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Dual action gels containing DsiRNA loaded gold nanoparticles: Augmenting diabetic wound healing by promoting angiogenesis and inhibiting infection

Ahmad Yasser Hamdi Nor Azlan [a, b], Hatice Katas [a, c], Norazlah Mohamad Zin [a], Mh Busra Fauzi [a]
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Abstract

Hyperglycemia induces the prostaglandin transporter (PGT) gene overexpression, leading to poor vascularization and wound healing. Dicer substrate small interfering RNA (DsiRNA) and gold nanoparticles (AuNPs) co-loaded into PF127 gel was developed to overcome the disturbance and infections. The AuNPs were biosynthesized using cold and hot water extracts of *Lignosus rhinocerotis* (abbreviated CLRE and HLRE, respectively). The wound healing efficacy of a PF127 gel containing DsiRNA-AuNPs -CLRE and -HLRE (assigned as F2 and F3, respectively) was evaluated in a diabetes-induced Wistar rat model. The F2 (DC) and F3 (DH) treated groups revealed a faster wound closure (92.67 ± 3.4% and 85.1 ± 7.3%, respectively) than the positive control (commercial gel, PTC(74.9 ± 13.3%). DH and DC groups presented an increased blood vessel density, along with decreased number of inflammatory cells. In comparison to positive control, higher prostaglandin E2 (PGE2) (495 ± 79 and 50 ± 131 pg/mL, for DC and DH group, respectively), vascular endothelial growth factor (VEGF) (49 ± 15 and 38 ± 3 pg/mL, for DC and DH group, respectively) and VEGF-A levels were detected in both groups (DC and DH), indicating the effectiveness of DsiRNA in enhancing PGE2 production and vascularization. On evaluating microbiomes adhered to the wound areas, Gram-positive bacteria Staphylococcus and Corynebacterium, as well as Gram-negative Pseudomonas, *Ralstonia pickettii*, and Acinetobacter, were found to be sensitive to the gel. Collectively, the gel was confirmed as a promising dressing for diabetic wound therapy, warranting further studies for clinical use.

Graphical abstract

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Introduction

Diabetes mellitus (DM) complicated by wound infections is considered an epidemic of multisystemic chronic disease [1]. A diminished ability to metabolize glucose results in a hyperglycemic condition, which impedes wound healing and results in stalled chronic wounds. Chronic wounds can be characterized as a condition in which tissue injuries do not heal in an organized manner or via a normal wound healing stage, extending more than 12 weeks to heal [2]. Despite many therapeutics have been developed, a therapy that effectively manages diabetic wounds by correcting biological abnormalities caused by hyperglycaemia is not currently available, especially when infections should be prevented. Hence, a dual action healing agent is needed to provide both effects.

In DM, hyperglycemia enhances prostaglandin transporter (PGT) gene expression, leading to reduced levels of prostaglandin E2 (PGE2). PGE2 plays an important role in inflammatory responses through eicosanoid signalling pathways that attenuate angiogenic responses and vasodilation, both of which are important during wound healing [3]. The abnormality could be rectified by administering an interfering RNA molecule (Dicer-substrate small interfering RNA, DsiRNA) to inhibit PGT production [4], [5], thus enhancing the production of PGE2. DsiRNA is a nucleic acid with the ability to silence target genes. Furthermore, DsiRNA is more potent than small interfering RNA (siRNA) as a lower dose is required to achieve therapeutic effects [4]. Nevertheless, the treatment of biological abnormalities alone is insufficient to comprehensively induce diabetic wound healing. Therefore, combination with an antibacterial agent is crucial for preventing bacterial infection at the wound site. Accordingly, a DsiRNA was recently combined with gold nanoparticles (AuNPs) synthesized from cold and hot water extracts of *Lignosus rhinocerotis*, abbreviated as CLRE and HLRE, respectively) that acted as an antibacterial carrier and formulation into a topical vehicle, PF127 gel. Gels containing DsiRNA-AuNPs are known to safely promote wound healing *in vitro* owing to their antibacterial and healing properties [6].

The present study aimed to elucidate the effects of PF127 gels containing DsiRNA-AuNPs -CLRE and -HLRE on the healing of excision wounds in a diabetes-induced Wistar rat model. Owing to the complexity of the diabetic wound, *in vitro* analyses alone, using cultured cells, are insufficient to generate comprehensive data. Therefore, additional studies using animal models are crucial. It should be noted that a wound infected with specific bacterial strains can be difficult to achieve and control, particularly in a non-sterile environment. Hence, to determine the gel efficacy in preventing or treating infection *in vivo*, the microbiome of bacteria adhered to wounds was analyzed via metagenomic studies (next gene sequencing [NGS] 16S rRNA gene sequencing). To assess wound healing rates, parameters such as the percentage of wound closure and tissue histology were determined.

In the present study, the fasting blood glucose of each animal was monitored throughout the study to confirm the diabetic model. To determine the safety and toxicity of the gels under investigation, serum biochemistry (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and creatinine [creat]) was analyzed. Moreover, vascular endothelial growth factor (VEGF) and PGE2 levels were determined using enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) to determine the efficacy in enhancing PGE2 and VEGF production, which are well-known mediators of angiogenesis during wound healing.

Section snippets

Materials

Gold (III) chloride hydrate (99.999% trace metal basis) was purchased from Sigma-Aldrich (Malaysia). Sodium hydroxide was purchased from R&M Marketing (Essex, UK). *L. rhinocerotis* sclerotial powder was provided as a gift from Lignas Bio Synergy Plt., Selangor, Malaysia. Low molecular weight chitosan (CS; molecular weight of 190 kDa, 75–85% degree of deacetylation) was purchased from Sigma-Aldrich (Ireland). Glacial acetic acid (99.7% purity) was purchased from R&M Chemicals (UK). Distilled...

Characterization of DsiRNA-AuNPs and gels

The synthesis of DsiRNA-AuNPs has been previously established by our group [6]. AuNPs were shown to range between 190 ± 33 and 208 ± 36 nm, with a negative surface charge (+30 ± 2 to +45 ± 8 mV). DsiRNA-AuNPs presented high entrapment efficiency (83 ± 2–85 ± 6%), with strong binding between DsiRNA and AuNPs obtained by gel electrophoresis. DsiRNA was also bound strongly to AuNPs after incorporating into the gel as no DsiRNA trailing band was detected Supplementary Fig. 1. The selected nanocomposites...

Conclusions

DsiRNA-AuNPs (particularly F3) were shown to be effective in treating diabetic wounds in a diabetes-induced (type 1) rat model. The percentage of wound closure was greater in the gel containing DsiRNA-AuNPs) than that in the positive (DTT) and normal (ND) control groups. Compared with other groups, histological analysis (H&E...

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper...

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