

# Proteolytically Degradable Hydrogels with a Fluorogenic Substrate for Studies of Cellular Proteolytic Activity and Migration

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We have developed proteolytically degradable hydrogels with covalently immobilized fluorogenic protease substrates to visualize extracellular proteolytic activity and cell migration in three dimensions. Dye quenched-bovine serum albumin (DQ-BSA), a quenched, proteolytically activated fluorogenic substrate, was conjugated to poly-(ethylene glycol) (PEG)-monoacrylate, and the product (DQ-BSA-PEG) was then covalently incorporated into proteolytically degradable and cell adhesive PEG hydrogels via photopolymerization. The DQ-BSA-PEG substrate in solution and incorporated into hydrogels exhibited significantly enhanced fluorescence after exposure to enzymes. Fibroblasts seeded within this hydrogel spread in three dimensions and extended lamellipodia. Cell migration and proteolytic activity were visualized using confocal microscopy. Proteolytic activity was concentrated near cell surfaces and remained present in the tracks where cell migration had occurred.

## Introduction

Cell migration is an important biological function that impacts such processes as embryonic development, wound healing, tumor metastasis, and chronic inflammation (1). Two factors that critically affect migratory behavior are cell surface adhesion receptors and proteolytic enzymes such as the matrix metalloproteinases (MMPs). The former mediates adhesive interactions with the extracellular matrix (ECM) (2), whereas the latter is involved in the degradation and remodeling of the ECM (3).

The concept of cell migration is generally based on three interdependent phases (2, 4). First, migrating cells protrude the leading edge (e.g., lamellipodia, filopodia) for adhesive cell-substratum interaction, which is mediated by multiple cell adhesion receptor families. Next, a gradient of binding and traction forces is generated by differential distribution of actin bundles between the front and rear of cells, followed by contraction of the cell body. Finally, migrating cells release or detach the rear end of their cell body from the substratum and propel forward. Pericellular proteolysis of the ECM component and cell surface molecules regulates cell migration by aiding detachment and by degrading and remodeling ECM to create pathways for migration. Classes of proteinases known to be involved in cell migration are cysteine proteinases, serine proteinases, and MMPs (5). Thus, proteolysis is highly intertwined with cellular migration, and understanding proteinases is critical to delineate the mechanisms behind cellular migration. Proteinases also play important roles in many pathological processes such as cancer, hepatitis, and HIV (6–8).

To investigate MMP activity in solution, various fluorescent substrates that are able to report proteolytic activity have been used (9–13). Among these substrates, DQ-BSA, a highly quenched fluorescent derivative of bovine serum albumin (BSA), has been extensively used

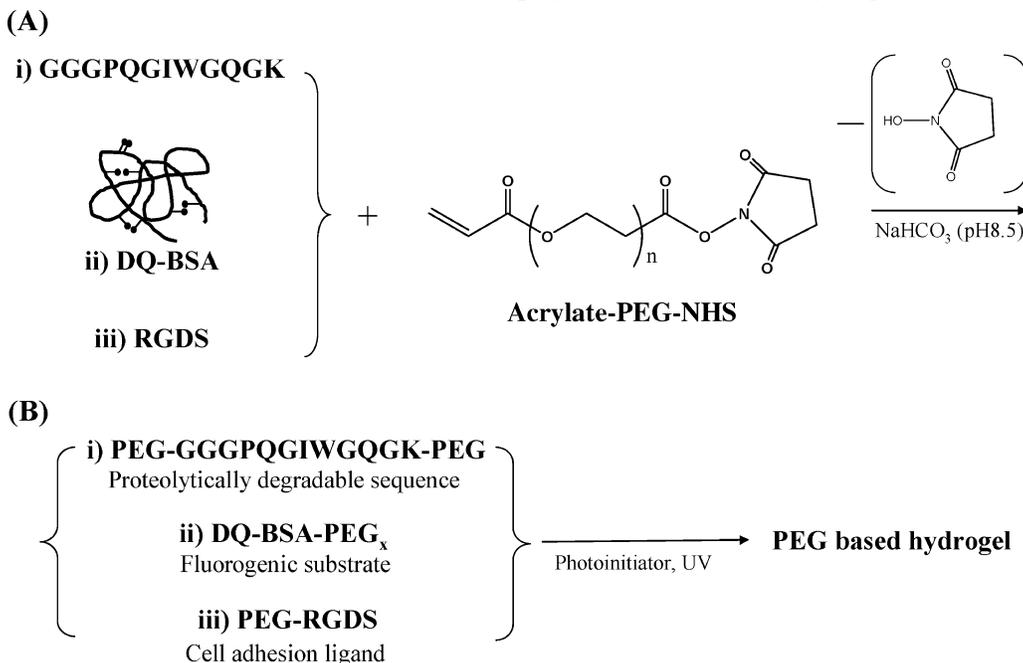
due to its dramatic increase in fluorescence intensity following proteolytic degradation (14–17). The fluorescence of DQ-BSA is largely quenched by several adjacent Bodipy dyes until DQ-BSA is hydrolyzed by proteases, producing fluorescent products (18). Kindzelskii et al. visualized neutrophil locomotion on the surface of gelatin hydrogels mixed with Bodipy-BSA and dihydrotetramethylrosamine (14). They demonstrated that migrating neutrophils showed an alternating pattern of proteolytic and oxidative functions. However, use of their system is limited to short term studies (minutes to perhaps hours) using rapidly migrating cells such as neutrophils because the fluorogenic substrate is noncovalently mixed into the gelatin gel and thus rapidly diffuses out.

We have previously reported the development of proteolytically degradable hydrogels as an ECM-mimetic scaffold that is degraded in response to cellular proteases such as the MMPs (19, 20). When cells are seeded in these materials, they can degrade the hydrogel scaffolds proteolytically as they would the ECM. Recently, Kim et al. have reported a similar approach, introducing a MMP degradable peptide into poly(*N*-isopropylacrylamide-*co*-acrylic acid) hydrogels (21).

In our ECM-mimetic materials, we have targeted degradation by MMPs because MMPs play a critical role in ECM remodeling and cell migration. The peptide that was incorporated into the polymer backbone, GPQGIWGQG, is a MMP-sensitive sequence that can be degraded by several MMPs including collagenase I, collagenase II, stromelysin I, matrilysin, and gelatinases (22). The GPQGIXGQG (X = arbitrary amino acid) contains the four amino acid residues surrounding the scissile bond in the collagenase cleavage site in the  $\alpha$ 1(I)-chain of collagen. The GPQGIWGQG peptide has been reported as the most rapidly degradable sequence among GPQGIXGQG motif peptides (22).

In this study, we sought to investigate cell migration in more detail by visualizing proteolytic activity in a biomimetic hydrogel. DQ-BSA was modified by conjugation to acrylated PEG chains, which allowed the fluores-

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**Scheme 1. Reaction for PEG Modifications (A) and Photopolymerization for PEG hydrogel (B)**

cent substrate to be covalently incorporated into hydrogels. We have shown that covalent modification of DQ-BSA with our hydrogel precursors still allowed proteolytic degradation of DQ-BSA by proteinase K, plasmin, and collagenase as determined by measuring fluorescence intensity of the solution. We have also demonstrated proteolytic susceptibility of polymerized hydrogels from these precursors by measuring fluorescence of the hydrogels during incubation with enzymes. Finally, by encapsulating fibroblasts within these hydrogels, we were able to spatially localize proteolytic activity and visualize cell migration through our proteolytically degradable hydrogels.

### Materials and Methods

**Synthesis of the PEG-GGGPQGIWGQGK-PEG and PEG-RGDS Conjugates.** The proteolytically degradable peptide sequence GGGPQGIWGQGK was synthesized using a 431A solid-phase peptide synthesizer (Applied Biosystems, Foster City, CA). This peptide has been shown to be degraded by MMPs (22, 23). The peptide was reacted with acrylate-PEG-*N*-hydroxysuccinimide (acrylate-PEG-NHS 3400 Da; Nektar, Huntsville, AL) at a 1:2 (peptide/PEG) molar ratio in 50 mM sodium bicarbonate buffer (pH 8.5) for 2 h at room temperature (Scheme 1A). The product was dialyzed (MWCO 5 000, Spectrum Laboratories, Inc., Rancho Dominguez, CA), lyophilized, and stored frozen under argon until use. The reaction between proteolytically degradable peptide sequence (GGGPQGIWGQGK) and acrylate-PEG-NHS yielded a proteolytically degradable difunctional photoreactive cross-linker where the peptide is flanked on each end by an acrylate-terminated PEG chain. The adhesive peptide RGDS (American Peptide, Sunnyvale, CA) was reacted with acrylate-PEG-NHS in a 1:1 (peptide/PEG) molar ratio as described above to give acrylate-PEG-RGDS. The conjugation of products were analyzed by <sup>1</sup>H NMR (Advance 400, Bruker, Germany) and gel permeation chromatography (GPC, Polymer Laboratories, Amherst, MA) with UV-vis and evaporative light scattering detectors.

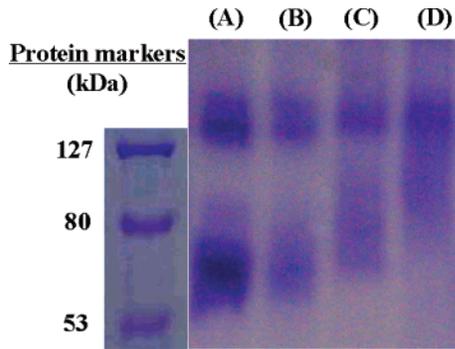
**Synthesis of the DQ-BSA-PEG Fluorogenic Substrate.** DQ-BSA (Molecular Probes, Eugene, OR) was

modified with a 2, 20, or 50 molar excess of acrylate-PEG-NHS by the same method as described above. <sup>1</sup>H NMR in D<sub>2</sub>O was used to analyze the conjugated products. Additionally, SDS-PAGE with a 10% polyacrylamide gel and Coomassie blue staining (Bio-Rad, Hercules, CA) were used to assess the DQ-BSA-PEG conjugation.

**Photopolymerization of Hydrogels.** Hydrogels were formed by combining 0.1 g/mL PEG-GGGPQGIWGQGK-PEG with or without 10 μg/mL fluorogenic substrate (DQ-BSA-PEG) in 10 mM HEPES buffered saline (HBS, pH 7.4). The solution was sterilized via filtration (MILLEX GP filter, 0.22 μm PES membrane, Millipore Corp., Bedford, MA). 2,2-Dimethoxy-2-phenyl acetophenone in *N*-vinylpyrrolidone (300 mg/mL, 10 μL/mL of polymer solution) was added as a photoinitiator. The solution was then exposed to long wavelength UV light (365 nm, 10 mW/cm<sup>2</sup>) to convert the liquid prepolymer into a hydrogel (20, 24).

**Proteolytic Degradation of PEG-GGGPQGIWGQGK-PEG Hydrogel.** A 100 μL portion of PEG-GGGPQGIWGQGK-PEG solution (0.1 g/mL in HBS) was placed in a 96-well plate and photopolymerized as described above. Resulting hydrogels were removed from the 96-well plate, weighed, and then allowed to swell in HBS with 1 mM CaCl<sub>2</sub> and 0.2 mg/mL sodium azide at 37 °C for 24 h. Each hydrogel sample was then incubated at 37 °C with HBS and 0.2 mg/mL proteinase K, 0.2 mg/mL collagenase I or 0.2 mg/mL plasmin, respectively. Degradation was evaluated by monitoring changes in the wet weight of hydrogels over time. The enzyme solution was refreshed every 24 h.

**Proteolytic Degradation of the DQ-BSA-PEG Substrates.** For degradation assays in solution, 10 μg/mL solution of the DQ-BSA-PEG<sub>50</sub> fluorogenic substrate in HBS was used. For hydrogel degradation assays, PEG-GGGPQGIWGQGK-PEG hydrogels were also prepared as described above with the addition of 10 μg/mL of the DQ-BSA-PEG<sub>50</sub> fluorogenic substrate before photopolymerization (Scheme 1B). We have verified that the photopolymerization conditions do not alter Bodipy fluorescence (data not shown). Proteinase K, plasmin or collagenase I (0.02 or 0.2 mg/mL) was added to the substrate solution and hydrogel, and each sample was incubated



**Figure 1.** Coomassie staining analysis of unmodified DQ-BSA and PEG-conjugated DQ-BSA: (A) DQ-BSA, (B) DQ-BSA-PEG<sub>2</sub>, (C) DQ-BSA-PEG<sub>20</sub>, (D) DQ-BSA-PEG<sub>50</sub> (subscript denotes the molar excess of PEG used for conjugation)

at 37 °C. Fluorescence of the solution and hydrogel was monitored over time using  $495 \pm 20$  nm excitation and  $528 \pm 20$  nm emission (FLx 800 microplate fluorescence reader, Bio-Tek Instrument, Inc., Winooski, Vermont).

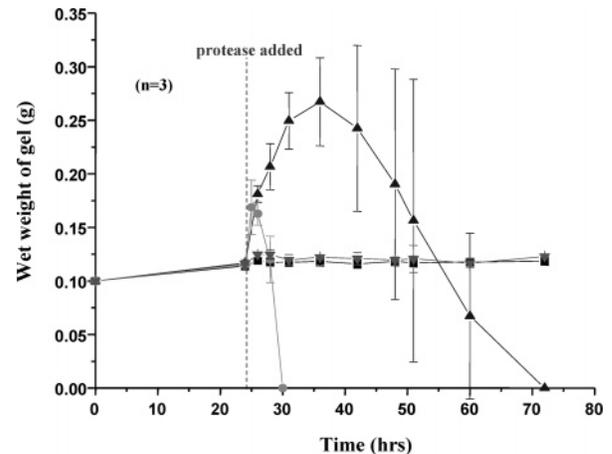
**Cell Maintenance.** Human dermal fibroblasts (HDFs; Clonetics, San Diego, CA) were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Bio-Whittaker, Walkersville, MD), 2 mM L-glutamine, 500 U penicillin, and 100 mg/mL streptomycin. Fibroblasts were incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. All experiments were conducted using fibroblasts at passage 3–6.

**Preparation of Hydrogels with Encapsulated Cells.** HDFs (500,000 cells/mL) were seeded inside the DQ-BSA-PEG<sub>50</sub> substrate hydrogel modified with PEG-RGDS (3.5 μmol/mL) by mixing cells with the hydrogel precursor solution, followed by photopolymerization (20). Hydrogels were allowed to swell in DMEM and were incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

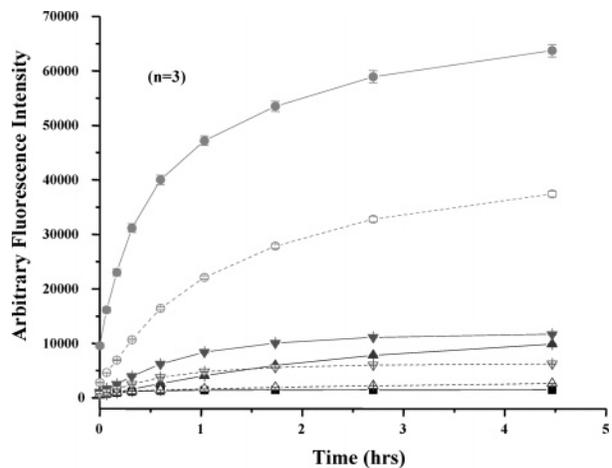
**Confocal Microscopy of Cells within Hydrogels.** Differential interference contrast (DIC) and fluorescence images of cells within hydrogels were acquired with an LSM 510 META confocal microscope (Carl Zeiss Inc., Oberkochen, Germany). The high degree of spectral overlap between the Bodipy fluorophore in DQ-BSA and fibroblast autofluorescence necessitated their separation by means other than physical band-pass filtering (25). This was achieved with the META detector, which can capture the entire spectral emission of the sample. At each time point, the "spectral fingerprints" of control samples of DQ-BSA hydrogels without cells or hydrogels with cells but lacking DQ-BSA were collected with the META detector from 520 to 710 nm. The META detector was then used to collect the spectral emission of the hydrogels containing both cells and DQ-BSA. The spectral fingerprints of each signal were used to linearly unmix the two signatures in combined samples. Following successful unmixing, the two signals were visually distinguished by assigning false colors to the emission data: green for the Bodipy fluorescence from DQ-BSA and red for fibroblast autofluorescence. Thus, this unmixing and false color assignment allowed the localization of each source of fluorescence within each image (25).

## Results

**Characterization of PEG-Modified Materials.** <sup>1</sup>H NMR analysis demonstrated that acrylate-PEG-NHS was successfully conjugated to GGGPQGIWGQGK and RGDS, respectively. These conjugates showed the methylene



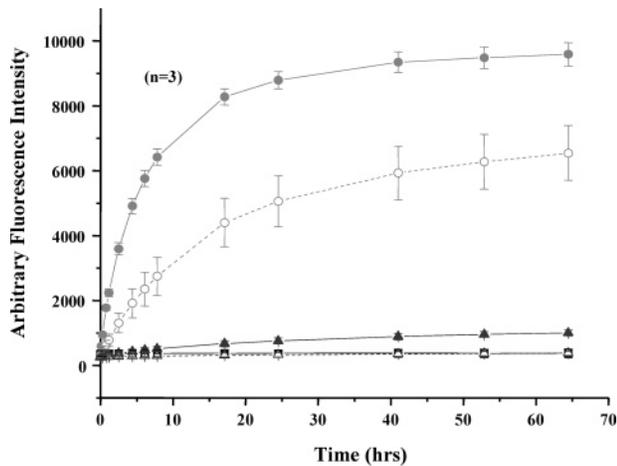
**Figure 2.** Degradation profiles of GGGPQGIWGQGK-derivative PEG hydrogels in enzyme solution: (●) 0.2 mg/mL proteinase K, (▲) 0.2 mg/mL collagenase I, (▼) 0.2 mg/mL plasmin, (■) no protease.



**Figure 3.** Increase in fluorescence with time mediated by proteolytic digestion of DQ-BSA-PEG<sub>50</sub> substrate solution: (●) 0.2 mg/mL proteinase K, (○) 0.02 mg/mL proteinase K, (▲) 0.2 mg/mL collagenase I, (△) 0.02 mg/mL collagenase I, (▼) 0.2 mg/mL plasmin, (▽) 0.02 mg/mL plasmin, (■) no protease.

protons of PEG as a triplet at 3.6–3.7 ppm, as well as the acrylate protons at 6.0–6.5 ppm. The success of DQ-BSA conjugation with acrylate-PEG-NHS was confirmed by SDS-PAGE and Coomassie blue staining of DQ-BSA and DQ-BSA-PEG<sub>x</sub> (subscript denotes the molar excess of PEG used for conjugation) (Figure 1). Stained bands of modified DQ-BSA-PEG<sub>x</sub> were present at higher molecular weight compared to unmodified DQ-BSA. Furthermore, higher ratios of PEG/DQ-BSA used during the reaction yielded products with correspondingly higher molecular weight.

**Proteolytic Degradation of Hydrogels.** Hydrogels with the proteolytically degradable peptide GGGPQGIWGQGK were transferred to proteinase K or collagenase I solutions following equilibrium swelling in HBS (24 h, Figure 2). The incorporated sequence GGGPQGIWGQGK was selected as a proteolytic degradable peptide, which has a known MMP cleavage site between Gly(G) and Ile(I). Proteinase K has very high nonspecific proteolytic activity, whereas collagenase I is specifically targeted to degrade the GPQGIWGQG sequence (22). Initially, the wet weight increased as proteinases cleaved peptides, loosening the hydrogel network and allowing more water to penetrate. Eventually, the hydrogels were completely degraded as additional sequences were cleaved and



**Figure 4.** Increase in fluorescence with time mediated by proteolytic digestion of proteolytic degradable hydrogels containing DQ-BSA-PEG<sub>50</sub> substrate: (●) 0.2 mg/mL proteinase K, (○) 0.02 mg/mL proteinase K, (▲) 0.2 mg/mL collagenase I, (△) 0.02 mg/mL collagenase I, (■) no protease.

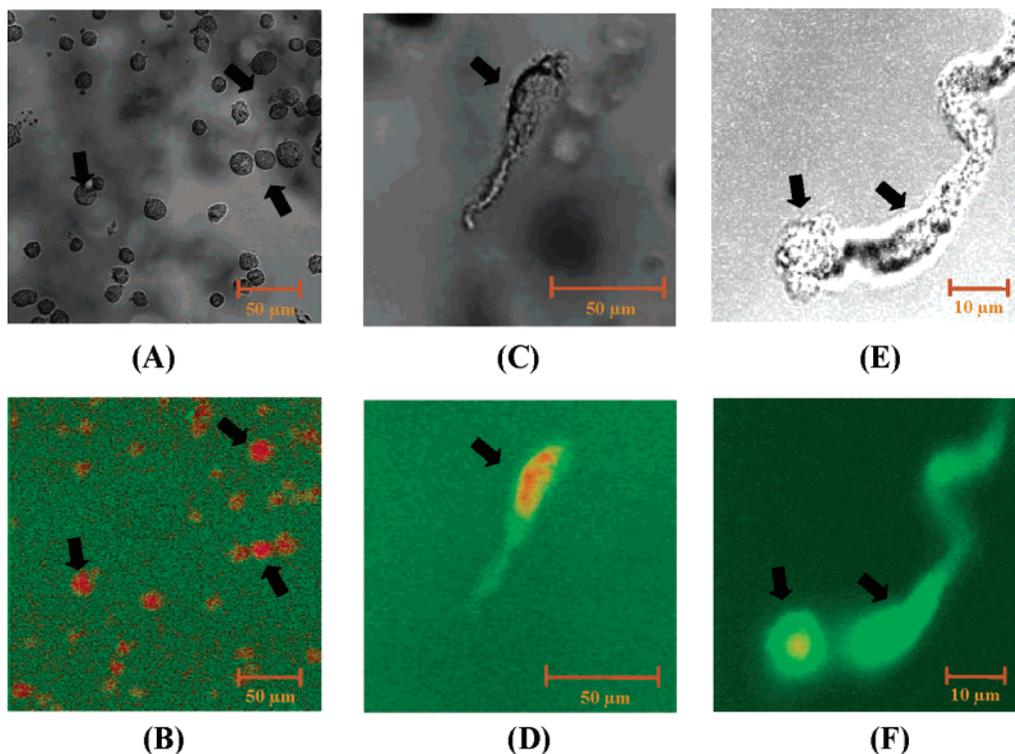
degraded moieties diffused out of the hydrogel (Figure 2). Proteinase K showed much stronger proteolytic activity compared to collagenase I, as expected, as evidenced by an accelerated hydrogel degradation profile. As shown in Figure 2, proteinase K (0.2 mg/mL) completely degraded hydrogels in 6 h while the same concentration of collagenase I took 48 hr. Meanwhile, plasmin did not degrade hydrogels as the GPQGIWGQG sequence is not sensitive to plasmin-mediated proteolysis. Hydrogels left in HBS alone did not significantly change in weight throughout the course of the experiment.

**Proteolytic Degradation of DQ-BSA-PEG Substrate.** Figure 3 shows the comparative results of proteolytic degradation of DQ-BSA-PEG<sub>50</sub> in solution using

proteinase K, plasmin, and collagenase I. Fluorescence intensity increased over time depending on the concentration and type of enzymes used. Degradation of the BSA with 0.2 mg/mL proteinase K increased fluorescence by 43-fold, whereas plasmin and collagenase I yielded lower values of 8- and 6.7-fold, respectively. This is expected since proteinase K cleaves many peptide sequences but plasmin and collagenase I are sequence-specific.

Having tested degradation and fluorescence enhancement of DQ-BSA-PEG<sub>50</sub> in solution, we investigated whether the substrate incorporated into a hydrogel system would exhibit similar degradation and fluorescence enhancement profiles. Hydrogels containing DQ-BSA-PEG<sub>50</sub> and the degradable peptide sequence along the backbone linker (PEG-GGGPQGIWGQGK-PEG) were also degraded with proteinase K and collagenase I. Proteinase K and collagenase I actively degraded DQ-BSA-PEG<sub>50</sub> substrate hydrogels and increased their fluorescence (Figure 4). As shown previously with the substrates in solution, proteinase K actively degraded DQ-BSA-PEG<sub>50</sub> incorporated into hydrogels and produced fluorescence stronger than that of collagenase I due to its much stronger and nonspecific proteolytic activity.

**Confocal Imaging of Fibroblasts in DQ-BSA-PEG Substrate Hydrogels.** Fibroblasts were encapsulated within these fluorogenic hydrogels to visualize cellular proteolytic activity. To differentiate extracellular proteolytic activity from intracellular autofluorescence, a META detector was used to collect the spectral fingerprint of fibroblasts and DQ-BSA-PEG. Using linear unmixing with the META detector, autofluorescence and DQ-BSA-PEG spectra were assigned false red and green colors, respectively, to aid in visualization and colocalization. Immediately after encapsulation, fibroblasts exhibited a rounded morphology (Figure 5A) and bright



**Figure 5.** DIC and confocal images of fibroblasts inside proteolytically degradable hydrogels containing DQ-BSA-PEG<sub>50</sub> substrate: (A) cell morphology immediately after seeding, (B) fluorescent images in panel A, (C) cell morphology after 7 days, (D) fluorescent image of cells in panel C, (E) cell morphology after 9 days, and (F) fluorescent image of cells in panel E. Green and red false colors illustrate the fluorescence acquired from DQ-BSA-PEG substrate and fibroblasts, respectively (Arrows indicate fibroblasts).

green fluorescence was not observed at the surface of cells compared to the background (Figure 5B). However, after 7 days, the fibroblasts inside the hydrogel extended many processes (Figure 5C). Bright green fluorescence was observed at the surface of cells (Figure 5D), suggesting that proteolytic enzymes secreted from fibroblasts were causing localized hydrogel degradation. Similar findings were also observed at 9 d (Figure 5E and 5F). These results demonstrate that DQ-BSA-PEG substrate hydrogel is a suitable system that allows one to investigate proteolytic activity and trace cell migration for more than a week.

### Discussion

In previous studies, we developed a biomimetic hydrogel system based on acrylated derivatives of PEG to investigate cell migration and tissue formation. This biomimetic hydrogel system was fabricated from acrylate-PEG-modified precursors containing cell adhesive peptides (in this case RGDS) and proteolytically degradable peptide sequences, which were cross-linked into the hydrogel during photopolymerization (26, 27). In this system, proteolytically degradable peptide sequences in the polymer backbone are subject to degradation by specific enzymes involved in cell migration. Moreover, migration of cells in this system can be further manipulated by immobilization of growth factors, either at a constant concentration or as a gradient (28).

DQ-BSA, a derivative of bovine serum albumin (BSA) labeled with the Bodipy fluorophore, has been extensively used because of its drastic changes in fluorescence intensity before and after proteolytic degradation. For examples, Petty et al. used gelatin gels mixed with DQ-BSA substrate to visualize neutrophil locomotion over a period of 2.7 min (14). Sameni et al. also used DQ-BSA substrate simply mixed into gelatin to image proteolysis by living breast cancer cells (16). However, the mixed DQ-BSA substrate used in these studies is not suitable to investigate proteolytic activity and cell migration on hydrogels for relatively long terms because fluorophores are able to diffuse out as the substrates are degraded. Thus, we covalently immobilized DQ-BSA to PEG hydrogel scaffolds.

In this study, DQ-BSA was conjugated to an increasing number of PEG chains as the molar ratio of acrylate-PEG-NHS added to the reaction mixture was increased. Increase in the molecular weight of the resulting products was confirmed by Coomassie staining of DQ-BSA-PEG in an SDS-PAGE gel (Figure 1). For cell studies, we used DQ-BSA-PEG<sub>50</sub> instead of DQ-BSA-PEG<sub>2</sub> and DQ-BSA-PEG<sub>20</sub> to increase the degree of incorporation. Comparing the fluorescence intensity of DQ-BSA-PEG substrate after proteolysis between solution (Figure 3) and hydrogel (Figure 4), the same concentration of protease did not increase the fluorescence intensity of hydrogels as rapidly as the fluorogenic substrate in solution, suggesting that the bulk of hydrogel is inaccessible to the enzymes and that hydrogels were gradually degraded from the surface by proteases.

To observe proteolytic activity and cell migration in three dimensions, fibroblasts were encapsulated into hydrogels incorporated with DQ-BSA-PEG substrate as previously described (29). In these materials, the encapsulated cells remain viable, undergo proliferation, and secrete matrix proteins such as collagen to begin tissue formation without a loss in cell viability (20). In addition, the encapsulated cells spread with leading edges as

shown previously during cell adhesion and migration in biomimetic PEG hydrogels as well as in the ECM matrix.

### Conclusion

DQ-BSA-PEG substrate was successfully incorporated into biomimetic hydrogels and applied to study proteolytic activity and visualize cell migration. DQ-BSA-PEG substrate solution and hydrogels showed increased fluorescence intensity depending on the type and concentration of proteases. Fibroblasts in biomimetic DQ-BSA-PEG substrate hydrogels gradually spread, extended lamellipodia, and simultaneously enhanced the extracellular DQ-BSA-PEG fluorescence intensity, indicating proteolytic activity. We believe that DQ-BSA-PEG substrate hydrogels will provide a useful tool to visualize proteolytic activity of cells, track cell migration, and understand the mechanisms of cell migration in two and three dimensions.

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### References and Notes

- Birkedal-Hansen, H. Proteolytic remodeling of extracellular matrix. *Curr. Opin. Cell. Biol.* **1995**, *7*, 728–735.
- Friedl, P.; Brocker, E. B. The biology of cell locomotion within three-dimensional extracellular matrix. *Cell. Mol. Life Sci.* **2000**, *57*, 41–64.
- Cox, E. A.; Huttenlocher, A. Regulation of integrin-mediated adhesion during cell migration. *Microsc. Res. Tech.* **1998**, *43*, 412–419.
- Lauffenburger, D. A.; Horwitz, A. F. Cell migration: a physically integrated molecular process. *Cell* **1996**, *84*, 359–369.
- Wolfsberg, T. G.; Straight, P. D.; Gerena, R. L.; Huovila, A. P.; Primakoff, P.; Myles, D. G.; White, J. M. ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* **1995**, *169*, 378–383.
- Barrett, A. J.; Rawlings, N. D.; Woessner, J., J. F. *Handbook of Proteolytic Enzymes*; Academic Press: San Diego, 1998.
- Bianchi, E.; Steinkuhler, C.; Taliani, M.; Urbani, A.; Francesco, R. D.; Pessi, A. Synthetic decapeptide substrates for the assay of human hepatitis C virus protease. *Anal. Biochem.* **1996**, *237*, 239–244.
- Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer. *Science* **1990**, *247*, 954–958.
- Ashcom, J. D.; Jacobson, L. A. Self-quenched fluorogenic protein substrates for the detection of cathepsin D and other protease activities. *Anal. Biochem.* **1989**, *176*, 261–264.
- Reis, R. C.; Sorgine, M. H.; Coelho-Sampaio, T. A novel methodology for the investigation of intracellular proteolytic processing in intact cells. *Eur. J. Cell. Biol.* **1998**, *75*, 192–197.
- Grahn, S.; Ullmann, D.; Jakubke, H. Design and synthesis of fluorogenic trypsin peptide substrates based on resonance energy transfer. *Anal. Biochem.* **1998**, *265*, 225–231.
- Nagase, H.; Fields, C. G.; Fields, G. B. Design and characterization of a fluorogenic substrate selectively hydrolyzed by stromelysin 1 (matrix metalloproteinase-3). *J. Biol. Chem.* **1994**, *269*, 20952–20957.
- Neumann, U.; Kubota, H.; Frei, K.; Ganu, V.; Leppert, D. Characterization of Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH<sub>2</sub>, a fluorogenic substrate with increased specificity constants for collagenases and tumor necrosis factor converting enzyme. *Anal. Biochem.* **2004**, *328*, 166–173.
- Kindzelskii, A. L.; Zhou, M. J.; Haugland, R. P.; Boxer, L. A.; Petty, H. R. Oscillatory pericellular proteolysis and oxidant deposition during neutrophil locomotion. *Biophys. J.* **1998**, *74*, 90–97.

- (15) Jones, L. J.; Upson, R. H.; Haugland, R. P.; Panchuk-Voloshina, N.; Zhou, M. Quenched BODIPY dye-labeled casein substrates for the assay of protease activity by direct fluorescence measurement. *Anal. Biochem.* **1997**, *251*, 144–152.
- (16) Sameni, M.; Moin, K.; Sloane, B. F. Imaging proteolysis by living human breast cancer cells. *Neoplasia* **2000**, *2*, 496–504.
- (17) Voss, E. W.; Workman, C. J.; Mummert, M. E. Detection of protease activity using a fluorescence-enhancement globular substrate. *Biotechniques* **1996**, *20*, 286–291.
- (18) Haugland, R. P.; Zhou, M. Dye Labeled Polymers as Reagents for Measuring Polymer Degradation. U.S. Patent 5,719,031, 1998.
- (19) West, J. L.; Hubbell, J. A. Polymeric biomaterials with degradation sites for proteases involved in cell migration. *Macromolecules* **1999**, *32*, 241–244.
- (20) Mann, B. K.; Gobin, A. S.; Tsai, A. T.; Schmedlen, R. H.; West, J. L. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogues for tissue engineering. *Biomaterials* **2001**, *22*, 3045–3051.
- (21) Kim, S.; Chung, E. H.; Gilbert, M.; Healy, K. E. Synthetic MMP-13 degradable ECMs based on poly(*N*-isopropylacrylamide-*co*-acrylic acid) semi-interpenetrating polymer networks. I. Degradation and cell migration. *J. Biomed. Mater. Res. A* **2005**.
- (22) Parks, W. C.; Mecham, R. P. Substrate specificity and mechanisms of substrate recognition of the matrix metalloproteinases. In *Matrix Metalloproteinases*; Imper, V., Van Wart, H. E., Eds.; Academic Press: San Diego, 1998; pp 219–242.
- (23) Lutolf, M. P.; Lauer-Fields, J. L.; Schmoekel, H. G.; Metters, A. T.; Weber, F. E.; Fields, G. B.; Hubbell, J. A. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. *Proc. Natl. Acad. Sci.* **2003**, *100*, 5413–5418.
- (24) Gobin, A. S.; West, J. L. Cell migration through defined, synthetic ECM analogs. *FASEB J.* **2002**, *16*, 751–753.
- (25) Dickinson, M. E.; Bearman, G.; Tille, S.; Lansford, R.; Fraser, S. E. Multi-spectral imaging and linear unmixing add a whole new dimension to laser scanning fluorescence microscopy. *Biotechniques* **2001**, *31*, 1272, 1274–1276, 1278.
- (26) Nguyen, K. T.; West, J. L. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials* **2002**, *23*, 4307–4314.
- (27) Gobin, A. S.; West, J. L. Val-ala-pro-gly, an elastin-derived nonintegrin ligand: smooth muscle cell adhesion and specificity. *J. Biomed. Mater. Res.* **2003**, *67*, 255–259.
- (28) DeLong, S. A.; Moon, J. J.; West, J. L. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. *Biomaterials* **2005**, *26*, 3227–3234.
- (29) Mann, B. K.; West, J. L. Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds. *J. Biomed. Mater. Res.* **2002**, *60*, 86–93.

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