Synthesis of Enzyme-Degradable, Peptide-Cross-Linked Dextran Hydrogels

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Hydrogels derived from synthetic polymers have been previously engineered to degrade under the activity of matrix metalloproteinases (MMPs). It is believed that these systems can act as extracellular-matrix (ECM) equivalents mimicking the degradation and remodeling of the ECM through the activity of cell-secreted enzymes. In this study, MMP-sensitive hydrogels derived from dextran were developed. In order to avoid the incorporation of hydrolyzable esters often introduced in dextran modification strategies, the polysaccharide was modified with *p*-maleimidophenyl isocyanate (PMPI) thereby introducing maleimide functionalities in the backbone and resulting in dextran derivatized with *p*-maleimidophenyl isocyanate (Dex-PMPI). This strategy was favored to separate out the effects of random hydrolysis and enzymatic digestion in the degradation of the dextran hydrogels. A peptide cross-linker, derived from collagen and susceptible to gelatinase A (MMP-2) digestion, was synthesized with bifunctional cysteine termini and used to cross-link the Dex-PMPI. These hydrogels were found to be hydrolytically stable for more than 200 days yet degraded either within 30 h when exposed to bacterial collagenase or within 16 days when exposed to human MMP-2, demonstrating enzymatic-mediated digestion of the peptide cross-links. Further modification of the cross-linked hydrogels with laminin-derived peptides enhanced cell adhesion and survival, demonstrating the potential of these materials for use in tissue engineering applications.

INTRODUCTION

One aspect of tissue engineering is the development of multicomponent scaffolds that can elicit specific biological functions. Ideally, these scaffolds would act as extracellular-matrix (ECM) equivalents, mimicking the cellular environment as closely as possible. The natural ECM is not only a physical support for the cells but also plays a key role in signal transduction by presenting adhesion molecules and serving as a reservoir for other molecules, such as cytokines, that influence growth and cell function. The dynamic interactions between cell and ECM have been mimicked in scaffold design where both physical and chemical stimuli have been incorporated to guide tissue regeneration both spatially and temporally (1-4); however, these scaffolds have not been optimized for nerve repair strategies.

Most biodegradable synthetic polymers studied for tissueengineering applications, such as polycaprolactone, polylactide, polyglycolide, and poly(lactide-*co*-glycolide), rely on random ester hydrolysis of the backbone chain instead of tailoring the degradation to specific cellular activity. Over the years, advances in molecular biology have provided a better understanding of the cellular environment which has been used to design scaffolds that mimic the degradation and the remodeling of the ECM. In this environment, growth, repair, and development are controlled by cell-secreted and cell-activated enzymes, such as matrix metalloproteinases (MMPs) and plasmin. These processes are regulated through enzymatic degradation and *de novo* synthesis of ECM components, making the degradation of this natural support dynamic. Due to their involvement in tissue remodeling, the activity of these enzymes is highly localized in the cellular periphery and is tightly regulated.

MMPs, which are calcium-requiring and zinc-dependent endopeptidases, constitute one of the major families of proteinases playing key roles in the responses of cells to their environment (5). They have the ability to hydrolyze one or several components of the ECM, as well as nonmatrix proteins thereby influencing cell migration, proliferation, differentiation, and death by both modifying the cellular microenvironment and regulating the activity of biological molecules. MMPs are secreted as inactive zymogens, and their activity is highly regulated at the transcriptional and posttranscriptional level. Expressed during development (6, 7), most MMPs have been found to be produced at very low or undetectable levels in the adult central nervous system (CNS); however, they are upregulated following spinal cord injury (8, 9) and may play a beneficial role in CNS repair strategies (5, 9). MMPs are expressed in the growth cones of numerous vertebrate neurons (10-12) and may regulate axonal guidance (13-15). Muir and co-workers previously reported that MMP-2 activity facilitated neurite extension of dorsal root ganglia (DRG) neurons within a reconstituted ECM (11) and promoted axonal growth by degrading inhibitory chondroitin sulfate proteoglycans (15). MMP-2 is therefore highly relevant to the enzymatic degradation of ECM analogues designed for neuronal applications.

Hubbell and West introduced the concept of mimicking the dynamic remodeling of the ECM through the development of telechelic peptide-poly(ethylene glycol) (PEG)-peptide block copolymers which were degraded either by plasmin or bacterial collagenase (16). Different hydrogels sensitive to proteases such as MMPs (3, 4, 17) and plasmins (17, 18) have been developed for various tissue engineering applications, mimicking some fundamental aspects of cell–ECM interactions by taking advantage of the time- and location-dependent ECM degradation activity exhibited during cellular outgrowth.

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Dextran is a natural polysaccharide which consists mainly of linear chains of α -1,6 linked D-glucopyranose residues. It has been widely used in biotechnology applications (19–22), studied as a drug delivery vehicle (23–26), and more recently has been investigated as a biomaterial (27–30). Dextran hydrogels are particularly compelling as scaffolds for soft tissueengineering applications because dextran is resistant to both protein adsorption (31, 32) and cell adhesion (27, 33, 34), allowing specific cellular behavior, such as adhesion (34, 35), to be dialed into the hydrogel design. Unlike PEG, which has modifiable groups only at the termini, dextran has three hydroxyl groups on each glucopyranose repeat unit that are available for chemical modification with either cross-linking agents for hydrogel formation (36, 37) or bioactive molecules (38–41).

In previous studies, we have reported methods to create macroporous, interconnected dextran scaffolds for tissueengineering applications using glycidyl-methacrylate derivatized dextran (Dex-MA) (36) and to render these hydrogels celladhesive by covalently immobilizing ECM-derived peptides (42). To create biodegradable hydrogels, we cross-linked the dextran with MMP-2-labile oligopeptides and characterized it for degradation and cytocompatibility in vitro. Specifically, dextran was first modified with p-maleimidophenyl isocyanate (PMPI), a sulfhydryl- and hydroxyl-reactive heterobifunctional cross-linker. The isocyanate terminus of PMPI reacts with the dextran hydroxyl functional group forming a hydrolytically stable carbamate linkage and provides a maleimide moiety on the other terminus, which reacts by Michael-type addition with a sulfhydryl functionalized cross-linker to form a thioether crosslinked hydrogel. The peptide cross-linker was specifically designed to be susceptible to MMP-2 degradation and to have a cysteine residue at each terminus, allowing conjugate addition to dextran derivatized with p-maleimidophenyl isocyanate (Dex-PMPI). To gain greater insight into the specificity of the protease activity, dicysteine peptides with high and low MMP-2-sensitive peptide sequences, GCRDGPQGIASQDRCG and GCRDG-PQGPAGQDRCG, were used to create the cross-linked gels and compared in terms of enzymatic degradation. Moreover, to explore these materials for tissue engineering applications, these dextran cross-linked hydrogels were modified with celladhesive peptides, CGDPGYIGSR and CQAASIKVAV, and compared in terms of neural cell adhesion.

EXPERIMENTAL PROCEDURES

Materials and Methods. All chemicals were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON) and used as received, unless otherwise noted. Water was distilled and deionized using Millipore Milli-RO 10 Plus at 18 M Ω resistance.

Synthesis of Functionalized Dextran. Synthesis of Dex-PMPI. Dextran 40 kDa (Leuconostoc mesenteroides) was dried under vacuum and dissolved under nitrogen in 12 mL of anhydrous dimethyl sulfoxide (DMSO) at predetermined reaction temperatures (25 °C or 45 °C), after which dibutylin dilaurate (DBTDL) was added. A range of catalyst concentrations from 0 to 5.4 mM was examined. PMPI (50 mg; Pierce Chemical Co., Rockford, IL) was dissolved in 3 mL of DMSO and then added to the dextran solution. The amount of dextran used varied from a molar ratio of 0.1 to 0.4 of PMPI to dextran glucopyranose residue. The reaction was conducted in the dark for 4 and 24 h. The solution was precipitated in 70 mL of cold ethanol, washed several times with ethanol to remove any residual traces of DMSO and catalyst, dried at room temperature under nitrogen, and stored as a yellow powder at -20 °C. Proton nuclear magnetic resonance (¹H NMR) spectra were collected on a Gemini 300 MHz spectrometer (Varian Associates, Inc. NMR Instruments, Palo Alto, CA) using HOD (4.8 ppm) as the reference. ¹H NMR (D₂O): δ 7.6 ppm (d, 2H, Ar–H ortho to maleimide), 7.4 ppm (d, 2H, Ar–H ortho to carbamate), 7.0 ppm (s, 2H, maleimide vinyl), 5.0 ppm (s, 1H, anomeric proton), 5.3 ppm (s, 1H, α -1,3 linkage) 3.3–4.2 ppm (br m, 6H, glucopyranosyl ring). The degree of modification (DS) was determined according to eq 1

$$DS = \frac{I_{\rm B}}{I_{\rm A}} \times 100 \tag{1}$$

where $I_{\rm B}$ is the integrated region of PMPI around δ 7.0–7.6 ppm (6H) and $I_{\rm A}$ is the integrated area of the glucopyranosyl ring of dextran at δ 3.3–4.2 ppm (6H).

Fourier transform infrared spectroscopy (FTIR) spectra were obtained on a Nicolet Avatar E.S.P. spectrometer (Thermo Electron Co., Waltham, MA). Samples of dextran and Dex-PMPI were ground separately with KBr powder (10-fold excess relative to sample mass) and pressed into pellets for FTIR examination. OMNIC software (Thermo Electron Co., Waltham, MA) was used for data acquisition and analysis. FTIR (KBr, in cm⁻¹): dextran 3398 (s, ν_{O-H}), 2915 (s, $\nu_{C-H/C-H2}$), 1013 (s, ν_{C-OH}); PMPI 3120 (s, ν_{sym} maleimide C–H), 3088 (s, ν_{asym} maleimide C–H), 3073 (s, $\nu_{aromatic}$ C–H), 2275 (s, ν_{asym} N=C=O), 1776 (w, ν_{sym} maleimide C=O), 1719 (s, ν_{asym} maleimide C=O), 1583 (W, $\nu_{maleimide}$ C=C), 1529 (s, ν_{sym} aromatic C–C), 1447 (W, ν_{asym} aromatic C–C), 1386 (s, ν_{sym} maleimide C=O), 1519 (s, ν_{n-H}), 1232 (s, $\nu_{carbamate}$ =C–O–C–).

Typical Synthesis of Dex-PMPI DS 10. Dextran 40 kDa (378 mg) was dissolved under nitrogen in 12 mL of anhydrous DMSO at 45 °C after which 45 μ L of DBTDL was added. PMPI (50 mg) was dissolved in DMSO (17 mg/mL) and then added to the dextran solution in order to obtain a DS of 10. The reaction mixture was stirred overnight under nitrogen and in the dark. Dex-PMPI was purified as reported previously and stored at -20 °C (recovery yield: 87%, DS: 10; based on ¹H NMR).

Peptide Synthesis. Two collagen-derived peptides containing octapeptide sequences cleavable by MMP (in **bold** and \ indicates the cleavage point) and capped by a tetrapeptide containing a cysteine residue at opposite ends were used as peptide cross-linkers: Ac-Gly-Cys-Arg-Asp-Gly-Pro-Gln-Gly-Ile-Ala-Ser-Gln-Asp-Arg-Cys-Gly-COOH (GCRDGPQG\IASQ-DRCG) and Ac-Gly-Cys-Arg-Asp-Gly-Pro-Gln-Gly\Pro-Ala-Gly-Gln-Asp-Arg-Cys-Gly-COOH(GCRDGPQG\PAGQDRCG). GPQGIASQ was selected as a highly MMP-2 sensitive sequence while GPQGPAGQ was chosen for its very low sensitivity to MMP-2 (43, 44). The GCRD sequence capping the peptides was used to improve peptide solubility and to introduce thiols as previously described by Lutolf et al. (3).

For the studies of hydrogel degradation, three peptides with fluororescent labels were synthesized. Two mono-cysteine collagen-derived peptides with sequences similar to the peptide cross-linkers but containing a fluorescent 5-carboxyfluorescein (5-FAM)-L-lysine residue were synthesized: Ac-Gly-Cys-Arg-Asp-**Gly-Pro-Gln-Gly\Ile-Ala-Ser-Gln**-Asp-Arg-Gly-Lys(5-FA M)-CONH₂ (GCRD-**GPQG\IASQ**DRGK-FAM) and Ac-Gly-Cys-Arg-Asp-**Gly-Pro-Gln-Gly\Pro-Ala-Gly-Gln**-Asp-Arg-Gly-Lys(5-FAM)-CONH₂ (GCRD**GPQG\PAGQ**DRGK-FAM). A nonfunctional monothiol peptide terminated by a fluorescent 7-methoxycoumarin (Mca)-L-lysine residue, Cys-Gly-Lys(Mca) (CGK-Mca), was also synthesized.

Finally, two laminin-derived peptides containing respectively the Tyr-Ile-Gly-Ser-Arg and Ile-Lys-Val-Ala-Val motifs (in italic) were synthesized to promote cellular adhesion and neurite outgrowth: H₂N-Cys-Asp-Pro-Gly-*Tyr-Ile-Gly-Ser-Arg*-COOH (CDPGYIGSR) and H₂N-Cys-Gln-Ala-Ala-Ser-*Ile-Lys-Val-Ala*- *Val*-COOH (CQAAS*IKVAV*), where the spacer group results in a more biomimetic conformation (45).

All peptides were synthesized by solid-phase synthesis using a Pioneer Peptide Synthesis System (Applied Biosystems, Foster City, CA) with standard Fmoc/ HATU chemistry. Amino acids including Fmoc-Lys(Mca)-OH were purchased from Novabiochem (EMD Biosciences, Inc., La Jolla, CA). Fmoc-Lys(5-FAM)-OH was from AnaSpec, Inc. (San Jose, CA). Activators and resins were purchased from Applied Biosystems and N,Ndimethylformamide (DMF) was from Caledon Laboratories Ltd. (Georgetown, ON). Laminin-derived peptides and fluorophorelabeled peptides were cleaved and deprotected from the resin for 2 h, and the peptide cross-linkers were treated for 4 h as previously described (42). The sulfhydryl content of peptide cross-linkers was determined photometrically using Ellman's reagent (5,5'-dithio-bis(2-nitrobenzoic acid). Peptide crosslinkers with free thiol content higher or equal to 80% were used for the experiments.

Degradation of Soluble Peptide Cross-Linkers. The substrate specificity of MMP-2 toward the cross-linkers was determined using a previously published fluorometric assay (46). Briefly, peptides were prepared as 540 mM stock solution in degradation buffer (50 mM Tricine, pH 7.5, 200 mM NaCl, 10 mM CaCl₂, 0.05% Brij-35). Digestion assays were carried out in degradation buffer by incubating a range of substrate concentrations (40, 80, 125, 160, 250, and 340 µM) with 40 nM MMP-2 at 30 °C. At predetermined time points, 20-µL aliquots of the degradation assay solution were withdrawn and added to 30 μ L of *o*-phenanthroline (20 mM) to quench the enzymatic reaction. Rates of hydrolysis were monitored through the formation of free amines resulting from the peptide cleavage by active human MMP-2 (recombinant MMP-2; Calbiochem, San Diego, CA). Fluorescamine solution (200 μ L, 5 mM in degradation buffer solution minus Brij-35) was added to the aliquots. The formation of fluorophores by the reaction of fluorescamine with the free amino groups was quantifed by fluorimetric measurement ($\lambda_{\text{excitation}} = 387 \text{ nm}, \lambda_{\text{emission}} = 480$ nm) using a Gemini EM microplate spectrofluorometer (Molecular Devices Corp., Sunnyvale, Ca). The kinetic parameters of the peptides were determined by Michaelis-Menten analysis as reported in Supporting Information.

Hydrogel Formation. Hydrogels were formed by the conjugate addition of the sulfhydryl groups on PEG-dithiol or peptide cross-linkers to the maleimides of derivatized dextran. Dex-PMPI was dissolved in 100 mM phosphate buffered saline (PBS) pH 6.5 to give a final desired concentration (2.5, 5, 10, or 20 wt %). For example, 2.5 mg of Dex-PMPI was dissolved in 12.5 μ L PBS buffer and mixed with 1.6 mg of peptide cross-linker dissolved in 12.5 μ L of PBS buffer to make 10 wt % dextran hydrogel. The reaction solution was quickly vortexed, transferred into a mold, and placed in an incubator at 37 °C overnight.

Hydrolytic Stability of Hydrogels. *Dextran Hydrogels Cross-Linked with PEG-Dithiol.* Hydrogel samples (100 μ L) of 2.5% dextran cross-linked with PEG-dithiol were immersed in 100 mM PBS solution (pH 7.4, 0.02% sodium azide) and incubated at 37 °C with buffer exchange every 2 days. The hydrolytic stability was determined by measuring the hydrogel water content (WC) according to eq 2. The hydrogels were weighed daily to determine the hydrated mass (W_h). The dry mass (W_d) was obtained for each hydrogel by freeze-drying the hydrogels and subsequently measuring the mass.

$$WC = \frac{W_h - W_d}{W_h} \times 100\%$$
(2)

Dex-Peptide Hydrogels. Hydrogel samples (50 μ L) of 20% Dex-PMPI cross-linked with GCRD**GPQGIASQ**DRCG were

immersed in 100 mM PBS solution (pH 7.4, 0.02% sodium azide), incubated at 37 °C with weekly buffer exchanges. Dex-MA (*36*) was also cross-linked with the peptide cross-linker through Michael-type reaction to form a 20% hydrogel and was used as comparison for stability. The hydrogels were weighed periodically to determine the normalized hydrogel weight (NW_h; eq 3) in the buffer.

$$NW_{h} = \frac{W_{t}}{W_{o}}$$
(3)

where W_t is the hydrated mass at time *t* and W_o the initial mass of the hydrogel after gelation.

Enzymatic Degradation. Collagenase Degradation. Hydrogel samples (50 μ L) of 20% Dex-PMPI cross-linked with GCRD**GPQGIASQ**DRCG were placed in PBS, pH 7.4, 100 mM with 0.2 mg/mL of sodium azide and 1 mM CaCl₂ at 37 °C for 24 h at which time their hydrated mass was measured. Methacrylated dextran hydrogels obtained by redox polymerization of Dex-MA were prepared as previously described (*36*) and were used as controls. Each sample was then immersed into 1 mL of PBS solution with 0.2 mg/mL of sodium azide and either without enzyme or with 2 ng/mL of bacterial collagenase (from *Clostridium histolyticum*) and incubated at 37 °C. At predetermined time points, the hydrogels were removed from the medium and their NW_h was determined.

MMP-2 Degradation. The degradation of hydrogels was followed with both bacterial collagenase (as described above) and with MMP-2. With bacterial collagenase, degradation was followed by measuring hydrogel wet mass, which is suitable when the enzyme concentration is maintained by the use of a large volume of degradation media. For practical reasons, with MMP-2, degradation was monitored by measuring the release of fluorescent peptides in the media, which is suitable for maintaining the enzyme concentration when small degradationmedia volumes are required. To study the degradation of the hydrogel through the activity of MMP-2, two mono-cysteine fluorescent peptides, CGK-Mca and either GCRDGPO-GIASODRGK-FAM or GCRDGPOGPAGODRGK-FAM, were covalently immobilized to Dex-PMPI followed by its crosslinking using either GCRDGPQGIASQDRCG or GCRDG-**PQGPAGQDRCG** peptides, respectively. Dex-PMPI (2.5 mg) was dissolved in 25 μ L of a solution of fluorescent peptides (0.4 mM/peptide). After 10 min, 25 µL of peptide cross-linker (27 mg/mL) was added. The solution was vortexed and transferred to a mold and incubated overnight. Hydrogels were washed for 1 week in degradation buffer to remove any unreacted fluorescent peptides. They were then placed in 1 mL of degradation buffer containing 40 nM of MMP-2. The enzymatic activity was correlated to the release of the dextran-CGK-Mca ($\lambda_{\text{excitation}} = 340$ nm, $\lambda_{\text{emission}} = 405$ nm) and the fluorescein-labeled peptide fragment ($\lambda_{\text{excitation}} = 494 \text{ nm}, \lambda_{\text{emission}}$ = 521 nm), **IASQ**DRGK-FAM or **PAGQ**DRGK-FAM, into the degradation buffer solution. At predetermined time points, the release of the fluorophore was quantified by transferring 100- μ L aliquots of supernatant to a 96-multiwell opaque plate and measuring the sample fluorescence. The aliquots were returned back to the degradation buffer solution. The degradation solution was refreshed daily until complete degradation of the hydrogel

Cytotoxicity Assay. *Macromer Cytotoxicity.* The cytotoxity of Dex-PMPI was investigated. Cells were incubated in presence of soluble dextran or Dex-PMPI in the medium solution. NIH-3T3 fibroblast cells were cultured at 37 °C in culture medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (FBS), 50 units/mL penicillin/50 mg/mL streptomycin; all from Invitrogen Corp., Grand Island, NY).

Dextran and Dex-PMPI DS 10 with Mw of 6 kDa and 40 kDa were added to medium at the following concentrations: 10, 1, 0.1, 0.01, 0,001, and 0 mg/mL dextran and 10, 1, 0.1, 0.01, 0,001, 0.0001, and 0 mg/mL Dex-PMPI. Cells were plated on collagen-coated 96-multiwell black-wall clear-bottom Costar plate (Corning Inc. Acton, MA) and cultured for 2 days. Samples were prepared in sextuplicate. To test for cell viability, a luminescent cell proliferation assay using CellTiter-Glo (Prome-ga Corp., Madison, WI) was used. The CellTiter-Glo assay quantif the amount of ATP present, indicating presence of metabolically active cells. The amount of ATP is directly proportional to the number of cells present in culture (*47*).

Hydrogel Cytotoxicity. The toxicity was also investigated on hydrogel extracts. Dex-PMPI hydrogels cross-linked with GCRD**GPQGIASQ**DRCG only or also containing CDPG*YIG-SR* and CQAAS*IKVAV* were prepared. Similar hydrogels with grafted laminin-derived peptides were used in the next section to briefly investigate cell adhesion on the dextran-peptide hydrogels. Dex-PMPI (1.25 mg) was dissolved in 25 μ L of a solution of laminin-derived peptides (0.8 mM/peptide). After 10 min, the solution was sterilized via centrifugation filtration using an Ultrafree MC sterile centrifugal filter (0.22 μ m; Millipore, Billerica, MA) and 25 μ L of filter-sterilized peptide cross-linker (27 mg/mL) was added. The solution was vortexed, transferred to a mold and incubated overnight at 37 °C.

Hydrogels were washed for 1 week with daily changes with DMEM supplemented with 10% FBS, 2 mM L-glutamine, and 50 units/mL penicillin/50 mg/mL streptomycin at 37 °C to remove any unreacted laminin-derived peptides or any leach-ables. The medium collected daily was pooled together and store at 4 °C. The collected medium was mixed with fresh culture medium (DMEM supplemented with 10% FBS, 2 mM L-glutamine, and 50 units/mL penicillin/50 mg/mL streptomycin) in order to have sufficient volume for cell culture (25% diluted in DMEM).

Embryonic day (E)15 dissociated rat DRG neurons were isolated from embryonic day 15 (E15) rats as reported in Supporting Information. NIH-3T3 fibroblast cells and DRGs were exposed to the tested gel extracts at 37 °C (50 ng/ mL nerve growth factor (NGF; Invitrogen Corp.) was added for DRGs), plated on collagen-coated 96-multiwell black-wall clearbottom plate, and cultured up to 3 days (1 plate/day) at 37 °C. Cells were also cultured into medium without extract as control. Samples were prepared in dodecaplicate. Once a day, cell culture was terminated and cell viability was assessed using the CellTiter-Glo assay.

Cell Adhesion. On the basis of previous results (42), cell adhesion was briefly investigated using laminin-derived peptides, CDPGYIGSR and CQAASIKVAV. The cell-adhesive peptides were introduced into the hydrogel to promote cellular adhesion. Dex-PMPI (0.88 mg) was dissolved in 17.5 μ L of a solution of cell-adhesive peptides (0.8 mM/ peptide). The solution was vortexed for 10 min and sterilized via centrifugation filtration, and 17.5 μ L of filter-sterilized peptide cross-linker (27 mg/mL) was added. The solution was vortexed and transferred to the bottom of well of a 96-multiwell half-area plate and incubated overnight. Hydrogels were washed for 1 week with culture medium (DMEM supplemented with 10% FBS, 2 mM L-glutamine, and 50 units/mL penicillin/50 mg/ mL streptomycin) at 37 °C to remove any unreacted laminin-derived peptides.

NIH-3T3 fibroblast cells and DRGs were cultured separately at 37 °C in culture medium. NGF (50 ng/mL) was added to the culture medium used for DRGs. Cells were plated onto the gels at a cell density of 3200 cells/gel. Cells were incubated at 37 °C for 2 days and then fixed in 3.7% formaldehyde for 20 min, rinsed with HBSS, and incubated in 0.1% Triton X-100 for 90 s. Finally, each well was rinsed with HBSS and incubated for 20 min in Alexa Fluor 488 phalloidin (Molecular Probes, Burlington, ON) solution (5 μ L in 200 μ L of HBSS). The phalloidin solution was replaced with HBSS, and the samples were stored at 4 °C until viewed under fluorescent microscopy. Samples were visualized with a Ziess Axiovert 100 inverted microscope (Carl Zeiss Canada Ltd., Toronto, ON), and images were captured using a digital camera.

Statistics. The statistics were performed with commercially available software program SigmaStat 3.11 (Systat Software Inc., Richmond, CA). All experimental results are reported as mean \pm 95% confidence interval. For multiple comparisons, one-way ANOVA was performed using Bonferroni *t*-test as posthoc test. Student's *t*-test was used to make pairwise comparisons. In all tests a *p*-value of <0.05 was regarded as significant.

RESULTS

Synthesis of PMPI-Derivatized Dextran. The heterobifunctional linker PMPI, introduced by Annunziato et al. (48), has been used to modify biomolecules (48) and PEG (49-51). In this project, it was used to derivatize natural polymers such as dextran for the purpose of forming hydrogels. Here, dextran reacts with PMPI in DMSO in the presence of the DBTDL as catalyst (Figure 1A). The successful conjugation of PMPI to dextran was confirmed by the FTIR spectrum of the Dex-PMPI adduct, which indicated the formation of carbamate linkages $(1714, 1519, \text{ and } 1232 \text{ cm}^{-1})$ and the disappearance of the isocyanate peak (2275 cm⁻¹). The ¹H NMR spectrum for Dex-PMPI showed distinctive peaks: the anomeric proton of the dextran glucopyranosyl ring at δ 5.0 ppm (singlet), the PMPI group at 7.0 ppm (singlet) for the maleimide carbon-carbon double bond, and at 7.4 ppm (doublet) and 7.6 ppm (doublet) for the aromatic protons. The DS of PMPI coupled to dextran was quantitatively determined using the integrated areas of the relevant chemical shifts, according to eq 1.

The coupling reaction between dextran and PMPI was studied in the presence of different amounts of DBTDL at either 25 °C or 45 °C for 4 h or 24 h. As shown in Table 1, successful coupling required the DBDTL catalyst, without which no or little product formation was observed. Under identical reaction conditions at 4 h and 25 °C, the coupling yield of 0% without DBTDL increased to 15% with 30 μ L of DBTDL. The importance of DBTDL was further confirmed when the reaction was repeated at 45 °C; the yield increased from 14% to 58% in the presence of 15 µL of DBTDL. At a temperature of 25 °C and in presence of 30 μ L of DBTDL, the coupling yield increased from 15% to 33% when the reaction time was extended from 4 to 24 h. The optimum coupling yield was obtained after reaction for 24 h at 45 °C in the presence of a minimum of 30 μ L of DBTDL, which yielded DEX-PMPI with a DS of 10 or 20 depending on the initial amount of PMPI used. These reaction conditions were used to synthesize DEX-PMPI DS10 (hereafter referred as Dex-PMPI), which was used for the remaining studies.

Hydrogel Formation and Hydrolytic Stability. Dextran hydrogels were first formed by cross-linking Dex-PMPI with PEG-dithiol via Michael-type addition. The PEG-dithiol was a useful model system to test the cross-linking system and the hydrolytic stability of the resulting hydrogels. The concentration of polymeric precursors was found to affect the rate of gelation: 20% Dex-PMPI gelled within seconds, whereas 2.5% Dex-PMPI only gelled after several minutes. The hydrogels were allowed to swell in PBS, and their WC was followed over 15 days (according to eq 2) during which time the WC was found to be constant at 94.9 \pm 0.2%. No excessive swelling was observed, suggesting that the cross-linked gels were hydrolytically stable.



Figure 1. (A) Reaction of dextran with *p*-maleimidophenyl isocynate (PMPI) and (B) preparation of dextran-peptide hydrogel through the Michaeltype reaction of peptide cross-linker to Dex-PMPI.

Table 1. Reaction Conditions for the Coupling of PMPI to Dextran

theoretical DS (%)	exptl DS (%)	DBTDL (mL)	time (h)	<i>Т</i> , °С	coupling yield (%)
10	0	0	4	25	0
37	5	0	4	45	14
38	22	15	4	45	58
38	6	30	4	25	15
15	5	30	24	25	33
15	15	30	24	45	100
10	10	45	24	45	100
20	20	45	24	45	100

Table 2. Degradation Kinetics of Soluble Peptides by MMP-2

peptide	$k_{\text{cat}}/K_{\text{M}}, \mathrm{M}^{-1} \mathrm{S}^{-1}$	$k_{\rm cat}, {f S}^{-1}$	$K_{\rm M}, \mu { m M}$
GPQGIASQ	583 ^a	2^a	3400 ^a
GPQGPAGQ	$<9^a$	ND^b	ND^b
GCRDGPQGIASQDRCG	2591 ± 570^{c}	0.47 ± 0.21^{c}	$189 \pm 106^{\circ}$
GCRDGPQGPAGQDRCG	20 ± 13^c	ND^{c}	ND^{c}

^{*a*} ND, not determined. ^{*b*} Netzel-Arnett, S., Sang, Q.-X., Moore, W., et al. (1993) *Biochemistry 32*, 6427–6432. ^{*c*} \pm 95% confidence interval (*n* = 3).

The hydrolytic stability of the dextran hydrogel cross-linked with dithiol peptides was similarly analyzed by evaluating the NW_h of 20% Dex-MA or Dex-PMPI hydrogels cross-linked with the peptide GCRD**GPQGIASQ**DRCG over a 200-day period (Figure 2). The Dex-PMPI-peptide hydrogel swelled to 2.25 times its original mass within the first 20 days and then did not change appreciably for the remaining 200 days, demonstrating the hydrolytic stability of these hydrogels. Both the thioether obtained by the conjugate addition of the thiol to the maleimide and the carbamate formed by coupling PMPI to dextran were resistant to hydrolysis. In contrast, Dex-MA-peptide hydrogel swelled to 3.25 times of their original mass within the first 8 days and then completely dissolved on day 9 due to the hydrolysis of glycidyl-methacrylate esters in Dex-MA.

Degradation of Soluble Peptide Cross-Links. As reported by Netzel-Arnett et al. (43), the **GPQGIASQ** sequence, derived from the calf and chick $\alpha 1$ (I) collagen chain, is highly sensitive to hydrolysis by MMP-2, whereas **GPQGPAGQ**, with Ile and Ser substituted by Pro and Gly, respectively, has low sensitivity (Table 2). Both peptides were extended on each terminus with the tetrapeptide sequence GCRD to improve solubility and to introduce the thiol groups required for cross-linking (3). The kinetic parameters for MMP-2 degradation of the peptides with and without GCRD were determined by Michaelis-Menten analysis. Substrate degradation was followed by measuring the fluorescence of fluorescamine adduct fragments of the peptide cleavage reaction ($\lambda_{\text{excitation}} = 387 \text{ nm}$, $\lambda_{\text{emission}} = 480 \text{ nm}$) and the kinetic parameters k_{cat} , K_m and k_{cat}/K_M were obtained using Lineweaver-Burk plots (eq 2S, Supporting Information) and by plotting the initial rate versus the product between the enzyme and substrate concentrations (eq 3S, Supporting Information). As it was previously reported (3), elongating the peptide sequence up to the P8 position changed the kinetic parameters and improved both the catalytic efficiency (i.e., higher k_{cat}/K_{M}) and the substrate affinity (i.e., lower $K_{\rm M}$). As shown in Table 2, elongation of the peptide sequences with GCRD fragments led to a greater than fourfold increase in k_{cat}/K_{M} for GCRDG-**PQGIASQDRCG** and more than doubled the k_{cat}/K_{M} for GCRD**GPQGPAGQ**DRCG. Despite the increase in k_{cat}/K_{M} values, the higher MMP-2 cleavage efficiency for GPQGIASQ relative to GPQGPAGQ was maintained, with GCRDGPQ-**GIASQDRCG** having a k_{cat}/K_M 2 orders of magnitude larger than that for GCRDGPQGPAGQDRCG.

Enzymatic Degradation. Collagenase Degradation. Collagenase-mediated degradation of 20% Dex-PMPI-GCRDGPQ-GIASQDRCG cross-linked hydrogels was followed by monitoring the hydrated mass of the hydrogel over time and compared to two controls: (1) identical hydrogels without collagenase and (2) a redox-polymerized poly(Dex-MA) hydrogel exposed to the same amount (2 ng/mL) of bacterial collagenase. As shown in Figure 3, dextran-peptide hydrogels degraded completely after 30 h of incubation with collagenase whereas without collagenase, the mass increased to a NWh of 1.2, demonstrating swelling but no degradation. These data demonstrate that the dextran-peptide hydrogels degraded only by an enzymatic process. Moreover, poly(Dex-MA) hydrogels obtained by redox polymerization did not degrade when exposed to collagenase, demonstrating that dextran-peptide hydrogels degrade by collagenase cleavage of the peptide cross-link and not the dextran backbone.

MMP-2 Degradation. MMP2-mediated degradation of the dextran-peptide hydrogels was assessed using a fluorimetric assay. Hydrogels were synthesized with 5% Dex-PMPI cross-



Figure 2. Hydrolytic stability of water dextran-peptide hydrogels; normalized hydrogel weight (NW_h) of 20% dextran-peptide hydrogels crosslinked through conjugate addition **GPQGIASQ** peptides to (\Box) Dex-MA and (\blacklozenge) Dex-PMPI (n = 3, mean \pm 95% confidence interval).



Figure 3. Bacterial collagenase degradation of dextran-peptide hydrogels; normalized hydrogel weight (NW_h) of (grey diamond) 20% dextran-peptide, no collagenase, (\blacksquare) 20% dextran-peptide, 2 ng/mL collagenase, (\triangle) 20% poly(Dex-MA) by redox polymerization, 2 ng/mL collagenase (peptide = GCRD**GPQGIASQ**DRCG, *n* = 3, mean \pm 95% confidence interval).

linked with GCRDGPOGIASODRCG. During hydrogel preparation, CGK-Mca and GCRDGPOGIASODRGK-FAM peptides were added in order to follow hydrogel degradation/ dissolution by Mca fluorescence ($\lambda_{\text{excitation}} = 340 \text{ nm}, \lambda_{\text{emission}}$ = 405 nm) independently of cross-link cleavage (measured by FAM fluorescence; $\lambda_{\text{excitation}} = 494 \text{ nm}$, $\lambda_{\text{emission}} = 521 \text{ nm}$) in the supernatant, assuming that the cross-link peptides and FAMmodified peptides degrade at similar rates. Hydrogels were incubated with 40 nM MMP-2, and the fluorescence of the supernatant was followed overtime. As shown in Figure 4, there was 100% release of both fluorophores and complete dissolution of the hydrogels after 16 days of incubation, which corresponds to the plateau observed in Figure 4. There was a near-linear increase in the release of the fluorescent IASQDRGK-FAM fragment into the degradation medium from dextran-peptide hydrogels due to MMP-2 digestion. Fluorescent dextran-CGK-Mca was released at a slower rate until close to the end of the

incubation period, indicating that a substantial amount of crosslinks must be digested prior to dextran dissolution. The dextranpeptide hydrogels appeared to decrease in size during degradation, suggesting a surface erosion process. The same experiment was repeated with dextran hydrogels cross-linked with GCRDG-PQGPAGQDRCG, the peptide with low sensitivity to MMP-2, and modified with GCRDGPQGPAGQDRGK-FAM to examine MMP-2 susceptibility (Figure 5). The degradation experiment with GCRDGPQGPAGQDRCG was pursued for 7 days due to the low enzyme sensitivity of the cross-links. As shown in Figure 6, the release of the fluorescent PAGQDRGK-FAM fragment was significantly slower than that of IASQDRGK-FAM, confirming the higher sensitivity to cleavage by MMP-2 for GCRDGPQGIASQDRCG compared to GCRDGPQG-**PAGO**DRCG in the cross-linked hydrogel. Moreover, this demonstrates that the rate of peptide-cross-linked dextran hydrogel degradation can be controlled by the choice of peptide cross-link and enzyme affinity.

Cytotoxicity Assay. The cytotoxicity of dextran and Dex-PMPI DS 10 with Mw of 6 kDa and 40 kDa was investigated by incubating cells in presence of soluble polysaccharides in the medium solution. Dextran and Dex-PMPI solutions were incubated with fibroblast cultures for 2 days at concentrations up to 10 mg/mL (Figure 7). The cytotoxicity was assessed by determining the presence of metabolically active cells by quantifying the amounts of ATP present. It was previously reported that the amount of ATP is directly proportional to the number of cells present in culture (47). As shown in Figure 7A, the presence of dextran 6 kDa and 40 kDa in the medium did not significantly impede the metabolic activity of the cultured fibroblasts. These results suggest that the polysaccharide, under its natural form, is not cytotoxic for the molecular weights and concentrations investigated. By contrast, as shown in Figure 7B, increasing the concentration of Dex-PMPI 6 kDa or 40 kDa in the culture medium to 1 mg/mL and 10 mg/mL resulted in a significant decrease in the production of ATP (p < 0.001 and p = 0.049 for 1 mg/mL and p < 0.001 for 10 mg/mL). This indicates that, above a Dex-PMPI concentration



Figure 4. MMP-2 degradation of dextran-peptide hydrogels; cumulative release of fluorophores from 20% dextran-peptide hydrogels exposed to 40 nM MMP-2: (\blacktriangle) IASQDRGK-FAM and (grey square) dextran-CGK-Mca (n = 3, mean \pm 95% confidence interval).



Figure 5. MMP-2 degradation of dextran-peptide hydrogels; cumulative release of fluorophores from 20% dextran-peptide hydrogels exposed to 40 nM MMP-2: (\triangle) **PAGQD**RGK-FAM and (grey diamond) dextran-CGK-Mca (n = 3, mean \pm 95% confidence interval).



Figure 6. MMP-2 degradation of dextran-peptide hydrogels; cumulative release of fluorophores from 20% dextran-peptide hydrogels exposed to 40 nM MMP-2: (\blacktriangle) **IASQ**DRGK-FAM and (\triangle) **PAGQ**D-RGK-FAM (n = 3, mean \pm 95% confidence interval).



Figure 7. Cell viability of 3T3 cells exposed to soluble dextran and Dex-PMPI in culture medium: (A) Dextran, (B) Dex-PMPI; \blacksquare 6 kDa, \Box 40 kDa; cell viability is expressed in ATP production (n = 6, mean \pm 95% confidence interval).

of 1 mg/ mL, the maleimide functionalities covalently immobilized to the dextran backbone can affect the cellular activity and even lead to some cytoxicity.



Figure 8. Cell viability of 3T3 cells and DRG neurons exposed to hydrogel extracts; control: \blacklozenge 3T3, \blacklozenge DRG, extracts from Dex-PMPI cross-linked with GCRDGPQGIASQDRCG: (titled gray square) 3T3, (gray circle), extracts from Dex-PMPI cross-linked with GCRDGPQ-GIASQDRCG and immobilized CDPGYIGSR and CQAASIKVAV: (grey square) 3T3, (grey circle) DRG (cell viability is expressed in ATP production) (n = 12, mean \pm 95% confidence interval).

The cytotoxicity of polysaccharide-based hydrogels was studied by exposing fibroblasts and DRGs respectively to culture medium containing extracts of Dex-PMPI hydrogels cross-linked with GCRDGPQGIASQDRCG. Two sets of hydrogels were used for the extraction assay; in one set, the hydrogels were simply Dex-PMPI cross-linked with GCRDGPQGIASQDRCG, and in the second set, the hydrogels were cross-linked with the same peptide but also contained CDPGYIGSR and CQAASIKVAV as cell-adhesive peptides. Both sets of hydrogels were extracted in cell culture medium for 7 days at 37 °C, and the extracts were incubated with fibroblast and DRG cultures for 3 days. Again the cytotoxicity was assessed by quantifying the amounts of ATP present. As seen in Figure 8, when compared to the controls, no cytotoxicity was detected from Dex-PMPI hydrogel extract, indicating that an insufficient amount of unreacted Dex-PMPI or peptide cross-linkers diffused from the hydrogels to cause any significant cytotoxicity to the fibroblasts or DRGs. The metabolic activity of 3T3 cells and DRG neurons significantly decreased when exposed to extracts obtained from hydrogels containing laminin-derived peptides. These results indicate that the cytotoxicity was probably caused by unreacted laminin-derived peptides binding and saturating the cell-surface receptors, thus interfering with the cell adhesion mechanism required for survival of these anchorage-dependent cells. These results are important because they underline the importance of thoroughly washing the hydrogels prior to cell culture.

Cell Culture. Cell adhesion of NIH-3T3 fibroblast cells and primary chick DRG neurons was investigated on MMP-degradable dextran hydrogels. Dex-PMPI hydrogels were modified with laminin-derived peptides CDPGYIGSR and CQAASIKVAV to provide a cell-adhesive substrate to the cells and cross-linked with GCRD**GPQGIASQ**DRCG. As shown in Figure 9, both fibroblasts and DRGs adhered, respectively, to dextran-peptide hydrogels containing the laminin-derived peptides but did not adhere to hydrogels lacking cell-adhesive peptides. This was not surprising given the high water content of dextran hydrogels, which are known to be non-cell adhesives (27, 33, 34) and based on the results previously (42). Imaging was difficult due to the uneven surface of the hydrogels.

DISCUSSION

Since the first MMP-sensitive telechelic peptide-PEG-peptide block copolymers were developed by West and Hubbell (16), polymers such as PEG and poly-N-isopropylacrylamide have been used to develop other MMP-degradable hydrogels (3, 4, 17). In the present study, a MMP-degradable dextran was synthesized and characterized. Dextran is the first naturally derived polymer to be tested for this type of application and is particularly compelling because it is inherently nonadhesive to



Figure 9. Cellular adhesion of NIH-3T3 fibroblast cells and DRG neurons to dextran-peptide hydrogels. Representative images of (A) NIH 3T3 fibroblasts cultured on unmodified dextran-peptide hydrogel demonstrates no visible adherent cells, (B) NIH 3T3 fibroblasts cultured on dextran-peptide hydrogel containing laminin-derived peptides after 2 days in vitro (2×10^5 cells/cm² were plated), (C) DRGs plated on unmodified dextran-peptide hydrogel demonstrate no visible adherent cells, and (D) DRGs cultured on dextran-peptide hydrogels containing laminin-derived peptides after 2 days of culture (2×10^5 cells/cm² were plated).

proteins (*31*, *32*) and cells (*27*, *33*, *34*), allowing cell adhesion to be "dialed-in" with peptide modification. In addition, dextran has multiple functional hydroxyl groups available for derivatization and conjugation. For example, there are significantly more reactive functional groups on dextran than on a multiarm PEG. Dextran 40 kDa derivatized to a DS of 10 has approximately 25 modified hydroxyls per polysaccharide molecule, whereas commercially available modified four-arm PEG has 4 reactive hydroxyls per molecule. These multiple sites allow efficient covalent modification with ECM-derived peptides to promote cellular adhesion or to immobilize bioactive molecules, such as growth factors, without having a significant impact on the degree of cross-linking required for gelation.

There have been several studies on dextran bioconjugation. However, most of these methods are either two-step conjugation procedures, such as carboxymethylation followed by carbodiimide conjugation (52) or periodate oxidation followed by Schiff base reaction (53, 54), or introduce hydrolyzable esters, such as meth(acrylates) (55–57). The dextran derivatization method described here with PMPI is a one-step reaction which introduces a stable carbamate bond and a maleimide group for Michael-type addition.

Prior to this work, PMPI had been primarily used to modify bioactive molecules (48) and PEG (49–51) or to immobilize biomolecules such as proteins (48), DNA (58), or oligonucleotides (51) on surfaces. In unpublished work, we have also extended the utility of this cross-linking agent to other polymers, including four-armed PEG-PMPI and PMPI-derivatized methylcellulose, demonstrating broad applicability as a heterobifunctional agent for hydrogel applications through Michaeltype addition. Moreover, by using the DBTDL catalyst common in the polyurethane literature (59, 60), we were able to achieve high yield for PMPI modification of dextran (Table 1). The Dex-PMPI cross-linked hydrogels were shown to be hydrolytically stable, demonstrating that the peptide dictates the degradation rate.

GPQGIASQ, an eight-amino-acid sequence derived from the α 1 (I) collagen chain of calf and chick, was selected as the MMP substrate (43, 44). Many MMPs, such as MMP-1, MMP-2, MMP-3, MMP-7 MMP-8, and MMP-9, can cleave this peptide at the Gly-Ile bond; however, MMP-2 has the greatest digestive efficiency (43, 44). GPQGPAGQ was selected as a control because it has been reported to have little degradation sensitivity to most MMPs (43, 44). Nagase et al., in studying the human MMP specificity using collagen-derived synthetic peptides, have assembled a database of more than 45 peptide sequences that have different degrees of sensitivity to MMP digestion (44). They reported that the efficiency of peptide hydrolysis varied for different MMPs, giving sequences that were more or less susceptible to cleavage by collagenases, gelatinases, matrilysins, or stromelysins. The kinetic characteristics of the 16-amino-acid GCRD-capped sequence used in this study follow the trend of their respective 8-amino-acid sequences (Table 2). On the basis of these data and on the synthetic peptide database built by Nagase et al., MMP-sensitive hydrogels can be designed to have higher affinity for one or more specific MMPs and this can be used, in turn, to control hydrogel degradation.

MMPs are highly involved in ECM remodeling processes such as tissue morphogenesis and wound healing. They are expressed following spinal cord injury and recent data suggest a beneficial role for MMP activity following SCI (5, 9). Enzyme-degradable hydrogels susceptible to MMP digestion have been previously investigated for bone, cartilage, and vascular applications where they are postulated to provide greater control over tissue regeneration. The MMP-sensitive hydrogels described in this report were designed for neural tissue-engineering applications and may be useful for investigating the mechanisms of cell migration and/or axonal outgrowth.

The Dex-PMPI-peptide hydrogel was found to degrade under the actions of both bacterial collagenase and MMP-2, as shown through the wet mass variation and the release of fluorophores into solution, respectively. Although hydrogels were exposed to lower concentration of bacterial collagenase than MMP-2, they degraded significantly more rapidly. This faster rate of degradation when exposed to bacterial collagenase likely reflects the fact that bacterial collagenases, unlike mammalian collagenases, are more active and less specific in cleaving collagen (61). As reported previously by Hennink and co-workers, the swelling behavior of dextran hydrogels degrading through bulk degradation is characterized by hydrogel swelling followed by rapid dissolution (62). It is believed that during bulk degradation, the cross-links are cleaved throughout the hydrogel to a point where the dextran chains only bear about two cross-links per chain, and further degradation of the cross-links results in the rapid disintegration of the hydrogel to soluble products. Similar observations were reported with MMP-degradable PEG hydrogels (3). In this study, hydrogels degraded by bacterial collagenase were characterized by an absence of significant swelling and a progressive mass loss (Figure 3). In addition, as shown in Figure 4, hydrogel degradation mediated by the activity of MMP-2 is accompanied by a continuous and progressive release of soluble dextran. These observations suggest proteolytic degradation of dextran-peptide hydrogels by a predominantly surface-erosion mechanism rather than by bulk degradation, which has been reported for MMP-degradable PEG hydrogels.

Interestingly, the activity of MMP-2 toward the **GPQGIASQ** sequence is 2 orders of magnitude greater than toward the **GPQGPAGQ** sequence for soluble peptides (Table 2). The difference in rates of hydrogel degradation by MMP-2 appear to be much closer for the respective dextran-peptide hydrogels (Figure 6). Under their soluble form, the peptides are very mobile and can adopt a conformation that will facilitate binding and cleavage by MMP-2. Once immobilized within the hydrogel degradation seems to be predominantly driven by surface erosion, thus limiting the access of MMP-2 to the surface of the hydrogel.

This study focused on preparing a new type of MMP-sensitive dextran-based hydrogel which could be used in neural tissue engineering. Although, many MMPs have been reported to be present following spinal cord injury and are believed to have a beneficial role in the healing process, MMP-2 was selected as a model enzyme. Along with previous observations reported by other groups (3, 4, 63-65) and our results, the degradation rate of the hydrogel can be tailored by enzyme specificity to the peptide sequence.

As reported, the gelation of Dex-PMPI hydrogels through Michael-type reaction is very fast, which may be advantageous for *in situ* gelation. Dex-PMPI was found to be non-cytotoxic as monomers at concentrations lower than 1 mg/mL. In addition, following gelation, the amount of un-cross-linked Dex-PMPI leaching out of the hydrogel was insufficient to cause any cytotoxicity. As shown in Figure 8, unreacted peptides leaching out of the Dex-PMPI hydrogel impeded cell viability. To pursue the *in situ* gelation use, peptide purification techniques have to be refined in order to improve the purity of peptides containing reactive thiols and optimized peptide immobilization.

Given that neural cells produce MMP-2 after injury and that MMP-2 degrades the dextran-peptide scaffolds, we were interested in cellular activity on the hydrogels and specifically cell adhesion. MMP-degradable hydrogels require the presence of cell-adhesive peptides for cell adhesion, survival, and ultimately invasion into the network (*17*, *65*, *66*). These data, coupled with previous reports, prompted us to explore modification of dextran-cross-linked hydrogels with laminin-derived peptides. Results reported previously showed that dextran hydrogels modified with CQAAS*IKVAV* and CDPG*YIGSR* promote greater adhesion of DRG neurons than those hydrogels

modified with CGRGDS (42). Interestingly, both NIH-3T3 and DRG cells adhered and survived when plated on dextran-peptide hydrogels modified with these laminin-derived peptides. Just as the cross-link density influenced the cell invasion (3), the ECM-peptide modification for cell adhesion must be optimized for cell migration (3, 17) and neurite outgrowth (67, 68). Interestingly, ECM peptides may also be manipulated to promote the expression of the MMPs (69).

CONCLUSIONS

MMP-sensitive dextran hydrogel systems were synthesized using peptide cross-linkers to control the rate of MMP-2 degradation and susceptibility to proteolytic remodeling. We have reported a new method to conjugate dextran through the use of the heterobifunctional PMPI molecule. Dextran-peptide hydrogels synthesized with Dex-PMPI degraded when incubated in the presence of bacterial collagenase or MMP-2 through enzymatic digestion of the peptide cross-links. Laminin-derived peptide-modified dextran hydrogels promoted cell adhesion and survival, allowing questions of cell-mediated expression of MMP for cell-based degradation to be pursued.

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Supporting Information Available: Degradation kinetics of soluble peptide cross-linkers and isolation of rat DRG neurons. This material is available free of charge via the Internet at http://pubs.acs.org/BC.

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