Affinity Peptides Protect Transforming Growth Factor Beta During Encapsulation in Poly(ethylene glycol) Hydrogels

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ABSTRACT: Transforming growth factor beta (TGFβ1) influences a host of cellular fates, including proliferation, migration, and differentiation. Due to its short half-life and cross-reactivity with a variety of cells, clinical application of TGFβ1 may benefit from a localized delivery strategy. Photoencapsulation of proteins in polymeric matrices offers such an opportunity; however, the reactions forming polymer networks often result in lowered protein bioactivity. Here, PEG-based gels formed from the chain polymerization of acrylated monomers were studied as a model system for TGFβ1 delivery. Concentrations of acrylate group ranging from 0 to 50 mM and photopolymerization conditions were systematically altered to study their effects on TGFβ1 bioactivity. In addition, two peptide sequences, WSHW (KD = 8.20 nM) and KRIWFIPRSSWY (KD = 10.41 nM), that exhibit binding affinity for TGFβ1 were introduced into the monomer solution prior to encapsulation to determine if affinity binders would increase the activity and release of the encapsulated growth factor. The addition of affinity peptides enhanced the bioavailability of TGFβ1 in vitro from 1.3- to 2.9-fold, compared to hydrogels with no peptide. Further, increasing the concentration of affinity peptides by a factor of 100–10000 relative to the TGFβ1 concentration increased fractional recovery of the protein from PEG hydrogels.

INTRODUCTION

Strategies to direct cellular functions in biomaterials via spatial and temporal delivery of proteins, such as growth factors, chemokines, and cytokines, are of growing interest in tissue engineering applications. These biomacromolecules can control cell differentiation, proliferation, migration, and even apoptosis.1–6 However, dosing and targeting of proteins to specific cell populations can pose significant challenges. For example, growth factors are eminently potent and can elicit a variety of cellular responses at picomolar concentrations.4 Further, many factors are cross-reactive across a multitude of cells and tissue types and are known to have short half-lives in vivo.5 To overcome some of these limitations, a biomaterial delivery platform was explored to facilitate greater control over the bioactivity and availability of growth factors, particularly transforming growth factor beta (TGFβ1), delivered locally to targeted cell populations or tissues.

TGFβ1, a member of the TGF superfamily, regulates many cellular processes including proliferation, differentiation, chemotaxis, and tumorigenesis.4 TGFβ1 is known to play a crucial role in promoting chondrogenic differentiation of human mesenchymal stem cells,7–10 guiding the organization of endothelial cells in angiogenesis,11 and regulating the extracellular matrix production of valvular smooth muscle cells.12 Because many cells express TGFβ1 receptors, a local delivery platform is often required for spatial and temporal control over its dosage. One method for controlling the delivery of growth factors is through encapsulation in polymeric matrices, such as poly(ethylene glycol) (PEG).

PEG hydrogels have been used to deliver a variety of growth factors; however, a great challenge facing PEG hydrogels cross-linked by chain growth polymerizations is the potential for irreversible protein damage.13 For many protein delivery applications, direct encapsulation of growth factors (i.e., through the inclusion of the target protein in the monomer precursor solution) is desirable due to its simplicity in preparation and a facile control of the total growth factor payload. Photoinitiated reactions are commonly used in cell encapsulation schemes, due to their mild reaction conditions, specifically physiological pH, temperatures, and osmolarity. While these characteristics render a photoinitiated polymerization system desirable for the formation of cell-laden hydrogels, they are known to create adverse reactions to protein therapeutics, which are usually unstable and can be easily denatured. For example, growth factors present in the formation of hydrogels are susceptible to damage during the reaction, primarily due to the presence of highly reactive radical species14 generated by cleavage of photoinitiator species. In addition to initiating polymerization reactions, these free radicals may undergo a number of nonspecific side reactions with functional groups associated with amino acids, including phenols, thiols, and disulfides,15 leading to either direct conjugation of the growth factor to the polymer.
backbone or loss of protein conformation and, therefore, bioactivity. The development of an encapsulation scheme to ameliorate potential radical damage would, therefore, be desirable to enhance the efficacy of polymeric growth factor delivery platforms.

A number of polymeric materials have been utilized as protein delivery vehicles, including alginate, collagen, PLGA, and PEG. Recent work demonstrates the use of novel polymeric materials incorporating affinity ligands for sustained protein release through mixed-mode, thiol-acrylate polymerizations.[19–21] These systems utilize ligands that noncovalently and reversibly interact with the target protein, with release being tuned by both diffusion and the binding kinetics unique to the ligand-protein pair. Specific peptide-ligand systems, where the peptide ligand has affinity for a unique protein, have been shown effective for controlled release of bFGF[19] and sequestration of MCP-1[20] and TNFα.[21] Nonspecific ligands such as heparin and alginate employ electrostatic affinity interactions present on numerous proteins;[22] such ligands, when added to hydrogel systems, have been utilized to govern release of many growth factors, including bFGF,[23] NGF,[24] VEGF,[25,26] PDGF-BB,[26,27] and TGFβ.[26–28]

We hypothesized that the presence of free radicals generated during photoinitiated polymerizations would induce TGFβ bioactivity and functional damage, and that the inclusion of affinity binding peptides during photopolymerization could prevent some of this damage. Using photo-cross-linked PEG hydrogels as a platform, we systematically studied the influence of photopolymerization conditions on TGFβ bioactivity and availability. Quantification of released TGFβ was determined by ELISA, while confirmation of TGFβ bioactivity was achieved via a TGFβ receptor reporter cell line. Further, we analyzed the binding affinity of the TGFβ peptide ligands, Trp-Ser-His-Trp[29] and Lys-Arg-Ile-Trp-Phe-Ile-Pro-Arg-Ser-Ser-Trp-Tyr,[30] using surface plasmon resonance studies. These affinity peptides were included in monomer solutions during photoencapsulation of TGFβ, and the enhancement of TGFβ recovery from photopolymerized PEG hydrogels was examined. The dose dependence of the peptide/TGFβ ratio on protein recovery and bioavailability from PEG hydrogels was also studied and quantified for both affinity sequences.

**EXPERIMENTAL SECTION**

**Materials.** All chemicals were purchased from Sigma-Aldrich unless otherwise noted.

**PEGDA Synthesis.** Poly(ethylene glycol) diacylate (PEGDA) monomers were prepared as previously described.[18] Briefly, hydroxyl-terminated poly(ethylene glycol) (Mn = 4600, 6000, or 10 000 Da) was reacted with acryloyl chloride in the presence of triethylamine under argon for overnight. The product solution was filtered through neutral alumina oxide and stirred for 2 h in sodium carbonate. After an additional filtration step, excess toluene was removed under reduced pressure and subsequently precipitated into cold ethyl ether. 1H NMR revealed a degree of acrylation of at least 95% for all material used in this study.

**Solid Phase Peptide Synthesis.** All peptides were synthesized using a solid phase peptide synthesizer (Applied Biosystems 433A) and standard Fmoc chemistry. Peptide cleavage solution was formed by dissolving 250 mg dithiothreitol and 250 mg phenol in a solution of 95% trifluoroacetic acid (TFA), 2.5% trisopropylsilane (TIS), and 2.5% deionized water. Synthesized peptides were cleaved in the solution for 2 h. Cleaved peptides were precipitated in cold ethyl ether and desiccated overnight, followed by reverse-phase HPLC (Waters Delta Prep 4000) purification. The collected fractions of purified peptides were identified by matrix-assisted laser desorption/ionization—time-of-flight (MALDI-TOF) mass spectrometry (Supporting Information).

**TGFβ Photodestruction Studies.** Recombinant human TGFβ solutions (Peprotech) (final concentration: 2 nM) were prepared in PBS in the presence of 1 mM lithium phenyl-2,4,6-trimethylbenzophosphinate (LAP) initiator and varying concentrations of poly(ethylene glycol) monoacrylate (Monomer-Polymer and Dajac Laboratories; Mn = 2000 Da). Solutions were exposed to UV light (Omnicure 365 nm) at an intensity of 10 mW/cm² for 3 min. Following UV exposure, TGFβ solutions were analyzed with a Human/Mouse TGFβ ELISA kit (eBioscience) to determine the recovery of intact TGFβ.

**TGFβ Release from PEG Hydrogels.** Human TGFβ at a final concentration of 25 nM was photoencapsulated in 10 wt% PEG (Mn = 10 000 Da) hydrogels. For affinity peptide formulations, TGFβ binding peptides (Promega) and KRIWFIPRSSWY were also incorporated. Peptides were included at a molar ratio, R, relative to TGFβ, equal to 100, 1000, or 10 000. Growth factor release studies were conducted in release buffer (1 mM EDTA and 0.05% BSA in PBS) in scintillation vials pretreated with SigmaCote to reduce nonspecific protein adsorption on the wall of the vials. Supernatants were collected at predetermined time points and replaced with fresh release buffer. Concentrations of released TGFβ were determined by ELISA.

**TGFβ Bioactivity Assay.** TGFβ bioactivity was confirmed with PE.25 cells stably transfected with a luciferase reporter gene. The assay was performed as reported previously.[31] Briefly, PE.25 cells were plated in 12-well plates (20000 cells/well) and incubated in serum-free DMEM media. PEGDA hydrogels (Mn = 10 000 Da) encapsulated with 25 nM TGFβ were placed in coculture with cells for 24 h at 37 °C and 5% CO2. Cells were lysed in lysis buffer (Promega) and frozen at −80 °C for greater than 2 h. Lysate was centrifuged at 15 000 rpm at 4 °C for 10 min and supernatant collected and added to luciferase substrate (Promega). Luminescence was measured using Perkin-Elmer 1420 spectrophotometer.

**Surface Plasmon Resonance Binding Studies.** A Biacore 3000 instrument (GE Healthcare) and research grade carboxymethyl-dextran functionalized (CMS) biosensor chips (GE Healthcare) were used for all studies. The flowcell surfaces were equilibrated in HBS-EP running buffer and preconditioned with NaOH, HCl, SDS and H2PO4. Flowcell surfaces were activated with a solution of 0.25 M n-hydroxysuccinimide and 0.5 M N-ethyl-N’-(dimethylaminopropyl) carbodiimide hydrochloride, followed by injection of 6.2 mM N-phenylde-thanolamine in 0.1 M borate buffer. Flowcells were then injected with ligand-functionalized affinity peptides in acetate buffer, and all surfaces were then deactivated with a solution of i-cysteine/NaCl in 100 mM sodium formate buffer.

After allowing the flowcell surfaces to equilibrate with HBS-EP running buffer, solutions of varying concentrations of TGFβ, diluted in HBS-EP buffer, were injected using kinetic analysis injection protocols through Biacore software. All data was analyzed using Scrubber2 software (BioLogic Software).

**Statistics.** All data are reported as a mean ± s.e.m. based on three repeats per experimental condition, unless otherwise noted.

**RESULTS AND DISCUSSION**

**Effect of Network Cross-Linking Density on TGFβ Recovery.** Varying cross-linking density (or mesh size) in hydrogel matrices provides a facile method to control protein diffusivity, and in general, the ratio of protein diffusivity in a network relative to that in a pure solvent scales with (1 − Rf/ξ). Here, Rf is the protein radius and ξ is the network mesh size. As an example, the mesh size of PEG hydrogels can be tailored by varying the molecular weight of PEGDA at a defined weight content.
Fractional release of TGF\(\beta_1\) as a function of time when entrapped in PEG hydrogels of varying molecular weight. All gels were formed from 10 wt % monomer systems. The final network mesh size affects TGF\(\beta_1\) release. While PEG 4600 and PEG 6000 gels had no appreciable TGF\(\beta_1\) release, PEG 10000 gels had 25% fractional release over 2 days.

Figure 1. Fractional release of TGF\(\beta_1\) as a function of time when entrapped in PEG hydrogels of varying molecular weight. All gels were formed from 10 wt % monomer systems. The final network mesh size affects TGF\(\beta_1\) release. While PEG 4600 and PEG 6000 gels had no appreciable TGF\(\beta_1\) release, PEG 10000 gels had 25% fractional release over 2 days.

shows fractional release of TGF\(\beta_1\) encapsulated in 10 wt % PEG hydrogels of varying molecular weights. Interestingly, hydrogels of PEGDA 4600 Da and 6000 Da released less than 5% of the encapsulated protein over a two day period, while 10 wt % PEGDA 10000 Da gels released approximately 25% of the TGF\(\beta_1\) payload over the same time frame. To determine if the lower TGF\(\beta_1\) release from PEGDA 4600 and 6000 Da gels was due to hindered diffusivity in the more cross-linked hydrogels, the average mesh size of these gels was estimated from equilibrium swelling ratios using a modified Flory–Rehner method.34 (Table 1).

While the hydrodynamic radius of TGF\(\beta_1\) has not been reported in the literature, proteins of similar molecular weight, including chymotrypsinogen35 and enhanced green fluorescent protein,36 have reported radii on the order of 28–35 Å (Supporting Information). However, because no appreciable amount of TGF\(\beta_1\) encapsulated in PEGDA 4600 and 6000 was released over a two day period, mesh size was not likely the principal determinant of TGF\(\beta_1\) release in this system. In comparing the three hydrogel formulations used, the photoinitiator concentration and UV exposure conditions were identical, as were the monomer concentrations relative to the TGF\(\beta_1\) concentration. Due to the use of a constant weight/volume formulation, acrylate concentrations were not held constant (Table 2), and the effect of this factor on TGF\(\beta_1\) release warranted further investigation, particularly because the rate of polymerization scales directly with the acrylate concentration to a first approximation.

Effect of Acrylate Concentration on TGF\(\beta_1\) Recovery in Solution. To characterize the effect of acrylate concentration on TGF\(\beta_1\) recovery during photoinitiated reactions, a PEG monoacrylate (PEGMA) monomer was selected. At low concentrations, similar to those used for hydrogel formation with the diacrylated PEG, monoacrylates do not form a cross-linked polymer when exposed to UV radiation in the presence of photoinitiators. Photoinitiated radicals can propagate through the acrylate group, and polyacrylate kinetic chains are formed via a chain polymerization, but the polymer remains soluble. This aspect makes PEGMA ideal for use in modeling the PEGMA–protein encapsulation reaction scheme.3,5,35 Proteins photoencapsulated in PEGDA may be covalently conjugated to the polymer, resulting in a loss of soluble protein in solution. Alternatively, irradiated solutions of PEGMA and TGF\(\beta_1\) may lead to radical mediated damage through chain transfer, and the solutions can be subsequently assayed for protein concentration or bioactivity. Reductions in either factor are attributed to the effects of reaction conditions.

Solutions of TGF\(\beta_1\) and photoinitiator, with varying concentrations of PEGMA, were exposed to UV dosages identical to that used for photoencapsulation with diacrylate PEGDA. The influence of the polymerization conditions on TGF\(\beta_1\) recovery after UV exposure was tested via ELISA on diluted solution samples, which showed increased growth factor recovery with increasing acrylate concentration. Maximum postirradiation recovery of TGF\(\beta_1\) greater than 90% was found for acrylate concentrations above 40 mM (Figure 2A). TGF\(\beta_1\) recovery data via ELISA (Figure 2a) supports results previously published on the so-called “protective effect” that increasing monomer concentrations afford proteins.13

Interestingly, bioactivity after UV exposure was maximized at 20 mM acrylate concentration, with lower activity at both higher and lower acrylate concentrations (Figure 2B). While loss of bioactivity of TGF\(\beta_1\) at low acrylate concentration is consistent with data from the ELISA assays, reduced bioactivity for samples with high acrylate concentrations is not. One explanation for this observation is potential PEGylation of the growth factor, resulting in a loss of bioactivity and increased hydrophilicity. Direct detection of PEGylated TGF\(\beta_1\) presents a challenge for traditional mass spectroscopic techniques, due to the biologically relevant nanomolar concentration range used. This concentration is several orders of magnitude below the limit of detection for HPLC, NMR, GC, and MALDI methods. PEGylated BMP-2 has been characterized qualitatively using SDS-PAGE,38 but this method is limited by low solubility of hydrophobic proteins, such as TGF\(\beta_1\), in SDS solutions. While we were unable to directly measure PEGylated TGF\(\beta_1\), others have observed PEGylated proteins during chain polymerization of PEG monoacrylate monomers in solution with a model protein, lysozyme.33 For solution studies using PEG monoacrylate, any PEGylated growth factor remains in the reaction solution and is potentially detectable by ELISA techniques. However, in diacrylate systems, PEGylated growth factors could be covalently conjugated to the hydrogel polymer. Any conjugation would lead to significant reduction in the total fractional release of soluble, bioactive TGF\(\beta_1\) from the polymer. This mechanism may explain, in part, the lower fractional release of TGF\(\beta_1\) from PEGDA 4600 and PEGDA 6000 hydrogels.

Surface Plasmon Resonance Studies Confirm TGF\(\beta_1\)/Peptide Affinity. Affinity peptides have previously been used to successfully control the release of encapsulated proteins.18 Further, a small soluble affinity ligand has been previously used to protect photoencapsulated bovine serum albumin in PEG hydrogels.37 Here, we aimed to test whether inclusion of affinity peptides in monomer solutions could help protect proteins from radical mediated damage and/or conjugation during photoencapsulation reactions. First, surface plasmon resonance (SPR) was used to characterize the binding affinity between each peptide sequence and TGF\(\beta_1\). SPR technology allows precise, label-free measurement of the formation of affinity-binding complexes between two interacting macromolecules39 and provides a useful way to analyze the affinity interactions between peptides and TGF\(\beta_1\). Two reported TGF\(\beta_1\) binding peptides were synthesized with a terminal cysteine separated from the binding sequence by two glycine spacers (CGGWSHW39 and CGGKRIWFIRPS-SWy40) and then covalently linked to a dextran-functionalized
SPR flowcell surface using standard ligand-thiol coupling chemistry. After equilibrating the chip in HBS-EP running buffer, TGFβ1 solutions of varying concentration, from 5 to 100 nM, were injected across flow cells, and the normalized response, proportional to the amount of peptide/TGFβ1 complex formed on the chip surface, is reported in Figure 3. Both the KRIWFIPRSSWY (Figure 3a) and WSHW (Figure 3b) functionalized flowcells exhibit the formation of affinity complexes with TGFβ1 and show binding in a dose-dependent manner, confirming peptide/TGFβ1 affinity interaction. Analysis of the association and dissociation regimes of the sensogram yielded $k_d$, the peptide/TGFβ1 dissociation rate constants, and $K_D$, equilibrium dissociation constants, as shown in Table 3. Each peptide sequence was found to have affinity binding capacity for TGFβ1 in the nanomolar range, qualifying each as a strong binder; however, the $K_D$ values for the two peptides do not differ significantly.

### Affinity Peptides Do Not Inhibit Bioavailability of TGFβ1.

After SPR confirmation of peptide TGFβ1 affinity, an inhibition study was conducted to determine if peptides incubated with TGFβ1 would interfere with extracellular TGF receptors, preventing growth factor signaling. The WSHW and KRIWFIPRSSWY sequences were originally reported as TGFβ1 inhibitors, but inhibition was demonstrated through growth factor pull-down studies, where solutions of growth factor are incubated with peptide tethered to a solid phase resin. A bioactivity assay was required to investigate whether soluble peptides, complexed to TGFβ1 in solution, would interfere with TGF receptor signaling. PE.25 cells, transfected with a luciferase reporter gene for SMAD signaling, were incubated with solutions of WSHW, KRIWFIPRSSWY, and TGFβ1 (Figure 4). At a 10,000 molar excess and a peptide with a nanomolar dissociation constant, over 99% of the growth factor in solution will exist in the peptide:TGFβ1 affinity complex. Luciferase activity of the cell lysate was
insignificant in TGFβ1 conditions for both peptide solutions and a control, indicating affinity peptides are incapable of binding TGFβ1 receptors. Luciferase activity of TGFβ1-solutions was 3 orders of magnitude greater than that of respective TGFβ1-solutions, with no statistical difference between the activity of the peptide solutions and that of the control media. Thus, the peptide/TGFβ1 affinity complex does not inhibit the growth factor receptor signaling, and TGFβ1 incubated with peptides in monomer solutions retains its bioactivity in the absence of photoencapsulation.

**Soluble Affinity Peptides Protect TGFβ1 During UV Exposure.** To further explore the effect of soluble peptides on protecting TGFβ1 during photopolymerization reactions, a monomeric solution study was employed, similar to that previously described. Solutions of PEGMA (Mn = 2000), photoinitiator, and TGFβ1 were exposed to UV radiation and, subsequently, assayed via ELISA for TGFβ1 concentration, as were solutions containing WSHW or KRIWFIPRSSWY (R = 1000) (Figure 5). For PEGMA solution exposed to UV radiation in the absence of affinity peptides, TGFβ1 recovery was 75% of the pre-exposure concentration, while solutions, including WSHW or KRIWFIPRSSWY peptides, had a recovery of approximately 100%, not significantly different from the non-UV exposure condition (t test, p < 0.05.) These results confirm affinity peptides offer a protective effect for the encapsulated proteins during photopolymerization reactions. The mechanism of this protection is unknown, but one might speculate that it helps shield reactive sites on TGFβ1, preventing undesirable protein–polymer conjugation. Further, the inclusion of these affinity peptides in monomer solutions should presumably increase the total fractional release of encapsulated growth factor from PEGDA hydrogels, as their presence in acrylic solutions provides an additional release of encapsulated growth factor from PEGDA monomer solutions should presumably increase the total fractional release of encapsulated growth factor from PEGDA monomer solutions.

**Affinity Peptides Increase Fractional Release of Encapsulated TGFβ1.** To characterize the effect of affinity peptides on TGFβ1 release from PEG hydrogels, the growth factor was encapsulated in monomer solutions of PEGDA (Mn = 10000) with or without affinity peptides. Control gels (no peptide) and affinity gels (WSHW or KRIWFIPRSSWY at R = 1000) were monitored over a four-day time frame (Figure 6a). Of the three conditions, control gels exhibited the lowest fractional release of growth factor, with only 12.7 ± 1.2% of the TGFβ1 payload released by day four. Affinity peptide gels released a larger fraction of the encapsulated growth factor; WSHW gels released 30.4 ± 5.3% and KRIWFIPRSSWY gels with 60.3 ± 5.8% of encapsulated TGFβ1 over the same time interval. Interestingly, the release from KRIWFIPRSSWY peptide gels is much greater than that of gels with WSHW, although the two peptides exhibit similar dissociation constants (Table 3). Because the two affinity peptides differ in size and isoelectric point, the difference in fractional release between the two affinity systems may be partially attributable to changes in the solubility of the TGFβ1–peptide complex, relative to unbound growth factor. However, TGFβ1 consists of two identical 112 amino acid chains, and the affinity peptides are 4 or 12 amino acids, so it is less clear of their effects on the overall protein solubility. While SPR techniques quantify binding strength between TGFβ1 and a given peptide, the photoencapsulation reaction is complicated by the inclusion of PEGDA monomer and initiator species. To confirm that differences in release between KRIWFIPRSSWY and WSHW gels were not due to bulk material differences, the shear modulus was measured for equilibrium swollen PEGDA gels to elucidate any potential differences in the cross-linking density. Rheometric data showed inclusion of either affinity peptide in monomer solution did not significantly affect the swollen shear modulus, G', of the resulting polymer (Supporting Information). Thus, increased TGFβ1 release from peptide gels was not attributed to bulk differences in hydrogel cross-linking density. These results provide confirmation that affinity peptide sequences WSHW and KRIWFIPRSSWY increase the amount of TGFβ1 recovered from PEGDA hydrogels.

The presence of soluble peptides (R = 1000) in monomer solutions of TGFβ1 and PEGDA (Mn = 10000) also increased bioactive growth factor release over a 24 h period, as seen in Figure 6b. While ELISA-based techniques allow for quantification of the total released growth factor, the method utilizes antibody-based recognition of specific binding epitopes on the target protein. To confirm that TGFβ1 released from PEGDA hydrogels was structurally functional, a cellular recognition assay was required. PE.25 cells incubated with control gels with no affinity peptide had luciferase activity twice that of negative control conditions, while gels with affinity peptides showed no significant increase in luciferase activity, demonstrating the functional release of TGFβ1 from affinity peptide gels.

### Table 3. Affinity Peptide/TGFβ1 Kinetic Parameters Calculated from SPR Analysis

| peptide sequence       | k_d × 10^4 (s⁻¹) | K_D  |
|------------------------|------------------|------|
| CGGWSHW                | 9.85             | 8.20 |
| CGGKRIWFIPRSSWY        | 8.22             | 10.41|

**Figure 4.** Affinity binding peptides WSHW or KRIWFIPRSSWY do not inhibit TGFβ1 receptor signaling in media supplemented with TGFβ1, and peptides do not elicit a response from PE.25 cells in TGFβ1 deficient media. Error bars represent standard error (n = 4).

**Figure 5.** Affinity peptides protect TGFβ1 during photoencapsulation. Soluble affinity peptides, added to a monomer solution of 20 mM PEG (Mn = 2000 Da) monoacrylate and photoinitiator, increase the amount of recovered TGFβ1 after UV exposure. * indicates p < 0.05 (n = 4) for each peptide concentration, relative to solution containing no peptide. Error bars represent standard error (n = 4).
control conditions (TGFβ1), while cells cocultured with gels encapsulated with WSHW (R = 1000) had three times higher activity, and KRIWFIPRSSWY gels produced 5 times greater luciferase activity in the reporter cells. This result correlates to the trend in amount of TGFβ1 released (as measured with ELISA) over the same 24 h time frame in Figure 6a. Soluble affinity peptides, when included in monomer solutions prior to polymerization, are shown to increase the amount of bioactive TGFβ1 released from PEG hydrogels. Further, fractional release of TGFβ1 was increased when the peptide concentration in the monomer formulation was increased (Figure 7). The growth factor was encapsulated at 25 nM in PEDGA (Mn = 10000) for all studies, and the relative molar ratio of soluble peptide/TGFβ1 was varied from a low concentration of 2.5 μM (R = 100) to a maximum peptide concentration of 250 μM (R = 10000). Gels encapsulated with affinity peptides WSHW or KRIWFIPRSSWY had the highest fractional release over a two-day timespan with a ratio of R = 10000, and lower fractional release was observed at lower ratios. At R = 10000, the inclusion of the WSHW peptide resulted in 58.8 ± 4.8% recovery, while KRIWFIPRSSWY (R = 10000) gel exhibited complete release (115 ± 15.5%) of encapsulated TGFβ1 over a two day timespan. In conjunction with the monoaclrylate studies on TGFβ1 recovery, these findings indicate that the presence of affinity peptides can be used to increase the amount of soluble and bioactive TGFβ1 in encapsulated hydrogels, resulting in more predictable delivery and higher total fractional release.

CONCLUSION

Conditions to maximize release of the human cytokine TGFβ1 from photopolymerized PEG diacrylate hydrogel encapsulation were studied systematically. In solution studies, high acrylate concentration, greater than 20 mM, showed an increase in recoverable TGFβ1 but a lowered bioactivity via cell activity assays. Inclusion of a affinity binding peptide sequences in monomer solutions, prior to photoencapsulation, allowed a higher total release of TGFβ1 from PEG hydrogels, as well as increased bioactivity of released protein. The inclusion of soluble peptides provides a facile method for increasing the net recovery of encapsulated TGFβ1 in applications demanding localized and sustained delivery, such as tissue regeneration and wound healing.

ASSOCIATED CONTENT

Supporting Information. (S01) MALDI-TOF spectra for peptides used; (S02) Hydrodynamic radius estimates from Stokes−Einstein at 20 °C; (S03) Selected protein molecular weights and hydrodynamic radii; (S04) Swollen shear modulus for peptide and control polymers. This material is available free of charge via the Internet at http://pubs.acs.org.
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