Local Delivery of PDGF-BB from Polyurethane Scaffold Enhances Tissue Regeneration in Rat Excisional Wounds

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Statement of Purpose: Biodegradable polyurethane (PUR) has been applied in tissue engineering extensively due to its excellent properties such as biocompability and tunable mechanical strength (1). Platelet-derived growth factor (PDGF) is a mitogenic and angiogenic protein which can promote fibroblast growth (2). It is desirable to provide both a supporting matrix and a growth stimulating drug in tissue engineering in order to accelerate new tissue formation. The present project aimed to develop a novel biodegradable scaffold, utilizing polyurethane scaffold as the matrix and incorporaing PDGF-BB to promote cell growth thus promote tissue regeneration. Both *in vitro* and *in vivo* validation on the scaffold were carried out. The ability of PDGF-BB to promote cell proliferation and new tissue formation early in the healing process is achieved through controlled release from PUR within one week.

Methods: PUR scaffolds were synthesized by one-shot reactive liquid molding of hexamethylene diisocyanate trimer (HDIt) with polyols (900-Da) (1). PDGF-BB was labeled with radioactive iodine (^{125}I) using IODO-BEADS Iodination Reagent. Microparticles made from amine-terminated PLGA were linked to heparin and PDGF-BB (3), followed by gelatin coating to yield granules of about 110 µm. PDGF powder or granules were incorporated in PUR composite scaffolds through mixing with the polyol before the foaming reaction. The internal morphology of the pores in the scaffolds were measured by SEM. *In vitro* release studies were carried out in α-MEM cell culture medium containing 1% BSA at 37 °C. Cell proliferation experiment was carried out through incubating MC3T3 cells with released PDGF-BB for 1 and/or 2 days, followed by CyQUANT assay. For the *in vivo* study, the scaffolds were cut into dimensions of 8x2 mm discs containing 1.8 µg PDGF-BB, and implanted into rat skin excisional wounds. The implants were then harvested at day 3, 7, 14, and 21 respectively, and then processed for histological analysis.

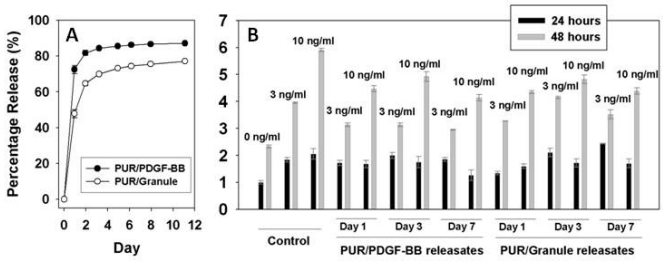

Figure 1. Release of ^{125}I-PDGF (A) from PUR scaffold and *in vitro* cell proliferation assay (B).

Results: The polyurethane scaffolds are porous and the pores were interconnected as evidenced by SEM imaging. The pore size was in the range of several hundred microns, and the polyurethane walls were ~20 µm thick. The PDGF-BB release from PUR was monitored by two methods. In the first approach, PDGF-BB was radiolabeled with iodine-125 (I-125) and a gamma reading machine was used to monitor the release kinetics. Release of PDGF-BB was also measured by ELISA assay using the liquid releasates. Both methods yield similar release profiles, characterized by a burst release on the first day (figure 1A). The ELISA assay detected a lower total release which is possibly due to denaturation of some fraction of the released protein which cannot be detected by its antibody. The releasates at day 1, 3, and 7 from both PUR/PDGF-BB and PUR/G-PDGF-BB were then incubated with MC3T3 cells. The released PDGF-BB was able to promote cell proliferation at both dosages tested (3 and 10 ng/ml) in a time dependent manner (figure 1B). After the PDGF-BB release and bioactivity was identified in vitro, the PUR/PDGF-BB implants were fitted into Sprague-Dawley rats skin excisional wounds. Little inflammatory response or cytotoxicity was evident. As shown in figure 2, the presence of PDGF in the scaffold enhanced scaffold degradation, presumably by attracting microphage cells, as well as new granulation tissue formed by infiltration of fibroblast cells. As the healing progressed, new extracellular matrix with dense collagen fibers filled the defect. At day 7, a remarkable level of new tissue infiltration and scaffold degradation was observed for the PDGF samples.

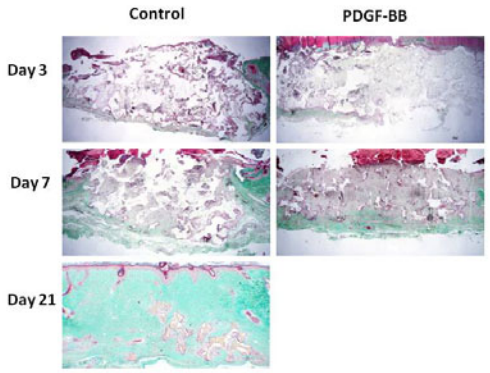

Figure 2. PDGF *in vivo* histology assay.

Conclusions: The ability of PDGF-BB to promote cell proliferation and new tissue formation early in the healing process is achieved through controlled release of PDGF-BB within one week. The ability of PUR implants to promote new tissue formation in rats' skin excisional wound model, achieved through local delivery of PDGF-BB, suggests a potential application of polyurethane scaffolds both as a supportive scaffold and as a protein delivery system in tissue engineering.

References:

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