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Delivery of basic fibroblast growth factor (bFGF) from photoresponsive hydrogel scaffolds

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Abstract

Exogenous growth factor therapy has shown a notable promise in accelerating the healing of acute and chronic wounds. However, their susceptibility to enzymatic degradation and short contact time with the wound bed warrant the use of sophisticated delivery vehicles that stabilize the encapsulated peptides and control their rate of release. Herein, we describe the synthesis of a nitrocinnamate-derived polyethylene glycol (PEG-NC) hydrogel system and study the release kinetics of basic fibroblast growth factor (bFGF) as a function of hydrogel properties. Long-wave ultraviolet irradiation (365 nm) was used to alter the physical properties of the gel scaffold (i.e. degree of swelling) and consequently control the release rates of the encapsulated bFGF. The degree of swelling (DS) decreased from 10.7 to 8 as the length of irradiation increased from 5 to 30 min. Similarly, the DS decreased from 17.5 to 11.5 by increasing the initial PEG-NC concentration from 10 to 30 w/v% while keeping the crosslinking irradiation at 10 min. Radiolabeled I^{125} studies were used to monitor the release of bFGF from PEG-NC hydrogels with variable swellabilities. By increasing the length of irradiation from 2 to 10 min the rate of bFGF release from PEG-NC gel scaffolds was decreased by 29% due to the enhanced crosslinking density. The bFGF-releasing PEG-NC hydrogels were not cytotoxic to human neonatal fibroblast cells and the released growth factor maintained its activity and induced fibroblast proliferation and collagen production in vitro. The addition of heparin within the gel scaffolds further increased the growth factor's activity.

Keywords: bFGF; Hydrogels; PEG; Growth factor delivery; Photosensitivity

1. Introduction

Exogenous administration of growth factors has been identified as a potential therapeutic approach in accelerating the rate of healing of acute and chronic wounds [1–5]. Nonetheless, their susceptibility to enzymatic degradation, short half-lives and the need of maintaining adequate pharmacological levels at the injured site have limited the clinical availability of peptide therapy in cutaneous repair [1,3]. In recent years, several strategies and controlled delivery vehicles have been considered to effectively deliver growth factors to the wound site, but an ideal method for their administration remains unclear [1,6–12]. Hydrogel networks are gaining increased clinical acceptance as a wound management modality. Attractive features of hydrogel dressings include their ability to maintain a moist wound environment, absorb exudate drainage, allow oxygen transport, and provide analgesia. In addition, because they are not strongly adherent to the wound, they do not cause discomfort during dressing changes. Hydrogels are currently used for the management of skin donor sites, superficial surgical wounds, and fresh and chronic damage to the epithelium [8,13]. Furthermore, bioactive gel scaffolds that deliver growth factors at controlled rates

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have demonstrated an enhanced effectiveness over conventional hydrogel dressings or simple peptide solutions injected into the wound site [1,2].

Basic fibroblast growth factor is a single chain peptide (MW = 16,000) composed of 146 amino acids, which promotes proliferation, differentiation, and numerous other cellular functions in cells derived from the mesoderm and neuroectoderm. It is a potent angiogenic factor in vivo and in vitro, and is mitogenic and chemotactic for both fibroblasts and endothelial cells. Administration of exogenous basic fibroblast growth factor has been proven beneficial in the healing of acute and chronic wounds in animals and clinical trials [3,12,14–17]. A number of controlled delivery vehicles have been developed to overcome the shortcomings of basic fibroblast growth factor therapy (i.e. susceptibility to enzymatic and thermal degradation, short duration of retention at wound sites, and the lack of ideal delivery vehicles) in a clinical setting [18–20]. The unique ability of heparin to bind to basic fibroblast growth factor and other basic polypeptides has been used to develop drug delivery systems for the controlled release of heparin-binding growth factors [21–24]. For example, Gospodarowicz and Cheng, showed that both acidic (aFGF) and basic (bFGF) fibroblast growth factors coupled with heparin were protected from heat and acid deactivation, while Vemuri et. al., described the stabilization of bFGF in the presence of heparin at high temperatures [22,23]. Edelman et al. reported that the binding of bFGF to heparin–Sepharose beads allowed prolonged storage in a microspherical controlled releasing matrix while Sakiyama-Elbert and Hubbell demonstrated that fibrin–heparin matrices can enhance nerve regeneration by controlling the release of bFGF at the injury site [10,20].

In a series of publications we have described the synthesis and characterization of various PEG-based photosensitive hydrogels systems and demonstrated their effectiveness as enzyme immobilization matrices, controlled delivery vehicles and potential antithrombogenic surfaces [25–27]. The mild conditions under which photogelation is achieved have propelled several investigators to utilize photopolymerization as a means for biomaterial preparation [28–32]. In theory, photopolymerization can provide an effective and benign method for in situ hydrogelation that could produce occlusive dressings that conform to the contour of the wound. Critical parameters in designing such a photosensitive gel system include fast gelation times and low-intensity irradiation. In this manuscript we will present the synthesis, properties and release kinetics of a bFGF-containing, photosensitive PEG-nitrocinnamate (PEG-NC) hydrogel system. In addition, we will report on the stabilizing effects of heparin on the activity of the releasing bFGF when it is incorporated within the gel scaffold. The PEG-NC gel scaffold utilizes non-toxic starting materials, mild reaction conditions and a non-harmful long-wave-ultraviolet light (365 nm) as means of photogelation, making it an attractive alternative to existing hydrogel-based delivery vehicles in cutaneous repair.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG, 8-arms, MW = 20,000) was purchased from Nektar Therapeutics (Huntsville, AL). Nitrocinnamic Acid (NCA), *N,N*-dimethylformamide (DMF), ether, heparin sodium salt (porcine intestinal mucosa, H3393), diisopropylcarbodamide (DIC), dimethylamino-pyridine (DAMP), and 1,4 dioxane were purchased from Sigma-Aldrich (St. Louis, MO). A Blak-Ray® monochromatic UV lamp (115 V, 60 Hz, 2.5 A, 365 nm), with an output of 7000 μW/cm^2 was used to induce photogelation. I^{125}-bFGF was purchased from Amersham (Piscataway, NJ) and used as a tracer to assess the release kinetics of bFGF. A gamma counter (Wallac 1480 Automatic Gamma Counter) was used to quantify the amount of bFGF released.

Fibroblast cells were purchased from American Type Culture Collection (ATCC®; CCD-1064SK; Neonatal Fibroblast; Manassas, VA). Phosphate buffered saline (PBS, pH = 7.4, 1×) trypsin-EDTA (0.25% Trypsin-EDTA), Iscove Modified Dulbeco's Medium (IMDM), Fetal Bovine serum (FBS), Hank's balanced salt solution (HBSS), trypan blue stain, and streptomycin–penicillin solution (10,000 μg/ml) were purchased from GibcoBrl (Grand Island, NY). Culture flasks and six-well plates were purchased from VWR (Swanee, GA). Cell counts were performed on a coulter particle counter (model: Z1KYBD).

2.2. Synthesis and characterization of PEG-NC macromers

PEG-NC was synthesized by reacting PEG (8 arms, MW = 20,000) with nitrocinnamic acid, as described in our previous publication [31]. Briefly, 5.023 g (26 mmol) of nitrocinnamic acid (NCA) was added to 60 ml of DMF in a three-neck flask, and sealed under vacuum. After the dissolution of NCA, 5 g of PEG (0.5 mmol) was dissolved in 20 ml of DMF and the solution was added to the three-neck flask under Nitrogen atmosphere. DAMP (0.124 g, 1.015 mmol) and DIC (4.07 ml, 25.98 mmol) were dissolved in DMF, added to the flask and the reaction was allowed to proceed for 24 h at 60 °C. Upon reaction completion, the solution was precipitated in ether, the yellowish precipitate was re-suspended in deionized water and the solution was filtered through a 0.2 μm filter to remove the unreacted NCA groups (insoluble in water). The final product was lyophilized and the dried PEG-NC was collected and kept at 4 °C. The reaction yield was 80% and the degree modification was 85% as determined by UV spectroscopy [26,31].

2.3. Storage stability of photosensitive PEG macromers

PEG (MW = 20,000, 8 arms) was modified according to methods described in our previous publications [26,31,32] with either nitrocinnamate, cinnamylidene, anthracene, or acrylate groups. The degree of modification for each derivative was around 80%. The photosensitive macromers were stored in the dark and at room temperature and at specified time points, aqueous solutions of each derivative at concentration of 3.0×10^{-5} M were prepared and absorbance readings were taken at their absorption maxima. Reduction in absorbance readings was indicative of the polymerization of the photosensitive groups in the absence of light, thus reducing the reactivity of the polymer.

2.4. Swelling studies

PEG-NC hydrogel membranes were prepared in quartz wells (latex insert dimensions: thickness = 0.62 mm, width = 10 mm, length = 20 mm) by varying the concentrations and UV exposure times. Exposure times were set at 5, 10, 20, 30, and 50 min. Following polymerization the gels