Panthenol citrate-based biomaterials accelerate diabetic wound closure and promote tissue regeneration
Huifeng Wang, Chongwen Duan, Guillermo Ameer
Department of Biomedical Engineering, Northwestern University, Center for Advanced Regenerative Engineering (CARE), Department of Surgery, Northwestern University Feinberg School of Medicine

Statement of Purpose: Diabetes mellitus (DM) is a prevalent chronic disease that is associated with persistent high blood glucose concentrations. Among diabetes patients, 19-34% develop diabetic chronic wounds on their lower limbs, which are exacerbated by oxidative stress, impaired angiogenesis, abnormal inflammation and bacterial infection. Citrate-based biomaterials (CBB) have shown significant promise in applications where tissue regeneration is important. We report the development of a new CBB comprised of panthenol citrate. The objective of this study was to synthesize and characterize panthenol citrate as an oligomer or a thermoresponsive hydrogel to assess whether it accelerates closure and promotes skin regeneration in diabetic wounds.

Methods: DL-panthenol and citric acid were reacted to create panthenol citrate (PC). PC was further incorporated into thermoresponsive hydrogel, PPCN, by reacting with polyethylene glycol, glycerol 1,3-diglycerolate diacylate, and N-isopropylacrylamide to synthesize PC-PPCN. Chemical structures were confirmed via 1H NMR and FT-IR spectroscopy. Phase transition temperatures were measured by rheology. Panthenol release from PAN-PPCN and PC were studied by HPLC. Antioxidant properties were characterized by iron chelation, ABTS free radical scavenging, and beta-carotene lipid peroxidation assays. Cell viability and proliferation were studied by alamarBlue assay. Cell migration was investigated by scratch assay. Tubulogenic properties were evaluated by tubule formation assay. To evaluate efficacy and safety in vivo, PC, PC-PPCN, PPCN and saline, were tested in splinted full thickness dermal wounds in diabetic db/db mice (Leprdb/J) (2 wounds per mice). Digital images of the wound were taken every 3 days until closure. Skin samples from the wound area were collected 3 days and 27 days post-wounding for histology and immunohistochemistry analysis.

Results: PC, PC-PPCN had antioxidant and antibacterial properties. Panthenol released from PC and PC-PPCN promoted dermal fibroblast, keratinocyte and endothelial cells migration and proliferation and endothelial tubule formation in vitro. Relative to the wounds treated with saline, all citrate-based materials exhibited faster wound closure rates. Wounds treated with PC and PC-PPCN displayed faster wound closure rate and exhibited enhanced granulation tissue and epithelial layer formation. Remarkably increased expression of fibroblast, keratinocyte and blood vessel marker were found in PC and PC-PPCN-treated wounds, indicating enhanced skin cell migration and proliferation and vascularization. Significantly less macrophages and pro-inflammatory cytokines and more anti-inflammatory cytokine markers were also observed in PC and PC-PPCN-treated wounds, suggesting modulation of the inflammatory response in wound environment. In addition, PC and PC-PPCN group demonstrated reduced oxidative stress and tissue damage.

Results: PC, PC-PPCN promoted wound closure in diabetic mice. (c) H&E and Masson’s trichrome staining of regenerated tissues. (d) Quantification of granulation tissue thickness. (e) Quantification of keratinocyte layer thickness. Granulation tissue and keratinocyte layer formed at the wound beds was significantly enhanced by PC and PC-PPCN. All data are presented as mean ± SD (n =5; *p < 0.05, **p < 0.01, ***p < 0.001).