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# The effect of the local delivery of platelet-derived growth factor from reactive two-component polyurethane scaffolds on the healing in rat skin excisional wounds

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ABSTRACT

A key tenet of tissue engineering is the principle that the scaffold can perform the dual roles of biomechanical and biochemical support through presentation of the appropriate mediators to surrounding tissue. While growth factors have been incorporated into scaffolds to achieve sustained release, there are a limited number of studies investigating release of biologically active molecules from reactive two-component polymers, which have potential application as injectable delivery systems. In this study, we report the sustained release of platelet-derived growth factor (PDGF) from a reactive two-component polyurethane. The release of PDGF was bi-phasic, characterized by an initial burst followed by a period of sustained release for up to 21 days. Despite the potential for amine and hydroxyl groups in the protein to react with the isocyanate groups in the reactive polyurethane, the *in vitro* bioactivity of the released PDGF was largely preserved when added as a lyophilized powder. PUR/PDGF scaffolds implanted in rat skin excisional wounds accelerated wound healing relative to the blank PUR control, resulting in almost complete healing with reepithelization at day 14. The presence of PDGF attracted both fibroblasts and mononuclear cells, significantly accelerating degradation of the polymer and enhancing formation of new granulation tissue as early as day 3. The ability of reactive two-component PUR scaffolds to promote new tissue formation *in vivo* through local delivery of PDGF may present compelling opportunities for the development of novel injectable therapeutics.



## 1. Introduction

Wound healing is a tightly orchestrated sequence of events that is driven by intercellular communication via cytokines and growth factors. When healing goes awry, many of the consequences can be attributed to the alteration of these signaling mechanisms, and deficient wound healing can be corrected, in part, by administration of signal molecules to sites of tissue repair. Reorganization of damaged tissue requires restoration of appropriate architecture and biomechanical properties. Supplementing the tissue defect with biocompatible scaffolds that may be derived from natural or synthetic sources can augment this aspect. A key tenet of tissue engineering is the principle that the scaffold — like the native extracellular matrix — can perform the dual roles of biomechanical and biochemical support through presentation of the appropriate mediators to surrounding tissue.

PDGF is a co-factor with vascular endothelial growth factor (VEGF) for stable angiogenesis [1]. It is chemotactic and mitogenic for bone cells, mesenchymal stem cells, ligament, dermal fibroblasts, and fibroblast-like cells derived from the periodontal ligament and gingiva [2–4]. PDGF-BB is the most widely understood and utilized isoform for wound healing applications, and it has been reported to stimulate and enhance new tissue formation in both bone and soft tissue models [5–8]. In as much as PDGF-BB is a universal ligand for the PDGF receptor, it has achieved moderate commercial success as a component of Regranex™, a carboxymethylcellulose (CMC) gel formulation of 100 μg/ml rhPDGF-BB (for simplicity referred to as PDGF). Due to the bolus release of

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PDGF from the CMC gel, this topical agent, which is approved for treatment of diabetic foot ulcers, must be applied daily or more frequently at high concentrations. In contrast, preclinical studies have shown that a sustained release of PDGF enhances cellular infiltration and new tissue formation relative to a bolus release. In a mid-sagittal dorsal incision model in the rat, a nearly linear sustained release (e.g., up to 14 days) of PDGF from nanofibrous scaffolds [9] was reported to enhance new tissue formation at days 7 and 14 [8], while a bolus release (e.g., complete release after 4 h) had a negligible effect [8,10]. Likewise, gene delivery of PDGF has produced positive effects by sustained, low level release [11].

To address the clinical need for an improved delivery device for sustained release of PDGF, we are investigating the potential of polyurethane (PUR) scaffolds. Porous, biodegradable, and biocompatible PUR scaffolds have been reported to support cellular infiltration and new tissue formation in sub-cutaneous [12–15], cardiovascular [16,17], and bone models [18,19]. Furthermore, these materials have been shown to biodegrade at a controlled rate to nontoxic products *in vitro* [16,17,20,21] and *in vivo* [14–16,22] to non-cytotoxic decomposition products. In addition to its function as a matrix supporting the ingrowth of cells and new tissue, the PUR scaffold can also function as a drug delivery system [23,24]. Biodegradable PUR scaffolds have been used for controlled release of growth factors such as bFGF [25] and PDGF-BB [12]. A distinct advantage of polyurethane scaffolds is the potential to inject the materials as a reactive liquid mixture which adheres to host tissue, conforms and expands to fill irregularly shaped wounds, and cures *in situ* to form an elastic porous scaffold [12,26]. A polyisocyanate (or NCO-terminated prepolymer) mixed with a polyol (hydroxyl functionality) reacts to form a polymer network incorporating urethane linkages. Water reacts with the polyisocyanate to form a disubstituted urea and carbon dioxide, which functions as a blowing agent, thereby introducing pores into the material.

In a previous study, we have reported the release of PDGF from PUR scaffolds, wherein radio-labeled PDGF was incorporated as a dry powder into PUR scaffolds that were prepared from hexamethylene diisocyanate trimer (HDIt). The release profile was characterized by a burst followed by a period of sustained release, and the cumulative release of PDGF was found to be independent of the dosage [12]. However, the bioactivity of the released PDGF was not assessed. Proteins such as PDGF contain active hydrogens, including primary amine and hydroxyl groups, which can potentially react with the polyisocyanate, thereby denaturing the protein. Therefore, in this study, we used two strategies for delivering PDGF from PUR scaffolds to address the need to preserve the bioactivity of the released PDGF. In the first approach, PDGF was incorporated into PUR scaffolds as a powder in the presence of excipients (heparin and glucose). In the second approach, PDGF was bound to PLGA microspheres through heparin, the microspheres were coated with a gelatin layer to form granules, and the granules were then incorporated into PUR scaffold. The gelatin coating was designed to protect active hydrogens (e.g., primary amine and hydroxyl groups) in the growth factors from reacting with the polyisocyanate component of the PUR. We have measured the *in vitro* release kinetics of PDGF from two-component reactive PUR scaffolds, as well as the *in vitro* biological activity of released PDGF, using both strategies. Considering that there were no substantial differences in either the release profile or the biological activity of released PDGF between the two strategies, the simpler approach of adding PDGF as a powder was selected for *in vivo* experiments. PUR scaffolds with and without PDGF were implanted in excisional wounds in Sprague-Dawley rats. We reasoned that the initial burst release of PDGF would attract mesenchymal cells into the scaffold, while the sustained release of PDGF would promote tissue remodeling in the later stages of the healing process. PUR + PDGF scaffolds implanted in rats promoted substantial ingrowth of cells as early as day 3 post-implantation, followed by extensive new tissue formation and scaffold degradation at day 7 and almost complete healing at day 14. Interestingly, PDGF not only enhanced ingrowth of cells and new tissue, but also accelerated scaffold degradation relative to the negative control. Thus the ability of PUR scaffolds to deliver biologically active PDGF at sustained low levels for several weeks presents potentially compelling opportunities for these materials as a combined scaffold and delivery system for regenerative medicine.

## 2. Materials and methods

### 2.1. Materials

Polyvinyl alcohol (PVA), glycolide, and D,L-lactide were obtained from Polysciences (Warrington, PA). The tertiary amine catalyst TEGOAMIN33, which comprises a solution of 33 wt% triethylene diamine (TEDA) in dirpropylene glycol, was received from Goldschmidt (Hopewell, VA) as a gift. Polyethylene glycol (PEG, 600 Da) was purchased from Alfa Aesar (Ward Hill, MA). Dichloromethane (DCM), methanol, trifluoroacetic acid (TFA) and glucose were from Acros Organics (Morris Plains, NJ). Hexamethylene diisocyanate trimer (HDIt, Desmodur N3300A) was received as a gift from Bayer Material Science (Pittsburgh, PA). PDGF-BB was received as a gift from Amgen (Thousand Oaks, CA). Sodium iodide ($Na^{125}I$) for radiolabeling was purchased from New England Nuclear (part of Perkin Elmer, Waltham, MA). Stannous octoate catalyst was received from Nusil technology (Overland Park, KS). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was from Advanced ChemTech (Louisville, Kentucky). Reagents for cell culture were all purchased from HyClone (Logan, UT). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO). Prior to use, glycerol and PEG 600 were dried at 10 mm Hg for 3 h at 80 °C, and ε-caprolactone was dried over anhydrous magnesium sulfate, while all other materials were used as received.

### 2.2. Radiolabeling of PDGF-BB

PDGF-BB was labeled with radioactive iodine ($^{125}I$) using IODO-BEADS Iodination Reagent (Pierce Biotechnology, Rockford, IL) in accordance with previously published techniques [12]. IODO-beads containing approximately 1 mCi $Na^{125}I$ were incubated in 1 ml of reaction buffer for 5 min under room temperature, followed by addition of 50 μg PDGF to the reaction solution and incubation for another 25 min. The solution was then removed from the IODO-BEADS reaction tube and the Iodine-labeled PDGF ($^{125}I$-PDGF) was separated in a Sephadex disposable PD-10 desalting column (Sigma–Aldrich). Eluted fractions were collected and Cobra II Autogamma counter (Packard Instrument Co, Meridien, CT) was adopted to determine the fractions containing the $^{125}I$-PDGF.

### 2.3. Amine-terminated poly(L-lactide-co-glycolide) (PLGA–$NH_2$) synthesis

Hydroxyl-terminated poly(L-lactide-*co*-glycolide) (PLGA–OH, 50/50 lactide/glycolide, MW 20,000) copolymers were synthesized by ring-opening polymerization of D,L-lactide and glycolide using a dried polycaprolactone triol (MW 300) starter in the presence of stannous octoate catalyst as described previously [20,22]. The polymer was then dissolved in DCM and reacted with N-t-Boc-glycine (4 mM) and DMAP (1.2 mM). To this mixture 4 mM of DCC was added, and the reaction mixture stirred in ice/water bath for 24 h. After filtering the dicyclohexylurea by-product, the filtrate was precipitated in methanol, filtered, and dried. The amine groups were deprotected by reacting the polymer in a solution of TFA in DCM for 3 h to remove the t-Boc groups, followed by precipitation in methanol, filtration, and drying. Structure of the the PLGA–$NH_2$ was verified by NMR (Bruker 300 MHz).

### 2.4. Fabrication of PLGA–heparin–PDGF microspheres

A microcapillary device was used to generate the PLGA–$NH_2$ microspheres [27]. An oil phase comprising a solution of PLGA–$NH_2$ in DCM (5% wt) was fed to the concentric tube (0.1 inch in diameter), while an aqueous carrier stream (2% w/v PVA) surrounded the emerging jet of the PLGA–$NH_2$/DCM solution, which was sprayed into a solution of 2% PVA. After stirring for ~3 h to evaporate the DCM, the particles were filtered, washed with Deionized water, and lyophilized for 24 h. The particle size was measured to be 50 μm using an Olympus BX60 microscope.

Heparin (activity: 170 USPU/mg) was covalently bound to the surfaces of PLGA–$NH_2$ microspheres using standard carbodiimide techniques [10,28]. Briefly, heparin (0.33 g/g PLGA–$NH_2$) was incubated with PLGA–$NH_2$ microspheres in a pH 5.5 buffer solution (0.1 M MES containing 0.5 M NaCl). To activate the COOH groups of the heparin, NHS (6 mmol) and EDC (6 mmol) were added to the solution, followed by reaction in an ice water bath for 24 h. The resulting PLGA-Hp microspheres were filtered, washed, and lyophilized. The amount of heparin conjugated to the microsphere surface was measured to be 0.15 wt% using the toluidine blue method [28].