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ARTICLE *in* BIOMACROMOLECULES · MARCH 2011

Impact Factor: 5.75 · DOI: 10.1021/bm101379v · Source: PubMed

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

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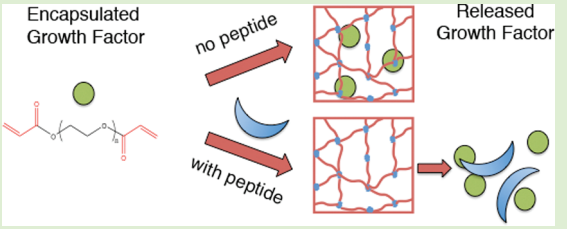
Supporting Information

ABSTRACT: Transforming growth factor beta (TGFβ_1) 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 TGFβ_1 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 TGFβ_1 delivery. Concentrations of acrylate group ranging from 0 to 50 mM and photopolymerization conditions were systematically altered to study their effects on TGFβ_1 bioactivity. In addition, two peptide sequences, WSHW (K_D = 8.20 nM) and KRIWFIPRSSWY (K_D = 10.41 nM), that exhibit binding affinity for TGFβ_1 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 bioactivity of TGFβ_1 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 TGFβ_1 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.[6] 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 (TGFβ_1), delivered locally to targeted cell populations or tissues.

TGFβ_1, a member of the TGF superfamily, regulates many cellular process including proliferation, differentiation, chemotaxis, and tumorigenesis.[4] TGFβ_1 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 TGFβ_1 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 species[14] 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

Received: November 17, 2010
Revised: January 19, 2011
Published: March 04, 2011

backbone or loss of protein conformation and, therefore, bioactivity.[13] 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,[16] collagen,[17] PLGA,[18] and PEG.[18] 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 sulfate 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_1.[26−28]

We hypothesized that the presence of free radicals generated during photoinitiated polymerizations would induce TGFβ_1 structural 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_1 bioactivity and availability. Quantification of released TGFβ_1 was determined by ELISA, while confirmation of TGFβ_1 bioactivity was achieved via a TGF-receptor reporter cell line. Further, we analyzed the binding affinity of the TGFβ_1 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_1, and the enhancement of TGFβ_1 recovery from photopolymerized PEG hydrogels was examined. The dose dependence of the peptide/TGFβ_1 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) diacrylate (PEGDA) monomers were prepared as previously described.[18] Briefly, hydroxyl-terminated poly(ethylene glycol) (M_n = 4600, 6000, or 10000 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 filtering step, excess toluene was removed under reduced pressure and subsequently precipitated into cold ethyl ether. ^{1}H 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% triisopropylsilane (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_1 Photodestruction Studies. Recombinant human TGFβ_1 solutions (Peprotech) (final concentration: 2 nM) were prepared in PBS in the presence of 1 mM lithium phenyl-2,4,6-trimethylbenzoylphosphinate[31] (LAP) initiator and varying concentrations of poly(ethylene glycol) monoacrylate (Monomer-Polymer and Dajac Laboratories; M_n = 2000 Da). Solutions were exposed to UV light (Omnicure 365 nm) at an intensity of 10 mW/cm^2 for 3 min. Following UV exposure, TGFβ_1 solutions were analyzed with a Human/Mouse TGFβ_1 ELISA kit (eBioscience) to determine the recovery of intact TGFβ_1

TGFβ_1 Release from PEG Hydrogels. Human TGFβ_1 at a final concentration of 25 nM was photoencapsulated in 10 wt % PEG (M_n = 10000 Da) hydrogels. For affinity peptide formulations, TGFβ_1 binding peptides WSHW or KRIWFIPRSSWY were also incorporated. Peptides were included at a molar ratio, R, relative to TGFβ, equal to 100, 1000, or 10000. 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 absorption on the wall of the vials. Supernatants were collected at predetermined time points and replaced with fresh release buffer. Concentrations of released TGFβ_1 were determined by ELISA.

TGFβ_1 Bioactivity Assay. TGFβ_1 bioactivity was confirmed with PE.25 cells stably transfected with a luciferase reporter gene. The assay was performed as reported previously.[32] Briefly, PE.25 cells were plated in 12-well plates (200000 cells/well) and incubated in serum-free DMEM media. PEGDA hydrogels (M_n = 10000 Da) encapsulated with 25 nM TGFβ_1 were placed in coculture with cells for 24 h at 37 °C and 5% CO_2. Cells were lysed in lysis buffer (Promega) and frozen at −80 °C for greater than 2 h. Lysate was centrifuged at 15000 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 (CM5) biosencor 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 H_3PO_4. 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*-phenyldiethanalamine 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 L-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_1, diluted in HBS-EP buffer, were injected using kinetic analysis injection protocols through Biacontrol 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_1 Release. 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 $(1 - R_h/\xi)$.[33] Here, R_h 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. Figure 1

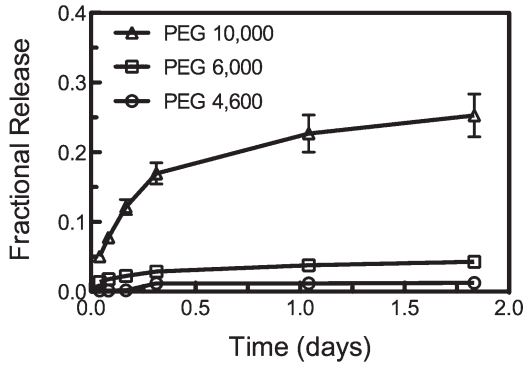


Figure 1. Fractional release of TGFβ_1 as a function of time when entrapped in PEG gels formed from the solution polymerization of PEGDA monomers of varying molecular weight. All gels were formed from 10 wt % monomer systems. The final network mesh size affects TGFβ_1 release. While PEG 4600 and PEG 6000 gels had no appreciable TGFβ_1 release, PEG 10000 gels had 25% fractional release over 2 days.

shows fractional release of TGFβ_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 TGFβ_1 payload over the same time frame. To determine if the lower TGFβ_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 TGFβ_1 has not been reported in the literature, proteins of similar molecular weight, including chymotrypsinogen[35] and enhanced green fluorescent protein,[36] have reported radii on the order of 28−35 Å (Supporting Information). However, because no appreciable amount of TGFβ_1 encapsulated in PEGDA 4600 and 6000 was released over a two day period, mesh size was not likely the principal determinant of TGFβ_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 TGFβ_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 TGFβ_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 TGFβ_1 Recovery in Solution. To characterize the effect of acrylate concentration on TGFβ_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 PEGDA−protein encapsulation reaction scheme.[13,37] 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 TGFβ_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 TGFβ_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 TGFβ_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 TGFβ_1 greater than 90% was found for acrylate concentrations above 40 mM (Figure 2A). TGFβ_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 TGFβ_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 TGFβ_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 TGFβ_1, in SDS solutions. While we were unable to directly measure PEGylated TGFβ_1, others have observed PEGylated proteins during chain polymerization of PEG monoacrylate monomers in solution with a model protein, lysozyme.[13] 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 TGFβ_1 from the polymer. This mechanism may explain, in part, the lower fractional release of TGFβ_1 from PEGDA 4600 and PEGDA 6000 hydrogels.

Surface Plasmon Resonance Studies Confirm TGFβ_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 TGFβ_1. SPR technology allows precise, label-free measurement of the formation of affinity-binding complexes between two interacting macomolecules[39] and provides a useful way to analyze the affinity interactions between peptides and TGFβ_1. Two reported TGFβ_1 binding peptides were synthesized with a terminal cysteine separated from the binding sequence by two glycine spacers (CGGWSHW[29] and CGGKRIWFIRPSSWY[30]) and then covalently linked to a dextran-functionalized

Table 1. Equilibrium Swelling and Calculated Average Mesh Size of Hydrogels Formed from the Solution Polymerization of Poly(ethylene glycol) Diacrylate Monomers of Varying Molecular Weight

PEGDA molecular weight (Da)	mass swelling ratio, q	volumetric swelling ratio, Q	mesh size, ξ (Å)
4600	17 ± 2	18 ± 2	200 ± 20
6000	24 ± 1	25 ± 1	300 ± 20
10000	29 ± 2	31 ± 2	380 ± 40

Table 2. Calculated Acrylate Concentrations in Formulations of Various Molecular Weight PEGDAs at 10 wt %, The Concentrations Used To Synthesize the Hydrogel Formulations

PEGDA molecular weight (Da)	acrylate concentration at 10 wt % monomer (mM)
4600	43.5
6000	33.3
10000	20

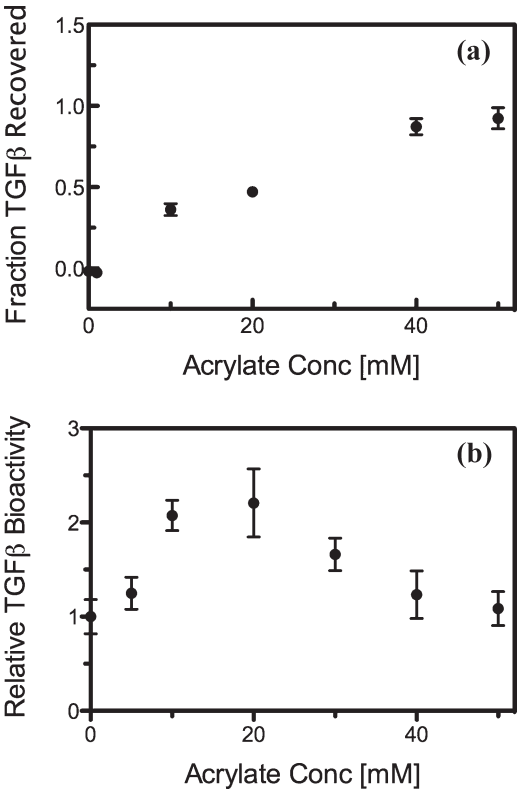


Figure 2. TGFβ_1 recovery from photopolymerized acrylate solutions. (a) In nongelling monoacrylate solutions, TGFβ_1 recovery is highest at high acrylate concentrations, as measured by ELISA. (b) Bioactivity assays across the same acrylate concentration range show a maximum at intermediate concentrations, as determined by reporter cell assay. Error bars represent standard error (n = 4).

SPR flowcell surface using standard ligand-thiol coupling chemistry. After equilibrating the chip in HBS-EP running buffer, TGFβ_1 solutions of varying concentration, from 5 to 100 nM,

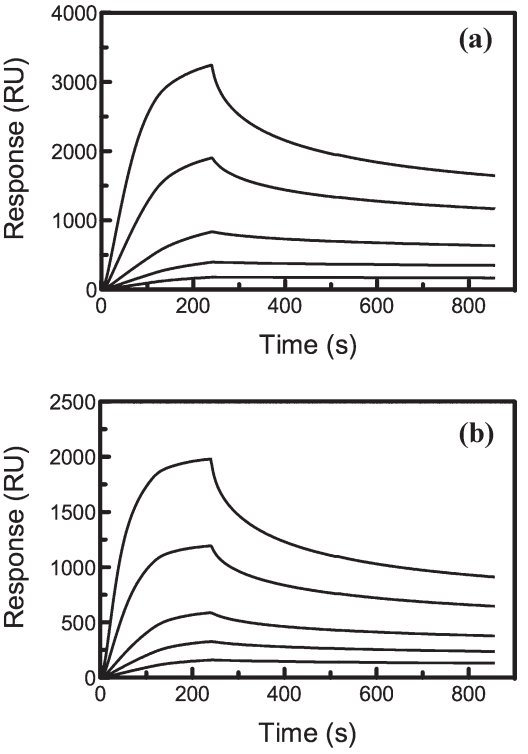


Figure 3. SPR sensorgrams for TGFβ_1 injection over surfaces functionalized with immobilized peptides: (a) CGGKRIWFIPRSSWY and (b) CGGWSHW. TGFβ_1 was injected at 100, 50, 20, 10, 5, and 0 nM in HBS-EP buffer at a flow rate of 50 μL/min. Sensorgrams represent the average signal for three injections.

were injected across flow cells, and the normalized response, proportional to the amount of peptide/TGFβ_1 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 TGFβ_1 and show binding in a dose-dependent manner, confirming peptide/TGFβ_1 affinity interaction. Analysis of the association and dissociation regimes of the sensogram yielded k_d, the peptide/TGFβ_1 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 TGFβ_1 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 TGFβ_1. After SPR confirmation of peptide TGFβ_1 affinity, an inhibition study was conducted to determine if peptides incubated with TGFβ_1 would interfere with extracellular TGF receptors, preventing growth factor signaling. The WSHW and KRIWFIPRSSWY sequences were originally reported as TGFβ_1 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 TGFβ_1 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 TGFβ_1 (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:TGFβ_1 affinity complex. Luciferase activity of the cell lysate was

Table 3. Affinity Peptide/TGFβ_1 Kinetic Parameters Calculated from SPR Analysis

peptide sequence	$k_d \times 10^4$ (s^{-1})	K_D
CGGWSHW	9.85	8.20
CGGKRIWFIPRSSWY	8.22	10.41

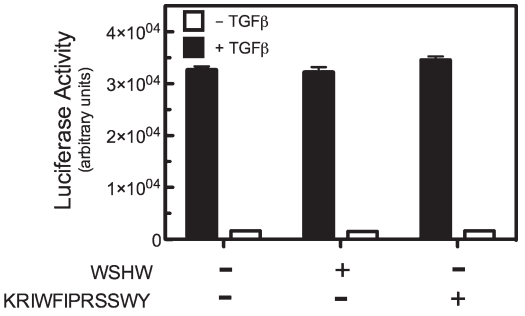


Figure 4. Affinity binding peptides WSHW or KRIWFIPRSSWY do not inhibit TGFβ_1 receptor signaling in media supplemented with TGFβ_1, and peptides do not ellicit a response from PE.25 cells in TGFβ_1 deficient media. Error bars represent standard error ($n = 4$).

insignificant in TGFβ_1- conditions for both peptide solutions and a control, indicating affinity peptides are incapable of binding TGFβ_1 receptors. Luciferase activity of TGFβ_1+ solutions was 3 orders of magnitude greater than that of respective TGFβ_1- solutions, with no statistical difference between the activity of the peptide solutions and that of the control media. Thus, the peptide/TGFβ_1 affinity complex does not inhibit the growth factor receptor signaling, and TGFβ_1 incubated with peptides in monomer solutions retains its bioactivity in the absence of photoencapsulation.

Soluble Affinity Peptides Protect TGFβ_1 During UV Exposure. To further explore the effect of soluble peptides on protecting TGFβ_1 during photopolymerization reactions, a monoacrylate solution study was employed, similar to that previously described. Solutions of PEGMA (M_n = 2000), photoinitiator, and TGFβ_1 were exposed to UV radiation and, subsequently, assayed via ELISA for TGFβ_1 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, TGFβ_1 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 TGFβ_1, 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 acrylate solutions provides an increase in recoverable TGFβ_1.

Affinity Peptides Increase Fractional Release of Encapsulated TGFβ_1. To characterize the effect of affinity peptides on TGFβ_1 release from PEG hydrogels, the growth factor was encapsulated in monomer solutions of PEGDA (M_n = 10000)

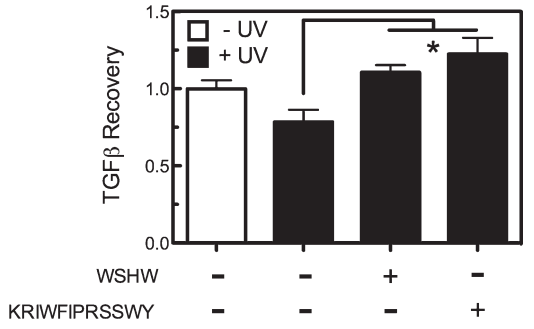


Figure 5. Affinity peptides protect TGFβ_1 during photoencapsulation. Soluble affinity peptides, added to a monomer solution of 20 mM PEG (M_n = 2000 Da) monoacrylate and photoinitiator, increase the amount of recovered TGFβ_1 after UV exposure. * indicates $p < 0.05$ ($n = 4$) for each peptide concentration, relative to solution containing no peptide. Error bars represent standard error ($n = 4$).

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 TGFβ_1 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 TGFβ_1 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 TGFβ_1−peptide complex, relative to unbound growth factor. However, TGFβ_1 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 TGFβ_1 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 TGFβ_1 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 TGFβ_1 recovered from PEGDA hydrogels.

The presence of soluble peptides (R = 1000) in monomer solutions of TGFβ_1 and PEGDA (M_n = 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 con-

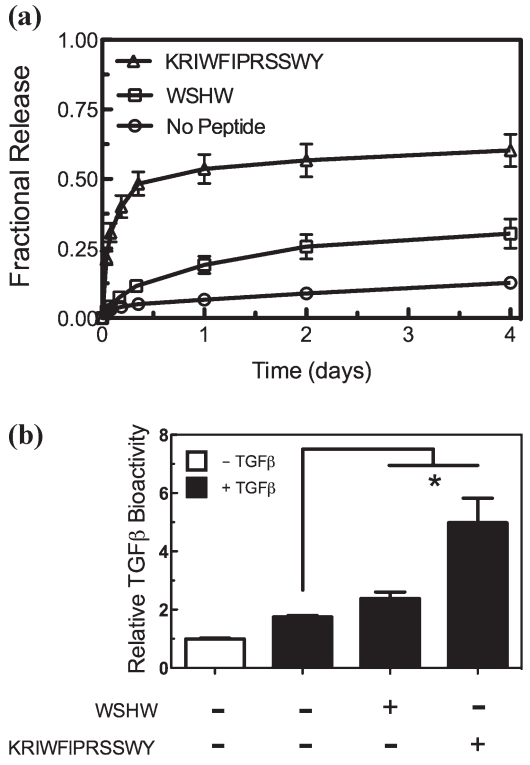


Figure 6. Affinity peptides increase release of encapsulated TGFβ_1 from PEG (M_n = 10000 Da) hydrogels. (a) Hydrogels encapsulated with TGFβ and affinity peptides (R = 1000) show greater release than gels encapsulated with growth factor alone. Error bars represent standard error (n = 4). (b) When peptides are encapsulated with TGFβ_1 in hydrogels, a higher cellular response is seen at 24 h, relative to hydrogels without TGFβ_1 or those with TGFβ_1 and no peptide. * represents p < 0.05 (n = 4) relative to TGFβ_1 positive gels with no affinity peptides.

trol conditions (TGFβ_1-), 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 TGFβ_1 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 TGFβ_1 released from PEG hydrogels.

Further, fractional release of TGFβ_1 was increased when the peptide concentration in the monomer formulation was increased (Figure 7). The growth factor was encapsulated at 25 nM in PEDGA (M_n = 10000) for all studies, and the relative molar ratio of soluble peptide/TGFβ_1 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 TGFβ_1 over a two day timespan. In conjunction with the monoacrylate studies on TGFβ_1 recovery, these findings indicate that the presence of affinity peptides can be used to increase the amount of soluble and bioactive TGFβ_1 in encapsulated hydrogels, resulting in more predictable delivery and higher total fractional release.

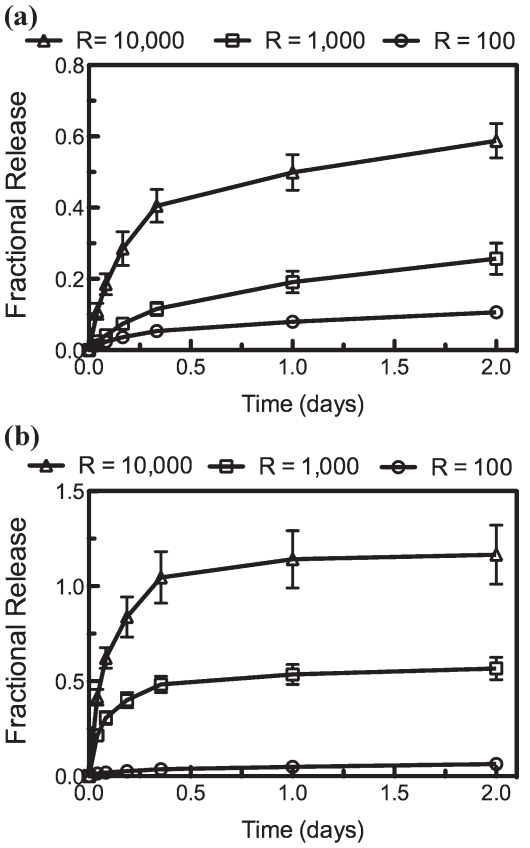


Figure 7. Increased concentration of affinity peptides increases release of encapsulated TGFβ_1 from PEG (M_n = 10000 Da) hydrogels. For both (a) WSHW and (b) KRIWFIPRSSWY peptides, high concentrations of peptide (R = 10000) results in maximum release of encapsulated TGFβ_1. For KRIWFIPRSSWY, 100% fractional release is achieved in 1 day at R = 10000, while R = 100 gels only released 6.4 ± 1% over a 2 day time span. For WSHW gels, R = 10000 gels achieved 58.8 ± 5% release in 2 days, while R = 100 gels only released 10.6 ± 1% of their payload in the same time. Error bars represent standard error (n = 4).

CONCLUSION

Conditions to maximize release of the human cytokine TGFβ_1 from photopolymerized PEG diacrylate hydrogel encapsulation were studied systematically. In solution studies, high acrylate concentration, greater than 20 mM, showed an increase in recoverable TGFβ_1 but a lowered bioactivity via cell activity assays. Inclusion of affinity binding peptide sequences in monomer solutions, prior to photoencapsulation, allowed a higher total release of TGFβ_1 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 TGFβ_1 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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ACKNOWLEDGMENT

PE.25 cells were a kind gift from Dr. Xui Dong Liu. The authors thank Shaun Bevers of the Biophysics Core Lab at the University of Colorado Health Sciences Center for support with SPR studies and Jake Luoma of the University of Colorado at Boulder for performing rheometric measurements. This work was funded through NIH grant AR053126 and the Howard Hughes Medical Institute.

REFERENCES

(1) Eswarakumar, V. P.; Lax, I.; Schlessinger, J. *Cytokine Growth Factor Rev.* **2005**, *16*, 139–149.
(2) Tayalia, P.; Mooney, D. J. *Adv. Mater.* **2009**, *21*, 3269–3285.
(3) Favoni, R. E.; De Cupis, A. *Pharmacol. Rev.* **2000**, *52*, 179–206.
(4) Cross, M.; Dexter, T. M. *Cell* **1991**, *64*, 271–280.
(5) Berk, B. C. *Physiol. Rev.* **2001**, *81*, 999–1030.
(6) Lee, S. J. *Yonsei Med. J.* **2000**, *41*, 704–719.
(7) Erickson, G. R.; Gimble, J. M.; Franklin, D. M.; Rice, H. E.; Awad, H.; Guilak, F. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 763–769.
(8) Johnstone, B.; Hering, T. M.; Caplan, A. I.; Goldberg, V. M.; Yoo, J. U. *Exp. Cell Res.* **1998**, *238*, 265–272.
(9) Salinas, C. N.; Anseth, K. S. *Biomaterials* **2008**, *29*, 2370–2377.
(10) Salinas, C. N.; Cole, B. B.; Kasko, A. M.; Anseth, K. S. *Tissue Eng.* **2007**, *13*, 1025–1034.
(11) Beck, L.; DAmore, P. A. *FASEB J.* **1997**, *11*, 365–373.
(12) Mann, B. K.; Schmedlen, R. H.; West, J. L. *Biomaterials* **2001**, *22*, 439–444.
(13) Lin, C. C.; Sawicki, S. M.; Metters, A. T. *Biomacromolecules* **2008**, *9*, 75–83.
(14) Dean, R. T.; Fu, S. L.; Stocker, R.; Davies, M. J. *Biochem. J.* **1997**, *324*, 1–18.
(15) Elias, R. J.; McClements, D. J.; Decker, E. A. *J. Agric. Food Chem.* **2005**, *53*, 10248–10253.
(16) Gombotz, W. R.; Wee, S. F. *Adv. Drug Delivery Rev.* **1998**, *31*, 267–285.
(17) Olsen, D.; Yang, C. L.; Bodo, M.; Chang, R.; Leigh, S.; Baez, J.; Carmichael, D.; Perala, M.; Hamalainen, E. R.; Jarvinen, M.; Polarek, J. *Adv. Drug Delivery Rev.* **2003**, *55*, 1547–1567.
(18) Baldwin, S. P.; Saltzman, W. M. *Adv. Drug Delivery Rev.* **1998**, *33*, 71–86.
(19) Lin, C. C.; Anseth, K. S. *Adv. Funct. Mater.* **2009**, *19*, 2325–2331.
(20) Lin, C. C.; Boyer, P. D.; Aimetti, A. A.; Anseth, K. S. *J. Controlled Release* **2010**, *142*, 384–391.
(21) Lin, C. C.; Metters, A. T.; Anseth, K. S. *Biomaterials* **2009**, *30*, 4907–4914.
(22) Seal, B. L.; Panitch, A. *Biomacromolecules* **2003**, *4*, 1572–1582.
(23) Wissink, M. J. B.; Beernink, R.; Pieper, J. S.; Poot, A. A.; Engbers, G. H. M.; Beugeling, T.; van Aken, W. G.; Feijen, J. *Biomaterials* **2001**, *22*, 2291–2299.
(24) Sakiyama-Elbert, S. E.; Hubbell, J. A. *J. Controlled Release* **2000**, *69*, 149–158.
(25) Tae, G.; Scatena, M.; Stayton, P. S.; Hoffman, A. S. *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 187–197.
(26) Freeman, I.; Cohen, S. *Biomaterials* **2009**, *30*, 2122–2131.
(27) Drinnan, C. T.; Zhang, G.; Alexander, M. A.; Pulido, A. S.; Suggs, L. J. *J. Controlled Release*, *147*, 180−6.
(28) Schroeder-Tefft, J. A.; Bentz, H.; Estridge, T. D. *J. Controlled Release* **1997**, *49*, 291–298.
(29) Young, G. D.; Murphy-Ullrich, J. E. *J. Biol. Chem.* **2004**, *279*, 47633–47642.
(30) Dotor, J.; Lopez-Vazquez, A. B.; Lasarte, J. J.; Sarobe, P.; Garcia-Granero, M.; Riezu-Boj, J. I.; Martinez, A.; Feijoo, E.; Lopez-Sagaseta, J.; Hermida, J.; Prieto, J.; Borras-Cuesta, F. *Cytokine* **2007**, *39*, 106–115.
(31) Fairbanks, B. D.; Schwartz, M. P.; Bowman, C. N.; Anseth, K. S. *Biomaterials* **2009**, *30*, 6702–6707.
(32) Clarke, D. C.; Brown, M. L.; Erickson, R. A.; Shi, Y. G.; Liu, X. D. *Mol. Cell. Biol.* **2009**, *29*, 2443–2455.
(33) Mason, M. N.; Metters, A. T.; Bowman, C. N.; Anseth, K. S. *Macromolecules* **2001**, *34*, 4630–4635.
(34) Peppas, N. A.; Merrill, E. W. *J. Polym. Sci., Part A: Polym. Chem.* **1976**, *14*, 459–464.
(35) Rosenblatt, J.; Rhee, W.; Wallace, D. *J. Controlled Release* **1989**, *9*, 195–203.
(36) Zustiak, S. P.; Boukari, H.; Leach, J. B. *Soft Matter* **2010**, *6*, 3609–3618.
(37) Lin, C. C.; Metters, A. T. *Pharm. Res.* **2006**, *23*, 614–622.
(38) Hu, J. L.; Duppatla, V.; Harth, S.; Schmitz, W.; Sebald, W. *Bioconjugate Chem.* **2010**, *21*, 1762–1772.
(39) Johnsson, B.; Lofas, S.; Lindquist, G. *Anal. Biochem.* **1991**, *198*, 268–277.