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Supporting Materials and Methods

Monofunctionalized polyethylene glycols (PEGs). MonomethylPEG of molecular mass 750 and 5,000 Da and triethylamine were purchased from Aldrich and used without purification. Acryloyl chloride was purchased from Aldrich and used freshly after distillation over quinoline. Branched PEGs were purchased from Shearwater Polymers (Huntsville, AL) and functionalized at the OH terminus: 4arm-PEG, molecular mass 10,000 (nominal), 4arm-PEG, molecular mass 15,000 (nominal), and 4arm-PEG, molecular mass 20,000 Da (nominal). Divinlyl sulfone was also from Aldrich. All standard peptide synthesis chemicals were analytical grade or better and were purchased from Nova Biochem.

Synthesis of PEG derivatives. PEG-monoacrylate was prepared according to a protocol described elsewhere (1). Branched PEG vinyl sulfones were produced under argon atmosphere by reacting a dichloromethane solution of the precursor polymers (previously dried over molecular sieves) with NaH and then, after hydrogen evolution, with divinyl sulfone (molar ratios: OH 1:NaH 5:divinyl sulfone 50). The reaction was carried out at room temperature under argon with constant stirring. After the neutralization of the reaction solution with concentrated acetic acid, the solution was filtered through paper until clear. The derivatized polymer was isolated by precipitation in cold diethylether. The product was redissolved in dichloromethane and reprecipitated in diethylether (with thorough washing) two times to remove all excess divinyl sulfone. Finally, the product was dried under vacuum. The derivatization was confirmed with ^{1}H NMR ($CDCl_3$). Characteristic vinyl sulfone peaks at 6.1, 6.4, and 6.8 ppm were observed. Degree of end group conversion was found to be 95–98%.

Peptide synthesis and purification. All peptides were synthesized on solid resin by using an automated peptide synthesizer (PerSeptive Biosystems, Farmington, MA) with

standard F-moc chemistry. Hydrophobic scavengers and cleaved protecting groups were removed by precipitation of the peptide in cold diethyl ether and dissolution in deionized water. Peptides were purified by C18 chromatography (Perceptive Biosystems Biocad 700E) and analyzed by matrix-assisted laser desorption ionization--time-of-flight (MALDI-TOF) MS.

PEGylation of peptides. The matrix metalloproteinase (MMP)-sensitive peptide Ac-GCRD-GPQGIWGQ-DRCG was PEGylated in order to test the influence of PEG chains on the kinetics of proteolytic breakdown by MMPs, i.e. the test substrate in a local environment similar to that within the crosslinked hydrogel. Monomethyl PEG-acrylate of two molecular masses was used: 750 and 5,000 Da. PEGylation was performed with a high excess (50-fold) of acrylate over thiol groups to prevent multimerization. The Michael-type reaction was performed by mixing a PEG and a peptide precursor at 37°C for 1 h to assure complete reaction. Both molecules were dissolved in 0.3 M triethanolamine buffer at pH 8. After reaction, the PEGylated peptides were purified by preparative HPLC (Perceptive Biosystems Biocad 700E) on a C_{18} column, lyophilized, and the molecular masses were measured using MALDI-TOF MS.

Kinetic measurements of hydrogel degradation by MMP-1. Degradation of hydrogels by MMP-1 (40 nM, 30°C) was determined by measuring the volume change during degradation and by using a modified version of the fluorescamine assay described above. Fluorescamine reaction of quenched and partially degraded hydrogel samples were conducted after nonenzymatic degradation of gels, i.e., cleavage of the ethyl sulfone linkage by addition of 1 M NaOH and incubation at 70°C for 3 h.

Cell culture and 3D cell invasion. Human foreskin fibroblasts (HFF) (neonatal normal human dermal fibroblast; Clonetics) were grown in fibroblast cell culture medium (DMEM, with 10% FBS and 1% antibiotic-antimycotic, GIBCO/BRL, Life Technologies, Grand Island, NY) at 37°C and 5% CO_2. Cells were removed from culture substrates by using trypsin/EDTA (GIBCO/BRL), centrifuged at 500 × *g* for 5 min, and

resuspended in culture medium. For invasion experiments, cells were used at passages 10-14.

Cell-induced proteolytic activity of the gels was studied by a previously described cell invasion assay. Two microliters of droplet cell-loaded fibrin clots (3×10^7 cells per ml) were embedded within 10 μl PEG-based hydrogels by placing clusters into precursor solution prior to gelation (n = 9–12 per condition). Samples were cultured in serum-containing DMEM in the 12-well tissue culture plates for up to 20 d. Cell invasion from the cluster was quantified by measuring the area of the original HFF-fibrin cluster in the center plane, as was the area of the HFF outgrowth, defined by the tips of the HFF branches in the center plane of focus. These two areas were approximated as circular areas, and their theoretical radii subtracted from each other to give an average HFF invasion length. For all sets of data, three independent experiments were performed. Cells were imaged by inverted phase contrast microscopy (Zeiss Axiovert 135) and confocal scanning laser microscopy, which was performed with a HCPLAPO ×10/0.4 N.A. objective by using a MRC 600 confocal system (Bio-Rad). Cell nuclei were stained with 4',6-diamidino-2-phenylindole, F-actin of the cytoskeleton with rhodamine-phalloidin. Twenty sections were taken at 23-μm intervals, and a composite image was assembled by using IMARIS (Bitplane, Zurich) image-processing software on an Indigo2 extreme Silicon Graphics Workstation (Silicon Graphics, Mountain View, CA).

Gel degradation behavior. Under the assumption of a pure bulk degradation mechanism and zero-order kinetics, a model for the degradation behavior [based on the model developed by Metters *et al*. (2)] was applied to fit to the experimental results. The individual MMP substrates within the gel are cleaved according to the zero-order kinetic equation:

$$\frac{d[S]}{dt} = -k_{cat}E \qquad [2],$$

and hence, $[S]_t = [S]_o - k_{cat}Et \qquad [3].$

$[S]_o$ is the concentration of substrate within the intact gel. The limit where degradation of substrates has advanced sufficiently, such that the gel can no longer form a network, can be calculated by using a statistical network formation model such as the one derived by Macosko and Miller (3) in a reversed sense. Hence, we can look at this process as the opposite of gel formation (2). The fraction of intact (noncleaved) substrates within the gel as a function of time, $X_{(t)}$, can be calculated as:

$$X_{(t)} = \frac{[S]_{(t)}}{[S]_o} = 1 - \frac{k_{cat} E t}{[S]_o} \qquad [4].$$

Following the Macosko and Miller theory (3), the extent of reaction at the gel point of cross-linking polymerizations can be calculated according to:

$$X_c = \frac{1}{\left[r\left(f_{vinylsulfone} - 1\right)\left(f_{thiol} - 1\right)\right]^{1/2}} \qquad [5].$$

with $f_{\text{vinylsulfone}}$ and f_{thiol} as the average functionality for the PEG macromer and peptide, respectively, and r the stoichiometric ratio of the two functional groups. The conversion of crosslinks is less than or equal to the critical conversion needed to form an infinite gel; in other words, dissolution occurs when $X_{(t)}$ is less than or equal to X_c, respectively. By solving Eq. **4** for the time t_c at which $X_{(t)}$ passes through X_c and combining with Eq. **5**, the time at gel breakdown (t_c) can be predicted:

$$t_c = \frac{\left(1 - X_c\right)}{k_{cat} E}[S]_o = \frac{\left(1 - \frac{1}{\left[r\left(f_{vinylsulfone} - 1\right)\left(f_{thiol} - 1\right)\right]^{1/2}}\right)}{k_{cat} E}[S]_o \qquad [6]$$

Hence, t_c is a function of the network architecture ($f_{vinylsulfone}$ and f_{thiol}), the substrate concentration (i.e., the crosslinking density, as seen through $[S]_o$), the kinetic constant (k_{cat}), and the enzyme concentration (E).

1. Elbert, D. L. & Hubbell, J. A. (2001) *Biomacromolecules* **2,** 430–441.

2. Metters, A. T., Bowman, C. N. & Anseth, K. S. (2000) *J. Phys. Chem. B* **104,** 7043–7049.

3. Macosko, C. W. & Miller, D. R. (1976) *Macromolecules* **9,** 199–206.