

Tuning Reaction and Diffusion Mediated Degradation of Enzyme-Sensitive Hydrogels

Stacey C. Skaalure, Umut Akalp, Franck J. Vernerey,* and Stephanie J. Bryant*

Department of Chemical and Biological Engineering University of Colorado

 $\sum_{i=1}^n\sum_{j$ hydrogel) and as crosslinks are cleaved, hydrogel degradation والمستملح والموالين والمواطن والروابس والإرضاع والمواطن والمستند والمستند والمستوات والمتعاطفين

a crosslinked solid polymer (i.e., ß uorescent regions) to a soluble polymer where the polymer chains rapidly diffuse out into the bath (i.e., nonß orescent regions). This critical point is correlated to the degree of network connectivity during hydrogel formation (i.e., the gel point), which has been described for an ideal network using a statistical network formation model (Equation (S2), Supporting Information)^[9] Experimentally, we observe a degradation front where the region of the hydrogel adjacent to the enzyme source is completely degraded, which advances along the length of the hydrogel, away from the

Figure 1. Characterization of hydrogel degradation in 1D. A) Three hydrogels with differing $\epsilon_{\rm c}$, with the concentrations with the their properties of: \approx enzyme concentration (Ce °),

crosslinked hydrogel case, a wide front that propagates quickly is observed resulting in an overall decrease in the crosslink density of the bulk hydrogel for Þnite distances (e.g., at 5 mm) (Figure 1 Cvi). In the intermediate and high crosslink hydrogel cases, a sharp front is observed and the initial bulk hydrogel properties are largely maintained (Figure 1 Cvii, viii). We demonstrate that the model is able to capture the propagating front and front width, especially for the intermediate and high crosslink hydrogel cases (Figure S4, Supporting Information). However due to limitations in the experimental set-up, the ß uorescence in the low crosslinked hydrogel does not directly correlate to crosslink density and therefore the front width could not be matched to the model (see Figure S4, Supporting Information). Nonetheless, the model can be used to describe the spatiotemporal changes in crosslink density for each of the three hydrogel cases.

 We next extended the experimental and computational analysis to 3D, using a low and high crosslinked hydrogel with formulations given in Table S1 and initial properties in Figure 2A. Experimentally, we developed a Òcell-mimeticÓ platform (Scheme 1C) using collagenase-loaded PLGA microparticles encapsulated in the enzyme-sensitive PEG hydrogel. Prior to encapsulation, release of collagenase from the microparticles was characterized by a rapid burst release followed by a slow yet sustained release of enzyme

www.advhealthmat.de

Figure 2. Characterization in 2D. A) Two hydrogels with the same microparticles with the same micropartic of initial volumetric substitution $(\mathsf{Q})_{p_0, p_1, p_2}$ interaction parameter $(\mathsf{E})_{p_0, p_1}$ as $(\mathsf{E})_{p_0, p_2}$ as $(\mathsf{E})_{p_1, p_2, p_3}$ as $(\mathsf{E})_{p_1, p_2, p_3}$ as $(\mathsf{E})_{p_2, p_3}$ determined by the model, and initial crosslink density in the sweet μ , phosphate buffered saline buffered salines (i.e., phosphate buffered salines of $\left(\frac{1}{N}\right)$ $\left(\begin{array}{c} 0 & x \\ y & y \end{array} \right)$ ($\left(\begin{array}{c} x \\ y \end{array} \right)$. $\frac{1}{x}$. In experiments, the set-up s described in Scheme 1 D was used with a fl uorescently labeled (red) hydrogel. In simulations, the enzyme radius and Michaelis–Menten kinetic constants were used and the 1D experiments were used red. B) Degradation of the low crosslink hydrogel is show $r_{\rm{c}}$ representative conformation void spaces (i), $r_{\rm{c}}$ at $r_{\rm{c}}$ the overall time, the overall time, the overall uorescence (ii) decreased as well as the compressive modulus (iii) for the hydrogel containing the collagenase-loaded microparticles. Hydrogels with BSA-loaded microparticles showed no change in overall fl uorescence (ii) and a slow decrease in the compressive modulus (iii), corresponding to degradation of the microparticles. Simulation results matched experiments showing a diffuse front surrounding the microparticles (iv) and a rapid loss in the compressive modulus (v). C) Degradation of the high crosslink hydrogel is shown by representative confocal microscopy images of the hydrogen with vital with visit spaces (i), which at 0 h correspond to the microparticles. Over time, the void
The over time, the overall flat uorescence was maintained, but the void spaces was maintained, but the void sp became signifi cantly larger in the hydrogel containing the collagenase-loaded microparticles, as shown in the histogram plot (ii). Hydrogels with BSA-loaded microparticles showed no change in the size of the voids, as shown in the histogram plot (iii). Statistical analysis was performed using a nonparametric $\lfloor \frac{1}{2} \rfloor$ decreased ($\lfloor = 0.05, n = 800, 1300 \rfloor$ decreased slowly over time in the hydrogels (iv) decreased slowly over time in the hydrogels over time in the hydrogels over time in the hydrogels (i with the collagenase-loaded microparticles, but no change was observed in the BSA-loaded microparticles. Simulation results matched experiments showing a relatively sharp front surrounding the microparticles with a slight loss in hydrogel crosslinking (v) and correspondingly a slow loss in the compressive modulus (vi) over time.

Figure 3. A) و التوسط المعروفي المعروفي المعارضين المعارفين المعارفين المعارفين المعارفين المعارفين المعارفين
والتوسط المعارفين المعارفين المعارفين المعارفين المعارفين (Fickis second law) and reaction (Fickis second law)
 (Michaelis–Menten kinetics) for enzyme-sensitive hydrogels. These four quantities are based on initial hydrogel properties, enzyme characteristics, and enzyme-substrate kinetics. The parameters and table μ and μ of contour plots for front velocity (in mm h μ μ) An example of contour plots for front velocity (in mm h μ) and from the case with the form and e for the case with a second with a function of and characteristic length
(mm) as a function of and characteristic length of 2 mm, and characteristic length of 2 mm. A second of 2 mm.
(m

(Figure S5, Supporting Information). This release proÞle, as a degradation appeared to be restricted more locally in the region function of time, was input into the computational model to immediately surrounding the cell-mimetic. This observation is demonstrate the modelÖs capability of incorporating complex upported by the following results: (a) the hydrogel unescence enzyme release proÞles. Hydrogels were encapsulated withwas largely maintained over time, but the size of the nonßreseither collagenase-loaded microparticles or BSA-loaded micro-cent regions (originally correlating to the microspheres, which particles and the spatiotemporal degradation behavior and exhibited a distribution of sizes) increased statistically with time macroscopic properties were evaluated over time. In the low (Figure 2CiĐiii), (b) the compressive modulus decreased over crosslinked hydrogel, bulk degradation was evident by a rapid time, but the change was gradual (Figure 2Civ), and (c) hydrogel and overall decrease in hydrogel ßuorescence (Figure 2Bi,ii).wet weights were maintained over time (Figure S6, Sup-Further, the compressive modulus (Figure 2Biii) decreased expo-porting Information). Simulations showed similar results with nentially while the hydrogel wet weights (Figure S6, Supporting a relatively sharp boundary surrounding the cell-mimetic and a Information) increased over time, consistent with the occurrence modulus that gradually decreased over time (Figure 2Dv,vi). In of bulk degradation. Simulations showed similar madings with addition, dual labeling of the hydrogel and enzyme qualitatively respect to a diffuse boundary surrounding the cell-mimetic and showed the spatiotemporal distribution of the hydrogel and a rapid loss in the compressive modulus of the bulk hydrogel enzyme over time, further supporting the above observations (Figure 2 Biv,v). On the contrary in the high crosslink hydrogel, (Figure S7, Supporting Information).

degradation with enzyme transport. Due to the strong nonlinearity of the system, we sought a numerical solution of these equations with a fi nite-element procedure relying on a Newton–Raphson approach and an implicit, backward-Euler algorithm for time integration. A convergence analysis of the method was performed for both the 1D and 3σ and allowed us to select approximate time step size and discretization that ensured maximum accuracy and low computational cost. Algorithms were written in Matlab.

Supporting Information

 Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

 S . S.C.S. and U.A. contributed equality to this work. S.J.B. and F.J.V. a_{i_1} a_{i_2} a_{i_3} a_{i_4} a_{i_5} a_{i_6} a_{i_7} a_{i_8} a_{i_9} a_{i_1} a_{i_2} a_{i_3} a_{i_4} a_{i_5} a_{i_7} a_{i_8} a_{i_9} a_{i_1} a_{i_2} a_{i_3} a_{i_4} a_{i_5} a_{i_7} a_{i_8} a_{i_9

- $R \sim \mu \sim \sigma \sim \sqrt{1 \sigma^2 / 201}$ R_{R} evised: R_{R} 1, 2015 \sim Published online: $\Pi_{\text{em}} = 1$, 201
- [1] M. R. Lutolf , F. E. Weber , H. G. Schmoekel , J. C. Schense , $T: \mathbb{R}^2 \to \mathbb{R}^2$, $\mathbb{R}^2 \to \mathbb{R}^2$, $\mathbb{R}^2 \to \mathbb{R}^2$, Nat. Biotechnol. 2003, 21, 13.
- $[2]$ a) C. S. Bahney , C. S. Bahney , C. Hsu , J. L. U. Hsu , J. Hoo , J. J. J. J. V. J. J. V. J. J. V. J. J. J. J $FASEB$ $2011, 25, 1'$, \supseteq) \supseteq , \supseteq $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, 2, \overline{S}) $\left[\cdot, \frac{1}{2}, \cdots, \frac{1}{n} \right]$. $\left[\cdot, \frac{1}{2}, \cdots, \frac{1}{n} \right]$. $\left[\cdot, \frac{1}{2}, \cdots, \frac{1}{n} \right]$
- Proc. Natl. Acad. Sci. US 2015 , 112, $\sqrt{3}$. $\sum_{i=1}^{3}$ S. C. Skaalure , S. Skaalure , S. Skaalure , S. S. Skaalure , S. Skaalure Mater. 2015, 4, 20
- $\Delta \cdot \mathbf{S}_{\mathrm{max}}$, $\Delta \cdot \mathbf{S}_{\mathrm{max}}$ Biomater. Sci2014, 2, 102.
- [5] a) A. J. Keung , S. Kumar , D. V. Schaffer , in Annual Review of Cell and Developmental Biology, λ , 26 (Eds. Result λ , Respectively, Re
- $[3 \text{cm} 1 \text{cm} 1)$, 2010, $[3 \text{cm} 1 \text{cm} 1]$, $[4 \text{cm} 1]$, $[4 \text{cm} 1]$, $[4 \text{cm} 1]$ F. Ahmed 2007, 59, 132. The Ahmed Scher , Adv. Drug Delivery. Re2007, 59, 132. I $(L \rightarrow A \rightarrow B)$, $(L \rightarrow C \rightarrow A \rightarrow C)$, Cancer Cell 2005, 7, 1, . 2013