on the film preparation conditions, mainly on the concentration of the polymer solution in the spincoating process.



Figure S4: AFM measurements for a 90 minutes crosslinked hydrogel film (degree of benzophenone substitution= 0.04; degree of carboxymethylation= 0.16) before swelling, after one cycle of swelling and drying and after 3 cycles of swelling and drying (from the left to the right). The surface roughness along the black line indicated in every AFM picture is shown below.

Refractive Index – Thickness Profiles for a Nanoparticle – Dextran Composite Hydrogel Film



Figure S5: Swelling behaviour of the hydrogel film containing benzophenone-functionalized SiO₂-nanoparticles as supercrosslinking units with a content of 5 wt%. The rWKB refractive index-thickness profiles extracted from the angular scans are presented. From these rWKB profiles the values summarized in Figure 5 were extracted.

SEM measurements of a Nanoparticle – Dextran Composite Hydrogel Film



Figure S6: SEM micrographs of the composite hydrogel films containing 10 wt% of benzophenone-functionalized SiO_2 nanoparticles as cross section (left) and surface view (right). Scanning electron microscopy was performed using a Gemini field emission electron microscope (Zeiss), equipped with an InLense detector.

Film stability depending on degrees of substitution for COOH and BP groups

In addition to the stability data from the BP-CMD (**3**) with the amide linkage, also the stability results for the ether linkage derivative BP-CMD (**5**) are included in Figure S7, which show the same trend of increased stability with increasing BP content and decreasing COOH substitution.



Figure S7: Degree of benzophenone substitution versus degree of carboxymethylation for photocrosslinked (90 min) hydrogels (type (3) - amide linkage - in black and type (5) - ether linkage - in green), 100 nm - 1 μ m dry thickness, on a gold surface with a self-assembled monolayer of BPSH as adhesion promoter. Stable hydrogel films that are represented by filled circles, while the empty circles indicate hydrogel films that delaminate from the substrate.

Variation of carboxymethylation conditions

The obtained degree of COOH substitution by varying the temperature and time in the carboxymethylation reactions is provided in Figure S8 below:



Figure S8: Degree of COOH substitution for various times and temperatures in the carboxymethylation reaction.

¹H NMR spectroscopic characterization details

The content of benzophenone and the carboxymethyl groups were determined by ¹H NMR in D₂O. In this solvent the hydroxyl groups are invisible in the NMR spectrum due to the proton-deuterium exchange resulting in a free and easily integrable CH(1) proton signal. The overall integral of the CH(1)-protons of the dextran backbone (5.24 ppm (CH(1) α -1,3), 5.08 ppm (CH(1)' α -1,6), 4.89 ppm (CH(1) α -1,6)) was set to one and used as reference. The different CH(1) signals result from different branches present in the backbone.¹² For the determination of the degree of carboxymethylation and the benzophenone content the proton signal of the two diastereotope methyl protons of the carboxymethyl group (4.27 ppm) and the nine aromatic benzophenone protons (7.80 ppm) respectively, were integrated relatively to the CH(1) proton.



Figure S9: ¹H NMR spectra of carboxymethylated dextran (CMD) (2) with varying degree of functionalization ($d_s = 0.06$ (A) and 0.15 (B)) in DMSO. Due to the peak broadening all integrations for the determination of degree of substitution were done with NMR in D2O (Figure S10).



Figure S10: ¹H NMR spectrum of the carboxymethylated and benzophenone -derivatized dextran synthesized by the amide linkage route (compound 3).



Figure S11: ¹H NMR spectrum of the photocrosslinkable carboxymethylated dextran salt with 4-aminomethyl benzophenone, termed "ammonium salt" (compound **6**).

In order to ensure that the carboxymethylation reaction did not affect the integrity of the dextran backbone (no chain scission or crosslinking), GPC (Suprema 3000 Linear 10 μ m SN 7032913 with precolumn SN 7052133) was employed in a buffer system (0.01 M phosphate buffer + 0.0027 M KCl + 0.13 M NaCl) in order to screen the charge effect in the carboxylated dextrans, which would inflate the polymer coils and lead to reduced retention times (corresponding to an apparent increased molecular weight, see Figure S13). The GPC traces in Figure S14 (reaction temperature 50 °C) and Figure S15 (reaction temperature 60 °C) show only a minor decrease of the retention time with increasing reaction time, which indicates that the hydrodynamic radius of the polymer coil increases slightly with increasing carboxylation. These results document that the molecular weight is not affected by the carboxymethylation reaction even for extended reaction times up to 6 h.



Figure S12: GPC of the commercially available and used dextrans Dextran 200T (nominal molecular weight of 200 000 g/mol) and Dextran 2Mio (nominal molecular weight of 2 000 000 g/mol) in comparison to the GPC standards of pullulans (Pull 710000 (= 710 000 g/mol), Pull 5600, and Pull 1080) and Dextran broad (all GPC standards from PSS).



Figure S13: GPC of the commercially available dextran Dextran 200T (nominal molecular weight of 200 000 g/mol, measured in water and in buffer) in comparison to the corresponding carboxymethylated dextran CMD with a degree of COOH substitution of 0.33 in water. The buffer has only minimal effect on the dextran elution, while the CMD in water elutes substantially earlier due to charge-induced inflation of the COOH-modified polymer coil.



Figure S14: Comparison of the GPC traces (in the buffer system) of the commercially available dextran Dextran 200T and the corresponding carboxymethylated dextran CMD derivatives after various times for the carboxymethylation reaction at 50 °C. The buffer leads to a similar elution profile for the CMD derivatives as for the parent dextran.



Figure S15: Comparison of the GPC traces (in the buffer system) of the commercially available dextran Dextran 200T and the corresponding carboxymethylated dextran CMD derivatives after various times for the carboxymethylation reaction at 60 °C. Again, the buffer leads to a similar elution profile for the CMD derivatives as for the parent dextran.

IR determination of degree of COOH substitution

Besides ¹H NMR characterization also IR spectroscopy allows the determination of the degree of COOH substitution by comparison of the peak integrals of the COOH band I(CO) around 1600 cm⁻¹ and the OH band I(OH) around 3450 cm⁻¹.



Figure S16: IR spectrum of CMD with a degree of COOH substitution of 0.33.

The degree of COOH substitution can be calculated from the peak integrals I(OH) and I(CO) by the following equation, which takes into account the area ratio of 3.02 between the absorptions for the hydroxyl group and the COOH function with a maximum of three functionalization sites per sugar unit:

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$$DS = 3 \times \frac{I(CO)}{3.02 \times I(OH) + I(CO)}$$

Comparison with the NMR results show a 1:1 correspondence with the calculated degrees of substitutions for the different CMD samples.



Figure S17: Comparison of the degree of COOH substitution as calculated from the IR spectra (DS calculated) and from the ¹H NMR spectra.

Optical microscopy of gelatin powder



Figure S18: Optical micrographs of pestled gelatin showing the polydisperse particle size between several micrometers to approximately hundred micrometers.

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