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Manipulating Cell Migration and Proliferation with a Light-Activated Polypeptide

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Polypeptide growth and differentiation factors modulate a wide variety of cell behaviors and can be used to manipulate cells in vitro for tissue engineering and basic studies of cell biology. To emulate in vitro the spatial aspect of growth factor function, new methods are needed to generate defined spatial gradients of activity. Polypeptide factors that are engineered to be activated with light provide a method for creating concentration gradients with the fine precision in space and time with which light can be directed. As a first test of this approach, we have chemically synthesized a polypeptide with the sequence of epidermal growth factor in which a critical glutamate is "caged" with a photoremovable group. Photolysis of this polypeptide afforded maximal mitogenic and chemokinetic activity at concentrations at which the caged factor was inactive. Spatially resolved photolysis of the factor resulted in spatial patterning of fibroblasts. This system will be useful for ex vivo tissue engineering and for investigating the interactions of cells with their matrix and the role of chemical gradients in biological pattern formation.

Introduction

Polypeptide growth factors modulate a wide variety of cellular behaviors. Initiating their effects by binding to extracellular receptors, they direct proliferation, migration, differentiation, matrix remodeling, and apoptosis.[1] They mediate communication between cells to coordinate their assembly into complex functional tissues during normal embryogenesis, tissue maintenance, and wound healing as well as in pathological conditions such as cancer.[2]

The behaviors elicited by growth factors are coordinated phenotypic programs. Growth factors stimulate groups of signaling pathways to influence large-scale cell behaviors. For example, cell migration in response to a growth factor involves coordinated cytoskeletal alterations, changes in cell–substrate adhesion, and extracellular matrix remodeling.[3] This ability to induce large-scale behavioral and phenotypic effects makes growth factors useful tools for manipulating cell behavior in vitro. Thus, basic studies of cell–cell and cell–matrix interactions have used growth factors to direct cell behaviors.[4–8] Furthermore, if efforts to engineer functional tissues ex vivo are to take advantage of the natural processes of tissue development,[9] they will utilize the phenotypic effects of the growth factors that naturally coordinate these processes.

In their native biological contexts, growth factors influence cell behavior with spatial dependence. Concentration gradients, which are formed by diffusion of the factors from localized sources create spatial patterns of their effects. A concentration gradient localizes the effect of a growth factor to the region of optimal concentration. Furthermore, in some cases, such as chemotaxis[10] and biological pattern formation by morphogens,[5,11] the gradient itself is required for activity.

To emulate these spatial effects in vitro, a variety of techniques have been developed for creating gradients of growth factors, including patterned immobilization,[12,13] controlled release,[4,14–18] and printing of growth factors on gel substrates.[19] However, the techniques available are limited by low spatial or temporal resolution, a dependence on specially formulated nonbiological substrates for cell growth, or creation of static, immobilized gradients that, once formed, are difficult to erase or amend. Thus, there is a need for methods that allow dynamic, high-resolution creation of growth factor gradients without dependence on a particular substrate. To fill this need, we are exploring the use of caged growth factors.

Photocaging is an approach to controlling protein activity with high precision in space and time.[20–32] In this approach, a photolabile protective group masks a critical functional element of the protein, yielding an inactive species. Photolysis of the masking group "uncages" the protein in its active composition. This activation can be controlled with the high spatial precision with which light can be directed ($<1\ \mu m$).[33] Thus, we consider creation and utilization of caged growth factors to be a promising approach to directing cell behavior. A photocaged factor that is delivered to a two- or three-dimensional culture of cells can be activated by photolysis in a defined region at the desired time and washed away when its effects are no longer desired.

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Results and Discussion

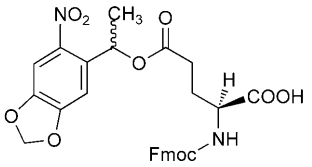
Synthesis of light-activated EGF-based polypeptide

Native human epidermal growth factor (EGF) is a polypeptide of 53 residues (Scheme 1 A).[34] It has mitotic, chemokinetic, and chemotactic effects on a wide range of cell types.[35–38] We have created a light-activated polypeptide by synthesizing the EGF sequence with a photolabile protective group on the side chain of glutamate 40. Mutation of this side chain to a glutamine has been shown to result in a fourfold decrease in receptor-binding activity.[39] Thus, we speculated that placement of a protective group on this glutamate would interfere with receptor binding by masking the carboxylate side chain of that residue.

A)

NSDSECPLSHDGYCLHDGVCMYIEALD
KYACNCWGYIGERCQYRDLKWWELR

B)



Scheme 1. Incorporation of caged glutamate into human EGF. A) Sequence of human EGF with the critical glutamate in bold type. B) Caged glutamate for incorporation into EGF at position 40 by solid-phase peptide synthesis.

We synthesized the protected peptide by incorporation of a photoprotected glutamate monomer (Scheme 1 B) during solid-phase peptide synthesis. The caged glutamate derivative that we employed was stable to the conditions of Fmoc solid-phase peptide synthesis. The presence of the α-nitropiperonyl protective group in the full-length product was confirmed by matrix-assisted laser desorption ionization mass spectrometry (MALDI MS).

Removal of this protective group was observed chromatographically (Figure 1 A). The HPLC trace of the starting material was a double peak with a shoulder, despite multiple rounds of HPLC purification. The double peak might be due to the presence of two poorly resolved diastereoisomers originating from the racemic protective group. The persistence of the shoulder through multiple rounds of purification suggests that it is an alternatively folded form of the polypeptide in equilibrium with the predominant fold. Irradiation with near-UV light (365 nm) produced a single product peak that appeared at approximately the same rate as the disappearance of the starting material, with a half-life of 36 s at 30 mW cm^{-2} (Figure 1). The molecular weight of the photolysis product was determined by MALDI MS and was consistent with removal of the protective group (see the Supporting Information). The following experiments demonstrate that the photolyzed material has biological activity that is qualitatively similar to that of EGF.

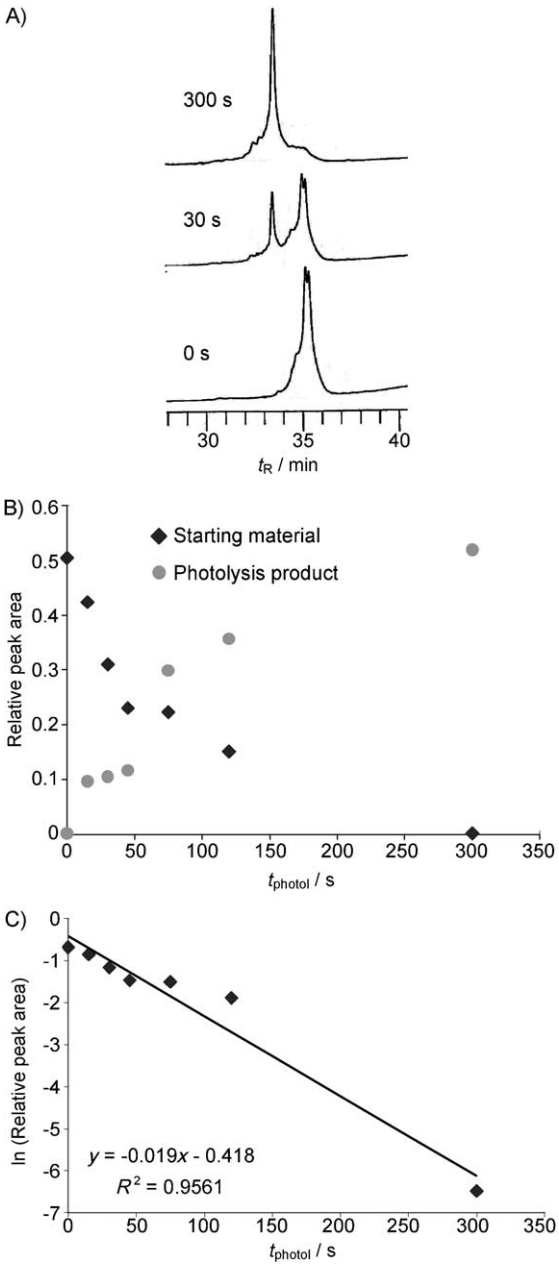


Figure 1. Photolysis of caged EGF. A) Chromatograms of caged EGF and photolysis products at 0, 30, and 300 s photolysis as indicated. MALDI-MS of the peak in the 0 s chromatogram *m/z* 6412, expected for caged EGF *m/z* 6414. MALDI-MS for product peak *m/z* 6222, expected for EGF *m/z* 6222 (In both cases expected masses assume no formation of disulfide bonds). B) Peak areas of starting material (◆) and photolysis product (●) relative to internal reference peak as a function of photolysis time; C) ln(relative peak area) of the starting material vs. photolysis time. The half-life of photolysis was calculated by linear regression analysis of this plot ($t_{1/2} = \ln 0.5/\text{slope}$).

Cell proliferation stimulated by light-activated EGF

To assess the effect of photocaging on the mitogenic activity of the polypeptide, we measured the factor's ability to stimulate proliferation of fibroblasts. The caged factor was added to a culture of fibroblasts, and it was added after complete photolysis to a separate culture of fibroblasts. In control experiments, a commercially obtained sample of native recombinant

human EGF or no growth factor was added to separate cultures. The mean cell density in each culture as a function of time was determined (Figure 2 A). The caged polypeptide did not stimulate proliferation of the fibroblasts at the concentration tested (50 ng mL^{-1}); the cell density decreased slightly as a function of time to an extent similar to that in the factor-free negative control. However, after photolysis, the uncaged synthetic growth factor stimulated proliferation of the fibroblasts to an extent similar to that stimulated by recombinant EGF.

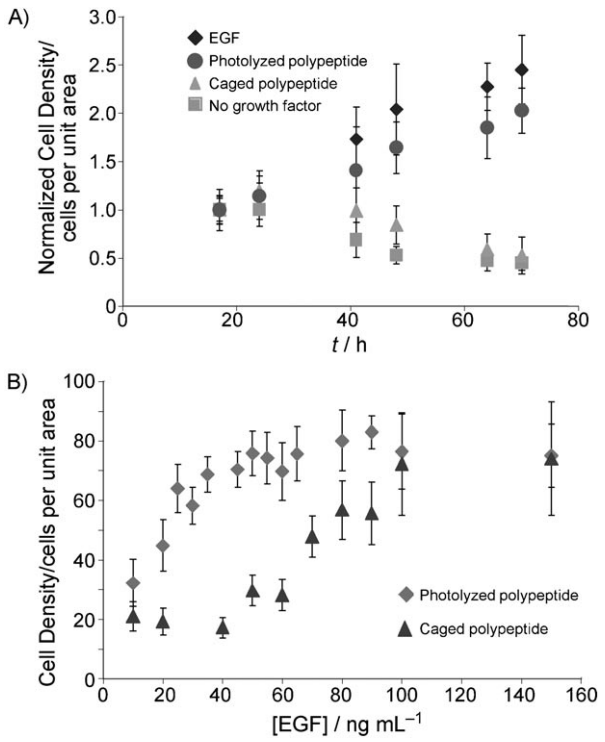


Figure 2. Cell proliferation was stimulated by caged polypeptide; before and after photolysis. A) Time course of proliferation of NIH/3T3 fibroblasts. Growth factor concentration was 50 ng mL^{-1} when present. B) Concentration dependence of polypeptide-stimulated proliferation of NIH/3T3 fibroblasts before and after photolysis. Error bars in A) and B) are 95% confidence intervals for mean cell counts in 20 microscopic fields.

The concentration dependence of polypeptide-stimulated proliferation before and after photolytic uncaging is shown in Figure 2 B. A portion of a stock solution of protected EGF was photolyzed to completion. Photolyzed and unphotolyzed factor were then added at varying dilutions to cultures of fibroblasts, which were incubated for 72 h prior to determination of the mean cell density. At sufficiently high concentrations (>80 ng mL^{-1}), both the caged polypeptide and its photolysis product stimulated proliferation of fibroblasts. However, over the concentration range of 25–50 ng mL^{-1}, the unphotolyzed growth factor displayed little or no activity, whereas the photolyzed factor displayed near-maximal activity.

Cell migration is stimulated by light-activated EGF

We have tested the activity of the caged polypeptide before and after photolysis for stimulating migration of human dermal fibroblasts. Cell migration was observed qualitatively with a "scratch–wound" assay[40,41] (Figure 3). A confluent culture of fibroblasts was scratched to create a cell-free region. Cell movement was observed as the cells migrated to fill in the scratch. The caged polypeptide stimulated little migration, comparable to a control experiment with no growth factor. However, the same material after photolysis stimulated substantially more "healing" of the scratch.

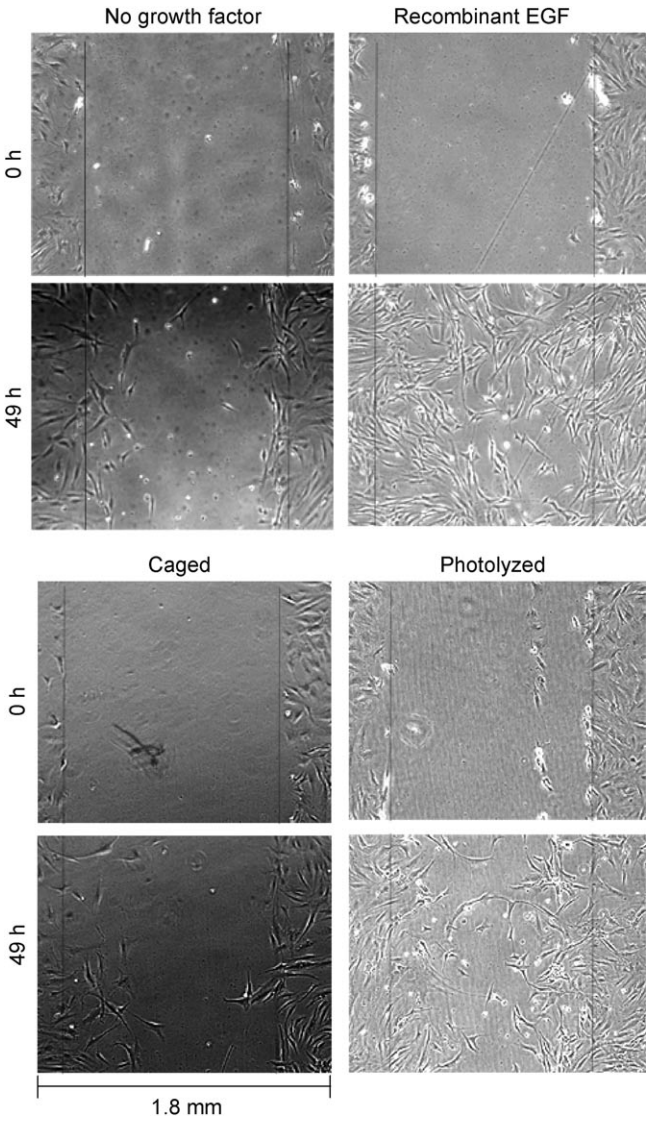


Figure 3. Scratch–wound assay for EGF-induced migration of human dermal fibroblasts. Photomicrographs of representative regions of scratch–wounds at the indicated times. Vertical lines indicate the alignment of the 0 h image and the 49 h image. Scratch-wounded cultures of fibroblasts were incubated with medium that contained the indicated growth factor: photoprotected (caged) polypeptide, caged polypeptide following photolysis, recombinant (commercial) EGF, or no growth factor. In all experiments that contained growth factor, it was present at 1 ng mL^{-1}, which was chosen because the chemokinetic activity of EGF toward fibroblasts decreases at concentrations above 1 ng mL^{-1}.[7]

Cell migration was assessed quantitatively by using the Boyden chamber method,[42] in which the number of cells migrating through a porous membrane is determined. Different initial growth factor concentrations on opposite sides of the membrane create a gradient to which the cells can respond chemotactically or chemokinetically. The chamber in which the cells were seeded initially contained growth-factor-free medium, and the chamber on the other side of the membrane initially contained 1 ng mL^{-1} polypeptide. Parallel experiments were done with caged polypeptide, caged polypeptide after complete photolysis, and in control experiments, recombinant native EGF, or no growth factor (Figure 4).

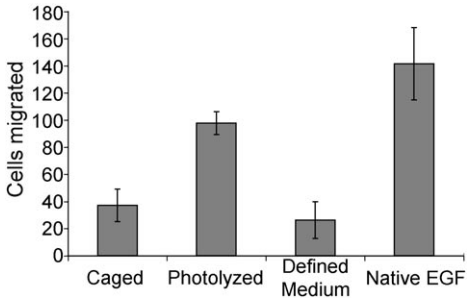


Figure 4. Boyden chamber assay for fibroblast migration stimulated by caged and light-activated polypeptide. Each value is the mean number for cells migrated on six separate membranes, and error bars are standard deviations in the mean.

Consistent with the scratch–wound assay, the caged polypeptide is almost completely inactive in the Boyden chamber assay; the number of cells migrating in its presence are within experimental error of the number of cells migrating in the absence of any growth factor. Furthermore, photolysis of the caged polypeptide resulted in a substantial increase ($P < 0.0002$) in the ability to stimulate cell migration. Though MS and HPLC analysis indicated that photolysis had been carried out to completion, the photolyzed material did not stimulate migration as effectively as the fivefold increase in cell migration brought about by native EGF. Nevertheless, photocaging clearly affords a method for triggering its ability to stimulate cell migration.

Light-directed spatial patterning of polypeptide activity

Whereas many of the effects that have been sought previously with light-triggered molecules are rapid, the large-scale phenotypic effects of growth factors, are apparent after time periods of hours to days. The active factor must be present, at least intermittently,[43] during this period. Therefore, to spatially control the effects of the light-activated polypeptide, it was necessary to maintain a gradient in its concentration over a time period of that magnitude, despite diffusion on a more rapid timescale. Continuous activation of the growth factor in the desired region was not possible, because continuous irradiation with near-UV light at relevant power levels killed the cells (data not shown). Therefore, we repetitively established a gradient by intermittently activating growth factor in the desired region.

Fibroblasts were seeded uniformly on a glass window of the culture device and allowed to adhere and spread. Defined medium that contained caged polypeptide (40 ng mL^{-1}) was delivered to the culture, and a region of the culture was illuminated intermittently. Illumination times for activation (see caption of Figure 5) were chosen based on the relationship of concentration to proliferative activity in Figure 2B, the expectation that the photolysis rate would be linearly related to the light power,[44] and an estimate of the diffusion coefficient of the EGF in aqueous medium of 10^{-6} cm^2 s.[45] Preliminary experiments confirmed survival and normal proliferation of the cells with the chosen light regimen (data not shown). In our model for this process, as activated growth factor diffused out of the illuminated area, thereby diminishing the gradient, caged growth factor diffused into the illuminated area from the surrounding volume of the culture chamber and became available for photolysis. As the caged factor was depleted, the gradient

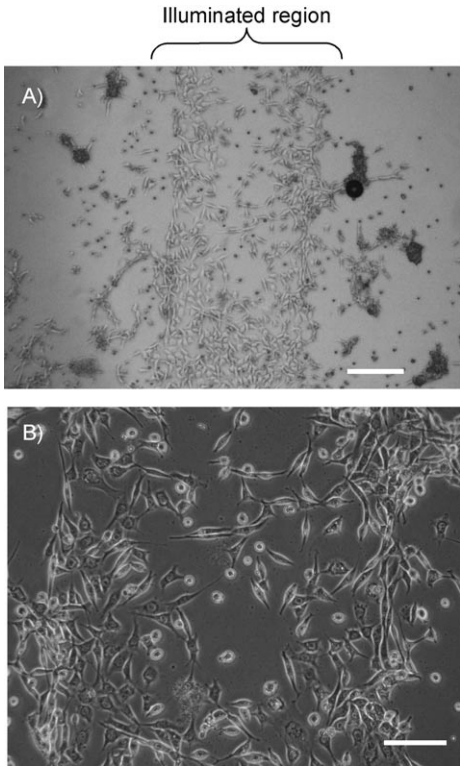


Figure 5. Spatially resolved activation of light-activated epidermal growth factor. A) Photomicrograph of a culture of NIH/3T3 fibroblasts treated with light-activated polypeptide. Cells were initially seeded at a density of 20 cells per mm^2 and allowed to adhere and spread before delivery of caged polypeptide and illumination. The indicated vertical strip, 340 μm wide, was illuminated for 90 s, followed by a 6 min wait, then a second pulse of illumination for 30 s. This regimen of illumination was repeated once every hour. The medium containing caged polypeptide was replaced every four hours, followed by a 30 min wait for the system to equilibrate before resuming the hourly illumination regimen. This regimen was maintained throughout the three-day experiment. The large dark features are aggregates of non-adherent spheroidal cells, presumed to be nonviable. Scale bar: 100 μm. B) Enlarged view of a portion of (A) with phase contrast. Scale bar: 50 μm.

that could be produced became shallower, so the medium was replaced regularly. The illumination and fluid replacement were automated to make the procedure reproducible and convenient.

The result after three days is shown in Figure 5. A clear band of cells (ca. 300 μm wide) is visible in the position of illumination. The nonuniformity of the cell density within this band is comparable to that typically seen in nonconfluent cultures of this cell type. A general alignment of cells along the edge of this band is noteworthy, though the origin of this effect is not clear. Outside of the illuminated region, few spread cells remain, and a low density of spheroidal cells decreases with increasing distance from the illuminated region. In control experiments, no illumination-dependent variations in cell distribution were observed when recombinant EGF was used instead of the caged polypeptide. The cells remained spread and proliferated uniformly across the surface. Also, when growth factor was omitted entirely from the medium, few cells survived for three days, instead a low density of spread and spheroidal cells was distributed without illumination-dependent variation. When the light-activated factor was used, but cells were omitted from the chamber until after the three day period of illumination, seeded cells did not adhere preferentially in the illuminated region; this suggests that the illumination-dependent density of cells was not due to immobilization of the polypeptide on the surface. Taken together, these control experiments indicate that the illumination-dependent variation in cell density is due to localized activation of diffusible polypeptide.

The pattern of cells created in this experiment confirms that repetitive, localized activation of a diffusible polypeptide factor can have localized effects on cell behavior. It suggests that activity is established after photolysis (including any conformational changes required for activity) on a timescale that allows a significant gradient in activity despite ongoing diffusion. The chemokinetic activity of EGF toward fibroblasts is partly chemotactic,[35] and both chemotactic migration of cells toward the illuminated area and increased survival and proliferation of cells within the illuminated region might contribute to the creation of this pattern.

Conclusions

A variety of proteins have been created previously in a light-activated form by caging critical functional groups, often to intervene within signal-transduction pathways,[21,27,46] to control rapid and transient processes such as ion channel gating[24] and cytoskeletal rearrangement,[47] or to control membrane permeability[20] (see refs. [48] and [49] for comprehensive reviews). Caged proteins have not been previously made that affect the global behavioral changes, such as migration and proliferation of populations of cells, that are influenced by growth factors. (For a short light-activated peptide designed to control lymphocyte migration see ref. [50].) Light-activation provides an approach to spatially resolved "remote-control" of such behaviors. Growth factors exert their effects extracellularly, obviating the need for intracellular delivery or endogenous expression of the modified protein.[51]

Both the mitogenic and chemokinetic activities of our polypeptide were clearly affected by the presence of the caging group on the masked glutamate. In both cases, concentrations were easily found where the caged factor had negligible activity and the photolyzed factor had substantial or near maximal activity. The concentration required for half-maximal activity in stimulating cell proliferation decreased by a factor of three upon photolysis; this is consistent with the fourfold difference of receptor-binding affinity between native EGF and the E40Q mutant of EGF.[39]

Other light-activated proteins and polypeptides that have been reported vary widely in the ratio of their uncaged-to-caged activity. Several examples have been reported for which this ratio is three- to fourfold,[26,52,53] which is similar to the threefold difference we observe in concentration for half-maximal activity. Other reported light-activated proteins also vary widely in the amount of activity recovered on photolysis. These parameters depend in part on the nature of the polypeptide being engineered and are best evaluated by the usefulness of the resulting polypeptide for manipulating biological response.[48] We have demonstrated the use of our light-activated peptide for patterning live cells on an unpatterned substrate, an important challenge in tissue engineering and studies of cell–cell and cell–substrate interactions. We were able to harness the threefold difference in concentration for half-maximal activity for this purpose, because the cell response is not linear with respect to concentration. Thus, at rationally chosen polypeptide concentrations, photolysis creates a steep gradient in activity between illuminated and unilluminated regions.

Other growth factors in light-activated form will be useful for spatial and temporal control of cell behavior for studies of cell–cell and cell–matrix interactions and in tissue engineering. This methodology can be applied to many different growth factors with a wide range of phenotypic effects and cell-type specificities. A considerable body of structural and mutagenesis data is available for many growth factors, allowing for rational choices of residues to mask. The nonlinear relationship between concentration and activity that enhances the attainable gradient in activity is characteristic of most growth factors. Though most growth and differentiation factors are larger than EGF, native chemical ligation can be applied to the synthesis of these polypeptides. The effectiveness of native chemical ligation for the synthesis of light-activated polypeptides[25] underscores the accessibility of this technique to growth factors that are too large for direct synthesis by solid-phase peptide synthesis, which requires ligation of caged fragments with other polypeptides or expressed proteins.[54]

In addition to extending this methodology to more growth factors, it will be desirable to extend it to three-dimensional contexts. In biological tissues, cells are normally found within a three-dimensional extracellular matrix, and it is well known that cells exhibit different behavior in three-dimensional cultures than when residing on flat surfaces.[55–58] Furthermore, the central challenges of ex vivo tissue engineering involve creation of three-dimensional tissue constructs.[59] A transpar-

ent matrix for cell growth, such as natural collagen or fibrin gels, will allow activation by light of a soluble factor and will have the benefit of slowing diffusion. High resolution of three-dimensional photochemical control has been demonstrated by using multiple caging groups[60] or a protective group with a high two-photon cross section.[61] Caging groups that can be removed with two photons of infrared light have the additional advantage that infrared light penetrates the compacted matrices of normal tissues more efficiently than ultraviolet light does.[62]

This report describes a light-activated protein that affords "remote control" of large-scale behaviors of cell populations. Cell migration and proliferation, behaviors that occur on the size scale of tissue organization, can be controlled in time and space with this light-activated growth factor. The factor is diffusible and presumably acts on an extracellular receptor. Thus, neither direct manipulation of individual cells, such as microinjection, nor chemical manipulation of the substrate, such as spatial patterning, is required. Because the growth factor may diffuse on a timescale faster than the timescale of the behavioral changes it evokes, effective spatial gradients of the active factor can be maintained by repetitive photolytic activation in the desired region. Light-activated growth factors and the general methodology of using light-activated growth and differentiation factors to manipulate cell behaviors have the potential to be useful in basic studies of the cell–cell and cell–matrix interactions that direct tissue growth and maintenance. In addition, the large number of growth factors and different growth-factor-induced cellular behaviors, the high degree of spatial and temporal resolution that can be obtained with photochemistry, and the dynamic nature of using soluble factors suggest potential for this technique to organize cells for tissue engineering.

Experimental Section

General: 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), hydroxybenzotriazole (HOBt), Fmoc arg(Pmc) Wang resin, and amino acids were from Novabiochem. *S*-Tritylmercaptopropionic acid was from Peptides International (Louisville, KY,USA). *N,N*-diisopropylethylamine (DIEA), diisopropylcarbodiimide (DIC), dicyclohexylcarbodiimide (DCC), *N*-methylmorpholine (NMM), 4-dimethylaminopyridine (DMAP), piperidine, trifluoroacetic acid (TFA), ethanedithiol, thioanisole, phenol, and triisopropylsilane were from Aldrich. Methylbenzhydrylamine (MBHA) resin was from Anaspec. 9-fluorenylmethyl chloroformate was from Fluka. Fmoc amide resin was from Applied Biosystems. *N*-methylpyrrolidinone (NMP), dimethylformamide (DMF) and HPLC grade acetonitrile (MeCN) and CH_2Cl_2 were from EMD (Gibbstown, NJ, USA). MALDI-MS was performed on a Micromass L/R reflectron instrument (Waters Corp.) or a Voyager DE Pro (ABI, Carlsbad, CA, USA), by using α-cyano-4-hydroxycinnamic acid (Agilent) as a matrix.

Synthesis of caged glutamate: Caged, Fmoc-protected glutamate for peptide synthesis (Figure 1B) was synthesized by using standard reactions, which are described in detail in the Supporting Information. Briefly, the protecting group, (*R,S*)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethanol ,[44] was coupled with *N*-Boc-glutamate-α-*tert*-butyl ester by using DCC and DMAP. The *t*-Boc and *tert*-butyl protective groups were removed with TFA. To finally protect the amine for Fmoc peptide synthesis, the photoprotected amino acid was reacted with 9-fluorenylmethyl chloroformate. The final product and each intermediate were characterized by ^{1}H NMR ([D_6]DMSO, 400 MHz, data in the Supporting Information). The final product was also confirmed by mass spectrometry; ESI MS *m/z* calcd for $C_{29}H_{26}N_2O_{10}$: 562.53 $[M+H]^+$; found: 563.32.

Synthesis of caged polypeptide: Synthesis of caged EGF was carried out in a single linear synthesis (50 μmol scale) by using standard methodology (details in the Supporting Information) and purified by reversed-phase HPLC (C4 column). The final purified product was characterized by MALDI-MS. Typical yield of purified material was 5–10 mg.

Photolysis of caged polypeptide: Photolysis of caged EGF for time course experiments and tissue culture experiments without patterned illumination was carried out in a quartz cuvette (1 cm path length) by using a Hg/Xe arc lamp with an interference filter that had a maximum transmission at 365 nm and peak width at half height of 10 nm. Light power at the position of the cuvette was measured with an OAI306 powermeter (Optical Associates, Inc., San Jose, CA, USA). The cuvette was positioned to provide power flux of 15–30 mW cm^{-2}. The concentration of the peptide solution was determined from its absorbance at 280 nm of an aliquot ($\varepsilon = 11\,200\ \text{M}^{-1}\,\text{cm}^{-1}$). The photolysis rate was determined for a 200 μg mL^{-1} solution of the EGF polypeptide in a total initial volume of 1.2 mL 20% (*v/v*) MeCN in H_2O. The solution contained an internal reference peptide with sequence WYPYDVPYA (0.5 mg mL^{-1}). Analysis was by HPLC (BioCad Sprint, C4 column). Buffer A was 0.1% TFA in H_2O; buffer B was 0.1% TFA in MeCN. A linear gradient was applied from 20% B to 80% B over 42 min (1 mL min^{-1}). Detection was at 220 nm. Peak areas for starting material and photolysis product were normalized relative to the internal reference. Photolysis for cell culture experiments was carried out in aqueous solution (30 min), and removal of the photolabile group was confirmed by MALDI-MS.

General cell culture: Tissue culture experiments used NIH/3T3 fibroblasts (ATCC, CRL-1658) or human foreskin fibroblasts (ATCC, CRL-2522). Fibroblasts were maintained at 37 °C in an atmosphere of 5% CO_2 in DMEM (Invitrogen) that contained 10% heat-inactivated fetal bovine serum (Invitrogen). Cells were removed from culture dishes by treatment with trypsin (Invitrogen). Human foreskin fibroblasts were used prior to 30 doublings. Defined medium was composed of RPMI (Invitrogen), 1× SPIT (Sigma), and 1× Serum Replacement 1 (Sigma). Recombinant human EGF was from Sigma.

Analysis of proliferative activity: Cells (NIH/3T3) were seeded in 10 cm polystyrene culture dishes at a density of 20 cells per mm^2, and incubated in defined medium without growth factor or serum for 16 h prior to addition of growth factor. Cells were counted by microscopic visualization (100× magnification) in 20 randomly selected fields for each dish. Only adherent, nonspheroidal cells were counted.

Cell mobility assays: For scratch–wound assays, cells were seeded in polystyrene tissue culture dishes (2.2 cm diameter) at a density of 80 cells per mm^2 in DMEM that contained 10% heat-inactivated fetal bovine serum, then allowed to proliferate to confluency. Each dish was subsequently washed with defined medium (no growth factor; 2×2 mL) and allowed to incubate under serum-starved conditions for 16 h at 37 °C. A scratch that was approximately 1.5 mm wide was made with the small end of a sterile pipet tip. The culture was washed with defined medium (3×2 mL) prior to addition of assay medium (1 mL).

A 24-well transwell device (Corning) was used to quantitate cell migration with the Boyden chamber method.[42] Human dermal fibroblasts were seeded in the upper chamber of the transwell device (5000 cells per well), that was separated from the lower chamber by a collagen-coated polycarbonate filter (8 μm pores). The upper chamber initially contained defined medium without growth factor. Defined medium that contained the factor to be tested was in the lower chamber. After 4 h at 37 °C, the cells on the upper surface of the filter membrane were removed with a cotton swab and cells remaining on the lower surface were fixed with EtOH. The membrane was removed from the chamber and stained with azure A, eosin Y, and methylene blue for counting.

Automated delivery and spatially defined activation of caged polypeptide: Spatially resolved activation of caged polypeptide was done in a device of our construction that coordinated delivery of media to tissue culture with spatially directed illumination. The tissue culture cell was a closed Teflon chamber with glass windows on opposite faces, which allowed the projection of light into the culture and microscopic examination. The lower window served as substrate for cell growth. The temperature of the chamber was regulated with resistive heaters thermostatically controlled, monitored with a thermocouple. Media were delivered to the chamber at a flow rate of 0.5 mL min^{-1} by peristaltic pump from refrigerated bottles equilibrated with 5% CO_2. The fluid volume in the culture cell was 1.5 mL, and covered an area of 3.1 cm^2. The culture was illuminated with the output from a Hg/Xe lamp with the power adjusted to deliver 12 mW cm^{-2} to the flow cell. The rectangular pattern of illumination was defined by a physical mask placed in contact with the outside of the substrate window of the flow cell.

Acknowledgements

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Keywords: caged proteins · EGF · growth factors · photochemistry · tissue engineering

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