Injectable, Biodegradable Polyurethane Scaffolds With Local Lovastatin Delivery For Enhanced Bone Regeneration

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Introduction: While autologous bone may be the ideal graft material for use in reconstructive orthopaedic surgery, its harvesting often causes donor site morbidity and has limited availability. Thus, synthetic or biological material substitutes have been increasingly studied for bone tissue repair. We have developed injectable biodegradable polyurethane (PUR) scaffolds with high porosity (>90%) that support cell migration and proliferation, and degrade to non-cytotoxic products [1].

Lovastatin (LV) has been shown to promote osteogenesis by upregulating BMP2 [2], and local delivery of LV could optimize its efficacy in vivo [3]. In this study, we have fabricated PUR scaffolds to simultaneously provide a template for cellular infiltration and new bone formation, as well as a vehicle for local LV delivery to further effective bone regeneration.

Materials and Methods: Porous PUR foams were synthesized by one-shot gas foaming of hexamethylene diisocyanate trimer and a hardener consisting of polyester triol, 600-MW PEG, water, catalyst, stabilizer, and pore opener using previously reported techniques [4].

In vitro biocompatibility of PUR was examined using preosteoblastic MC3T3-E1 cells. The cells were statically seeded onto thin foam discs (5x10^4 cells/implant) in 24-well plate, and cultured for 5 days under normal cell culture conditions. Cell viability was assessed qualitatively by fluorescent images using Live/Dead Viability/Cytotoxicity assay kit (Invitrogen Molecular Probes). In vivo behavior of the scaffolds (without LV) was preliminarily evaluated for biocompatibility, osteoconductivity, and tissue regeneration. Foam cores (6x3 mm) were implanted into rat tibial plug defects. The samples were removed at 3 weeks after implantation, and undecalcified sections were stained by toluidine blue.

**Lovastatin incorporation into PUR scaffolds:** Powdered LV was mixed thoroughly with the hardener before foam synthesis at 20 µg (LV-L), 200 µg (LV-M), and 800 µg (LV-H) per gram of foam. In vitro release of LV in PBS at 37 °C was measured daily from 1 to 30 days. The released LV was quantified by HPLC at 237 nm. We also examined in vitro biocompatibility of PUR scaffolds incorporated with LV: MC3T3-E1 cell attachment at 4 hours after seeding and cell viability at 5 days (5 x 10^4 cells/implant in 24-well dish) by MTT assay (Sigma-Aldrich).

The stimulatory activity of LV released from PUR scaffolds (r-LV) in BMP2 expression was evaluated. MC3T3-E1 cells were cultured with r-LV (0, 0.2, 1 µM) and LV (positive control: 1 µM) for 24 and 48 hours. Total RNA was isolated using TriZOL reagent, and mRNA was transcribed to cDNA using Superscript II (Invitrogen). To measure the expression of BMP2 expression, Taqman® gene expression assays (Applied Biosystems) were used to perform qRT-PCR, using 18S as an endogenous control. Statistical evaluations were conducted by ANOVA, followed by Tukey-kramer test. P values less than 0.05 were considered significant.

Results: MC3T3-E1 cells substantially permeated and adhered to the scaffold interstices, and viable cells were well visible at 5 days after seeding in fluorescent microscope images (Fig. 1A). The rat tibial plug implants showed good biocompatibility and osteoconductivity. After 3 weeks, undecalcified histological sections exhibited rich new bone formation within the foams (Fig. 1B). Evidence of scaffold biodegradation was also apparent by 3 weeks.

In the LV release assay, 10-20% of the incorporated LV eluted by 30 days, with a nearly linear release profile and nearly constant daily elution (Fig. 2). The dose did not appear to significantly affect the release profile. Cell attachment and cell viability in PUR-LV scaffolds was comparable to control PUR scaffolds without LV (Fig. 3A,B). BMP2 expression in MC3T3-E1 cells treated with r-LV was enhanced in a dose-dependent manner, although its effect was lower than LV (Fig. 3C).

Discussion: These biodegradable PUR scaffolds show promise as possible therapies for bone regeneration. Their elastomeric and resilient properties may improve adhesion and integration at the bone-material interface. With >90% porosity, cells readily infiltrated the materials and produced new tissue, and in vivo studies demonstrate the scaffolds’ osteoconductivity and biocompatibility, as well as preliminary biodegradation.

In vitro LV release experiments reveal a linear, controlled elution profile from the scaffolds. The relatively low release levels may result from the low water solubility of LV, since release from the scaffolds likely is diffusion-controlled. LV released from PUR enhances BMP2 expression and does not negatively affect cell attachment and viability. This scaffold therefore provides a structural support for bone ingrowth, and also a matrix from which biologicals can be released locally to enhance bone formation. Since preliminary studies support their application as an injectable scaffold, PUR scaffolds can offer a useful therapeutic approach in bone regeneration when incorporated with LV.


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