Versatile Platform for Sustained Gene Silencing Improves Excisional Wound Healing in Diabetic Rats

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ABSTRACT SUMMARY

We recently developed a platform for sustained, local gene silencing based on pH-responsive, micellar, small interfering RNA (siRNA)-loaded nanoparticles (si-NPs) loaded into biodegradable, tissue inductive scaffolds. Using this platform, we demonstrated that sustained, local silencing of prolyl hydroxylase domain protein 2 (PHD2) activated hypoxia induced factor 1α (HIF1α) controlled genes and increased angiogenesis in mouse subcutaneous poly(ester urethane) (PEUR) implants. Herein, we extend this technology to a new class of biodegradable scaffolds, poly(thioketal urethanes) (PTK-URs), which are specifically degraded by cell-generated reactive oxygen species (ROS). In a diabetic rat excisional wound model, PTK-URs improved wound stenting and tissue infiltration relative to PEURs. In this same pathological wound model, PHD2 si-NP delivery from PEURs and PTK-URs improved wound site angiogenesis. These combined data demonstrate that PTK-UR scaffolds are an effective material for healing of diabetic excisional wounds by both serving as a regenerative tissue template and by providing efficient, local delivery of PHD2 si-NPs.

Figure 1 (A) A polyester triol precursor, or (B) polythioketal diol precursor are reacted with (C) lysine triisocyanate (LTI) to produce an injectable reaction mix that can be loaded with embedded si-NPs during scaffold curing. (D) The reaction cures creating a porous scaffold loaded with si-NPs, as confirmed by (E) SEM and (F) confocal microscopy (fluorescent si-NPs visible in scaffold matrix). Scale = 200µm.

INTRODUCTION

The discovery of RNA interference (RNAi) has motivated development of technologies to overcome delivery barriers and harness therapeutic gene silencing. Regenerative medicine remains a relatively unexplored area of RNAi, as many applications have been directed at systemic delivery applications. Rapid nuclease degradation and poor cellular uptake necessitate that siRNA be packaged into an efficient carrier. siRNA has a short half-life in rapidly dividing cells (e.g., regenerating tissues), and local delivery must be sustained in order to achieve a long term effect.

We previously detailed a platform for the efficient local delivery of siRNA to sites of tissue regeneration utilizing sustained release of siRNA loaded endosomolytic nanoparticles (si-NPs) from biodegradable poly(ester urethane) (PEUR) scaffolds. The platform was adapted to have controllable release kinetics and applied to improve angiogenesis and tissue regeneration through the sustained silencing of prolyl hydroxylase domain protein 2 (PHD2), a regulator of the hypoxia induced factor 1α (HIF1α).

We have also recently tested new scaffold chemistry based on poly(thioketal urethane) (PTK-UR) polymers that specifically degrade through first-order degradation kinetics via a reactive oxygen species (ROS) dependent mechanism. The PTK-URs offer significant advantages over other materials, such as polyester based materials, by providing a cell-degradable template for guiding new tissue formation, whereas polyesters degradation acidifies the local environment and triggers a poorly-controlled autocatalytic degradation mechanism. PTK-URs have also been shown to better stent subcutaneous wounds in rats and have degradation rates that appropriately match tissue in-growth and cell-mediated degradation of the scaffold. Herein we demonstrate the enhanced wound healing capacity of PTK-UR scaffolds over PEUR materials by improved wound stenting and tissue infiltration in diabetic rat excisional wounds. In this same model, we also show an increase in the vascular density within both PTK-UR and PEUR scaffolds loaded for sustained delivery of PHD2 si-NPs.

EXPERIMENTAL METHODS

A diblock copolymer composed of 2-(dimethylamino)ethyl methacrylate (DMAEMA), 2-propylacrylic acid (PAA), and butyl methacrylate (BMA) was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. NPs were self-assembled by slow addition of the polymers from ethanol into diH2O and siRNA was electrostatically condensed onto the surface, forming si-NPs. Trehalose was added as an excipient to control the release kinetics and improve bioactivity of the released si-NPs. A condensation polymerization with 2,2-dimethoxy propane (DMP) and 2-mercaptoethyl ether (MEE) was used to synthesize the base PTK polymer. The PTK homobifunctional thiol end groups were converted to hydroxyl groups post-polymerization.

Lyophilized NPs containing either a scrambled siRNA (SCR) or an siRNA against PHD2 were resuspended into the polyester triol (900 Da, 60%PCL, 30%PGA, 10%PLA) or PTK diol (1000 Da), and scaffold curing was initiated by subsequent mixing with LTI. Water was added as a blowing agent for scaffold porosity.

PEUR loaded with siRNA were preformed into discs and implanted subcutaneously in mice. Scaffolds were...
evaluated for vascular response by μCT and CD31 immunohistochemistry (IHC). Sprague-Dawley rats were induced with diabetes through IP administration of streptozotocin (STZ). 8mm full thickness excisional wounds were made in the dorsal skin of rats, and PEUR or PTK-UR scaffolds loaded with SCR si-NPs, PHD2 si-NPs, or empty scaffolds were implanted. Wounds were excised at 7 and 14 days and analyzed for wound stenting, tissue infiltration, and vessel density using ImageJ.

All experiments with animals were approved by Vanderbilt University’s Institutional Animal Care and Use Committee (IACUC).

RESULTS AND DISCUSSION
The diblock copolymer DMAEMA71-b-BMA142-co-PAA84-co-DMAEMA79 (PDI = 1.41) was used to form micellar NPs (d0 = 39.6±12.6 nm, ζ-potential of +20.2 mV) that electrostatically complexed siRNA and were finely tuned to be membrane disruptive at endo-lysosomal pH. Trehalose was added to samples of si-NPs that were then lyophilized, resuspended into either polyester triols or PTK diols, and fabricated into scaffolds through a reactive foam processing with LTI. (Fig 1)

To evaluate PHD2 silencing, PEUR scaffolds were implanted subcutaneous in balb/c mice and an 80% silencing of PHD2 was observed which resulted in a HIF1α-mediated 200% increase in VEGF and 290% increase in FGF-2. CD31 histology and μCT confirmed a 3-fold increase in blood vessel density (Fig 2).

We recently showed that PTK-UR scaffolds more effectively stented subcutaneous pockets in rats than PEUR materials. This stenting behavior was also seen in the more mechanically challenging excisional rat wound model as PTK-URs. This stenting effects retain the open scaffold pore morphology and enhance the quantity and quality of granulation tissue formation within the wound site (Fig 3A,C). This effect was quantified, and PTK-UR scaffolds were both thicker and had a higher relative percentage of scaffold interior filled with granulation tissue relative to PEUR materials (Fig 3B,C).

Figure 2. Vascularization of PEUR scaffolds loaded with SCR siRNA or PHD2 siRNA was examined by μCT and CD31 IHC. Scaffolds with PHD2 siRNA are shown to have a 3-fold increase in vascular density. (Scale = 200 μm)

Figure 3. 7 days post implantation, PTK-URs in a rat excisional wound (A) more effectively stent the wound area (*p<0.05) and (B) promote more tissue growth into the scaffold interior than PEURs (**p<0.005).

Figure 4. 14 days post implantation, PHD2 si-NP delivery from PEUR and PTK-UR scaffolds improved vascular density in rat excisional wounds (†, p<0.05)

At day 14, wound areas were evaluated for vascular density and it was found that loading of PEUR and PTK scaffolds with PHD2 si-NPs increased vascular density relative to their respective SCR controls by 1.6 fold and 1.8 fold respectively.

CONCLUSIONS
We have adapted a biomaterial platform for sustained gene silencing, and we have also explored a new PTK-UR scaffold chemistry for effective wound stenting and tissue infiltration. Release of PHD2 si-NPs improved local vascularization within both PEUR and PTK-UR scaffolds through HIF1α mediated transcription of pro-angiogenic genes. These materials hold tremendous promise as clinically translatable treatments for improving chronic wound healing.

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