Nanoceria-miRNA as a modulator of inflammation in diabetic wounds

Irina Kalashnikova[1], Soumen Das[1], Carlos Zgheib[2], Junwang Xu[2], Kenneth W Liechty[2], Sudipta Seal[1]

[1] Nanoscience Technology Center, University of Central Florida, 12424 Research Parkway Suite 400 Orlando, FL 32826, USA
[2]Anschutz Medical Campus, 2TCH, Children's Hospital, University of Colorado Denver,13065 East 17th Avenue Aurora, CO 80045, USA

Statement of Purpose: Wounds complicated by diabetes are mainly characterized by impaired healing due to attenuated immune response and at the end chronic inflammation. These are caused by impaired balance of oxidants/anti-oxidants system, delayed response of macrophages and their suppressed phagocytic function [1-3]. It has lately been established diabetic wounds have decreased expression of miRNA-146a during the wound healing response. Correction of miRNA-146a expression level is able to normalize immune response and the inflammatory conditions of the diabetic wounds [3]. Recent studies have ascertained wound healing potential of cerium oxide nanoparticles (CNPs) based on its radical-scavenging properties [4, 5]. We hypothesize that CNPs conjugated with miRNA-146a (CNP-miR146a) will correct the diabetic wound healing impairment by regulation the inflammation response and oxidative stress. **Methods:** CNPs using simple wet chemistry methods as described previously [4, 5]. Mature single-stranded miRNA-146a was attached to hydroxyl groups of CNP using 1,1-carbonyldiimidazole. The CNP-miR146a was characterized by dynamic light scattering (DLS), high resolution transmission electron microscopy (HR-TEM), UV-Vis spectrophotometry, Fourier transform infrared spectroscopy (FT-IR) and cytotoxicity test (MTT). To test our hypothesis *in vitro* (primary murine dermal fibroblasts) and *in vivo* models of non-diabetic (Hz) and diabetic (Db) wounds were treated with CNP-miR146a and the expression profile of miRNA-146a and its target genes (NF-kb, IRAK1, TRAF6, IL6 and MIP2) were examined. MiRNA-146a, CNPs and CNP-scrambled miR served as the appropriate controls. *In vivo*, four groups of mice (Hz and Db) were wounded (8mm full-thickness wound) and treated intradermally with 50ul single injection of 10μM CNP, CNP-miR146a, or vehicle. Total cellular RNA was isolated from the samples, purified and expression level of miRNA-146a and genes were analyzed using real-time quantitative polymerase chain reaction. Photographs of the wounds were taken every other day to evaluate the time-course of wound closure. Inflammation status was checked by immunohistochemistry (F4/80). **Results:** HR-TEM and DLS results showed the size distribution 3-5 nm and 30-35 nm, respectively. Zeta potential of conjugated CNPs was negative and the value varied within range 8-29 depending on miR amount attached. UV-vis and FT-IR confirmed the presence of miR-146a and formation of covalent bonds with CNPs. The level of miR146a expression in the fibroblasts being treated with CNPs-miR146a was regulated in dose- and time-dependent manner (**Fig. 1 A, B**). Up-regulation of miR-146a resulted in decreased gene expression of inflammatory and pro-inflammatory genes (TRAF 6, IRAK1, NF-kB, and IL-6 and IL-8/MIP-2) as well as in increased macrophage cells number recruitment into wound site. **Conclusion:** The CNPs-miR146a up-regulated miRNA-146a expression and repressed expression of the inflammatory and pro-inflammatory genes in both wound models that shorten time of non-diabetic (33%) and diabetic wounds closure (21%) (**Fig.2). References: 1.** Knobel D, Crawford JL, Butala P, Davidson EH, Wetterau M, Sultan SM, et al. Local reactive oxygen species scavenging improves diabetic wound healing. J Am Coll Surg.211:S80; **2.** Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, et al. Macrophage Dysfunction Impairs Resolution of Inflammation in the Wounds of Diabetic Mice. PLoS One. 2010;5:e9539; **3.** Xu J, Wu W, Zhang L, Dorset-Martin W, Morris MW, Mitchell ME, et al. The role of microRNA-146a in the pathogenesis of the diabetic wound-healing impairment: correction with mesenchymal stem cell treatment. Diabetes.2012;61:2906-12; **4.** Chigurupati S, Mughal MR, Okun E, Das S, Kumar A, McCaffery M, et al. Effects of cerium oxide nanoparticles on the growth of keratinocytes, fibroblasts and vascular endothelial cells in cutaneous wound healing. Biomaterials. 2013; 34:2194-201; **5.** Das S, Singh S, Dowding JM, Oommen S, Kumar A, Sayle TXT, et al. The induction of angiogenesis by cerium oxide nanoparticles through the modulation of oxygen in intracellular environments. Biomaterials. 2012;33:7746-55.

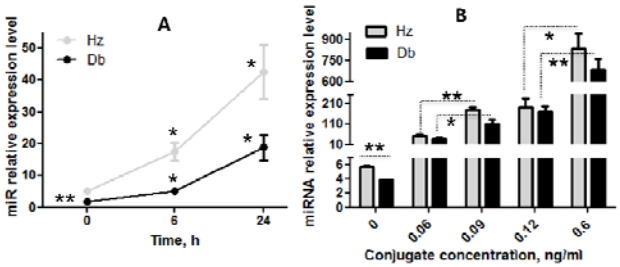

Figure 1. MiRNA-146a expression level in fibroblasts treated with vehicle or CNPs-miR146a: **A**-time-dependent profile of miRNA expression, **B**-concentration-dependent profile of miRNA expression.

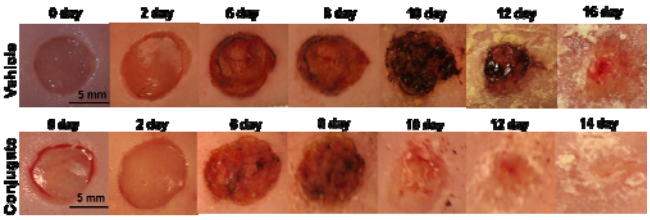

Figure 2. Time-course of wounds closure in diabetic mice treated with single injection of vehicle and CNPs-miR146a.