Biomaterials xxx (2014) 1–9

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Controlled release and gradient formation of human glial-cell derived neurotrophic factor from heparinated poly(ethylene glycol) microsphere-based scaffolds

Jacob L. Roam, Peter K. Nguyen, Donald L. Elbert*

Department of Biomedical Engineering and Center for Materials Innovation, Washington University in St. Louis, St. Louis, MO, USA

ARTICLE INFO

Article history:
Received 14 February 2014
Accepted 8 April 2014
Available online xxx

Keywords:
Microsphere
Hydrogel
Gradient
Release
Heparin
GDNF

ABSTRACT

Introduction of spatial patterning of proteins, while retaining activity and releasability, is critical for the field of regenerative medicine. Reversible binding to heparin, which many biological molecules exhibit, is one potential pathway to achieve this goal. We have covalently bound heparin to poly(ethylene glycol) (PEG) microspheres to create useful spatial patterns of glial-cell derived human neurotrophic factor (GDNF) in scaffolds to promote peripheral nerve regeneration. Labeled GDNF was incubated with heparinated microspheres that were subsequently centrifuged into cylindrical scaffolds in distinct layers containing different concentrations of GDNF. The GDNF was then allowed to diffuse out of the scaffold, and release was tracked via fluorescent scanning confocal microscopy. The measured release profile was compared to predicted Fickian models. Solutions of reaction–diffusion equations suggested the concentrations of GDNF in each discrete layer that would result in a nearly linear concentration gradient over much of the length of the scaffold. The agreement between the predicted and measured GDNF concentration gradients was high. Multilayer scaffolds with different amounts of heparin and GDNF and different crosslinking densities allow the design of a wide variety of gradients and release kinetics. Additionally, fabrication is much simpler and more robust than typical gradient-forming systems due to the low viscosity of the microsphere solutions compared to gelating solutions, which can easily result in premature gelation or the trapping of air bubbles with a nerve guidance conduit. The microsphere-based method provides a framework for producing specific growth factor gradients in conduits designed to enhance nerve regeneration.

1. Introduction

The importance of gradients in biological molecules is well recognized. Processes such as nerve regeneration, wound healing, embryogenesis, angiogenesis, and immunity have been found to depend significantly on biological gradients [1–12]. In chemotaxis cells follow concentration gradients in signaling molecules, with the steepness of the gradients greatly influencing cell movement more than the average concentration [5–7]. To replicate and improve upon developmental and repair processes to engineer tissues and organs, production of bioactive gradients along with spatial patterning will be essential.

In recent years an increasing number of researchers have proposed many methods to this end [13–28]. For example, Khademhosseini and colleagues have created gradients in adhesion peptides using inverse flows and photopolymerization in microfluidic channels to influence and study endothelial cell migration [25]. Shoichet et al. have immobilized nerve growth factor in concentration gradients and observed enhanced directionality of extending dendrites [4,6,57]. Bellamkonda et al. found increasing concentration gradients in laminin-1 could alter the direction of growing dorsal root ganglia and enhanced regeneration of sciatic nerves in rats with nerve growth factor [21–23].

Many of the current methods for the patterning and delivery of bioactive molecules use various forms of covalent attachment [13–18]. Irreversible coupling, however, may not be the optimal approach. Covalent attachment can potentially hinder the ability of cells to access the molecules, and chemical modification may result in a loss of activity. An alternative that our lab has explored recently is the use of heparin-decorated synthetic materials that can bind

* Corresponding author. Department of Biomedical Engineering, Campus Box 1907, One Brookings Dr., Washington University, St. Louis, MO 63130, USA. Tel.: +1 314 935 7519; fax: +1 314 935 7448.
E-mail address: elbert@biomed.wustl.edu (D.L. Elbert).

http://dx.doi.org/10.1016/j.biomaterials.2014.04.027
0142-9612/

electrostatically (reversibly) many useful proteins, including proteins that promote nerve regeneration [26–34]. GDNF, a heparin binding protein, has been shown to enhance motor and sensory nerve regeneration [35,36]. Synthetic polymer hydrogels have been extensively explored to create scaffolds for regenerative medicine, and have seen some promising results [37–39]. Functional peptides, proteins, or other biological molecules like heparin may be incorporated into these hydrogels imparting biological functions, such as cell adhesion or cell-initiated degradability [2,38,40–42]. However, bulk hydrogel scaffolds generally lack macroporosity or spatial anisotropy. To address these limitations we and others are seeking to produce heterogeneous scaffolds using modular assembly of hydrogel microparticles [28,38,43–48].

Gradient producing systems such as pulsatile application of picoliters of growth factor solutions, simple diffusion of molecules into a gel, gradient makers using two polymerizing solutions, and microfluidic devices have been used extensively [1,4,5,10,24–26]. However scaling issues and difficulties in pumping polymerizing solutions are only a few of the challenges faced by these methods due to the low volumes involved (e.g. about 70 μL of fluid per centimeter of conduit). The formation of gradients of growth factors, as well as addition of adhesion factors and degradability in bioactive scaffolds, is proposed to be improved by assembling microparticles in a modular manner [28,38,44,45,49,50]. To this end our lab has developed PEG hydrogel microspheres fabricated from multi-arm PEG derivatives in aqueous solution with kosmotropic salts via a thermally induced phase separation [28,38]. In this strategy, solutions are *not mixed* during microsphere formation, with size controlled by the length of time required for gelation [58]. We have already successfully imparted different functionalities, such as cell adhesion, degradability, heparination, and protein and drug delivery to these microspheres [28,38].

In a recent study we engineered gradients into scaffolds made from these PEG microspheres, most notably decorating the microspheres with heparin and creating a gradient of *covalently coupled* GDNF [28]. However, we had not demonstrated the release of electrostatically (i.e. reversibly) bound GDNF from these scaffolds. The challenges in the previous publication that did not allow release of GDNF were overcome and the results are presented here.

2. Materials and methods

Unless otherwise noted, all reagents were purchased from Sigma–Aldrich.

2.1. PEG synthesis

PEG_8-vinylsulfone (PEG_8-VS) and PEG_8-amine was synthesized from eight-arm PEG-OH (PEG_8-OH; mol. wt. 10,000; Shearwater Polymers, Huntsville, AL) as previously described [51]. PEG macromonomers were dissolved separately at 200 mg/mL in Dulbecco's phosphate buffered saline (PBS; 8 mM sodium phosphate, 2 mM potassium phosphate, 140 mM sodium chloride, 10 mM potassium chloride, pH 7.4) and sterile filtered with 0.22 μm syringe filters (Millipore).

2.2. Heparin attachment

A solution of 500 mM N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), 12.5 mM N-Hydroxy-succinimide (NHS), and 50 mg/mL heparin sodium salt (mol. wt. ~18,000, ~2.78 mM) in MES buffer (10 mM, pH 6.0) was incubated at room temperature for 30 min. The activated heparin solution was then added to a 200 mg/mL solution of PEG_8-amine at a 20:1, or 160:1 PEG_8-amine to heparin molar ratio and incubated at room temperature for another 30 min before refrigeration. For microsphere formation, heparin-conjugated PEG_8-amine was mixed with PEG_8-VS in a 1:2 ratio of the two PEG types (see Fig. 1).

2.3. Microsphere formation

PEG_8-amine (with or without bound heparin) solutions were combined with PEG_8-VS solutions at a 1:2 ratio. The PEG solutions were diluted to 20 mg/mL PEG with PBS and 1.5 M sodium sulfate (in PBS) to a final sodium sulfate concentration of 0.6 M. The PEG_8-VS/PEG_8-amine solutions were then incubated above the cloud point at 70 °C for various times. Suspensions of microspheres were subsequently buffer exchanged into 8 mM sodium phosphate twice to remove the sodium sulfate by: (1) diluting the microsphere solution 3:1 with PBS and titurating, (2) centrifuging at 14,100 *g* for 2 min, and (3) removing the supernatant.

2.4. GDNF labeling

Dylight-488 NHS-ester (Pierce) was dissolved in dimethyl formamide at 10 mg/mL. Recombinant human GDNF (Peprotech, Rocky Hill, NJ) was dissolved in 8 mM sodium phosphate buffer (pH 7.4). Dylight-488 was added to the solution for a final GDNF concentration of 10 μg/mL and a final Dylight-488 concentration of 50 ng/mL and incubated overnight at 2 °C. The solution was then dialyzed using Slide-A-Lyzer MINI Dialysis Units (Thermo Scientific, Rockford, IL, 3500 MWCO) in 8 mM sodium phosphate buffer (pH 7.4) to remove unbound Dylight-488.

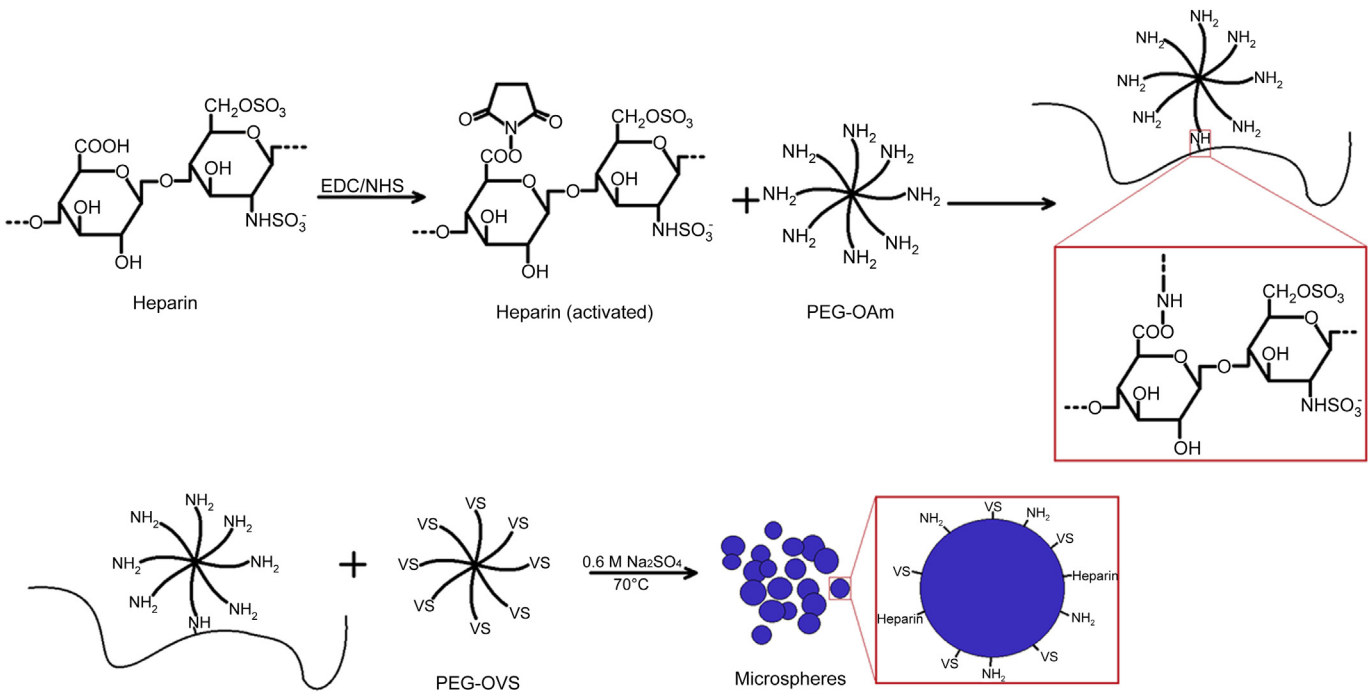

Fig. 1. Heparin attachment.

Please cite this article in press as: Roam JL, et al., Controlled release and gradient formation of human glial-cell derived neurotrophic factor from heparinated poly(ethylene glycol) microsphere-based scaffolds, Biomaterials (2014), http://dx.doi.org/10.1016/j.biomaterials.2014.04.027