

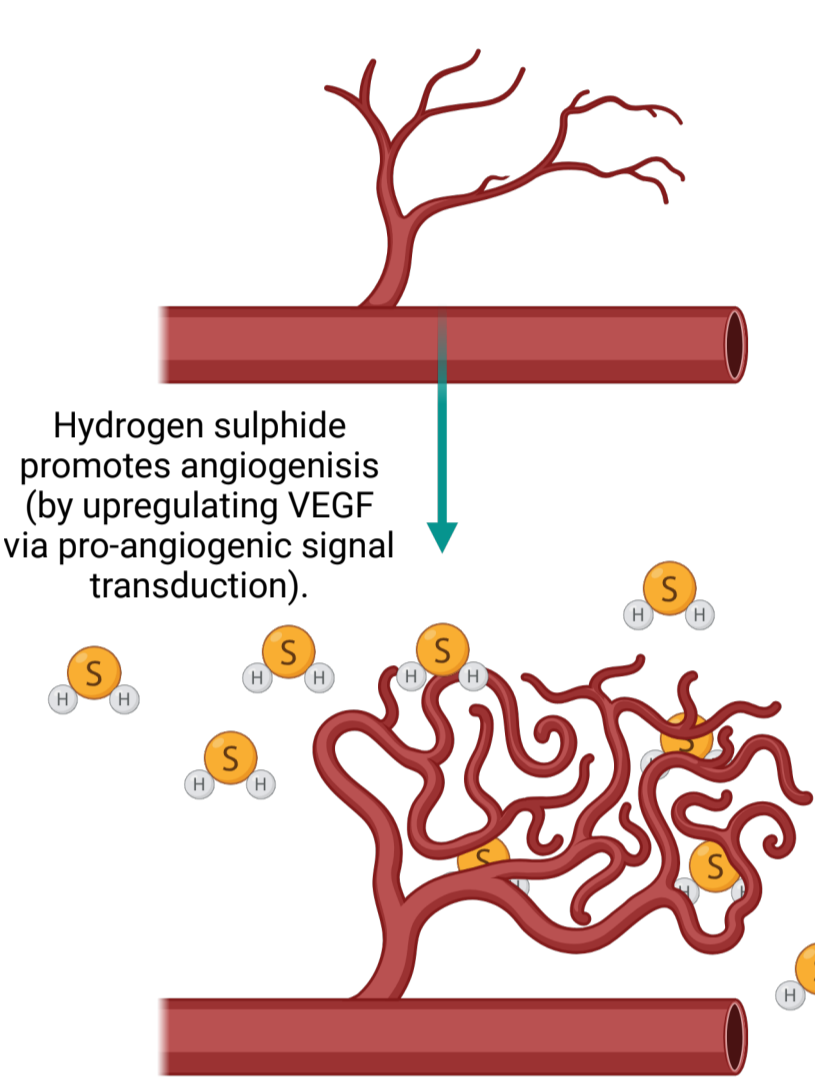
Assessing Therapeutic Efficacy of AP39-Loaded Hydrogels in Mitigating Inflammation and Cellular Senescence to Improve Diabetic Wound Healing

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INTRODUCTION

Diabetic wounds such as foot ulcers are a major cause of morbidity globally affecting 26million patients annually [1]. Wounds take longer to heal due to chronic inflammation and impaired tissue repair, often exacerbated by cellular senescence—a state of irreversible cell cycle arrest that contributes to chronic inflammation and hindered healing. Wounds are prone to infection leading to further complications such as sepsis, amputations and significant social dysfunction [2, 3]. Angiogenesis is essential during wound healing during which cells proliferate and migrate into the wound. However, these events are impaired in wound healing processes involving diabetic patients.

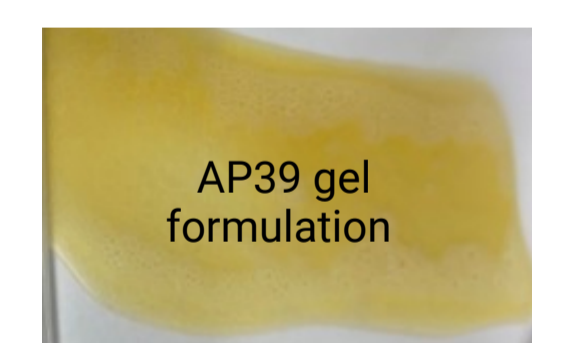
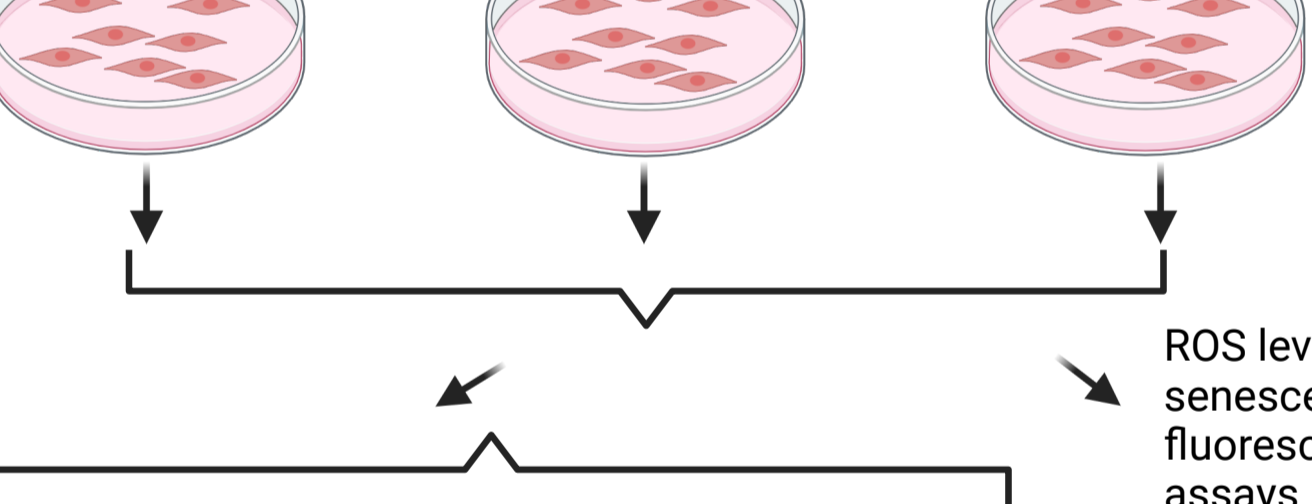


AP39, a hydrogen sulphide-donor, can reduce oxidative stress, promote angiogenesis, and modulate inflammation, processes that are disrupted in diabetic wounds [4, 5]. Hydrogels can maintain a moist wound environment and support autolytic debridement, thus offer an effective platform to deliver AP39. This project evaluates therapeutic potential of a novel topical hydrogel containing AP39 to enhance wound healing and reduce inflammation in diabetic wound models.

METHODS

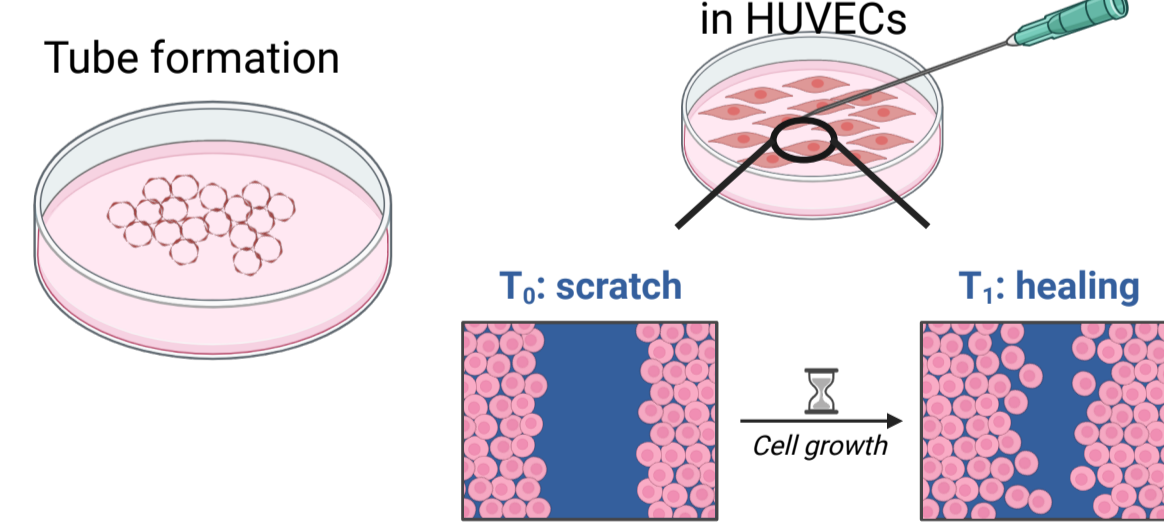
HUVEC/HDFa cells cultured in a hyperglycaemic, proinflammatory environment to mimic senescent cells in a diabetic wound

Control Glucose (20mM) and TNF- α (10ng/mL) Glucose (20mM) and TNF- α (10ng/mL) + AP39 gel



A 2-acrylamido-2-methylpropane sulfonic acid-based (NaAMPS) skin adhesive hydrogel was formulated with up to 5% v/v propylene glycol, 18% w/v Pluracare L 64 G, 0.01% w/v Photoinitiator and 0.1% w/v AP39. The inclusion of AP39 had minimal impact on the tack (2.48 ± 0.66 gf). AP39 stability in formulation, determined by HPLC-UV, was maintained over 28days

Test potential of AP39 to modulate pro-inflammatory response in HUVEC/HDFa cells, by measuring protein levels of IL-6 in culture media by ELISA
Test potential of AP39 to modulate extracellular matrix remodeling by measuring protein MMP9 in HDFa culture media by ELISA



Tube formation and wound healing assays were performed as indicative of angiogenesis.

RESULTS

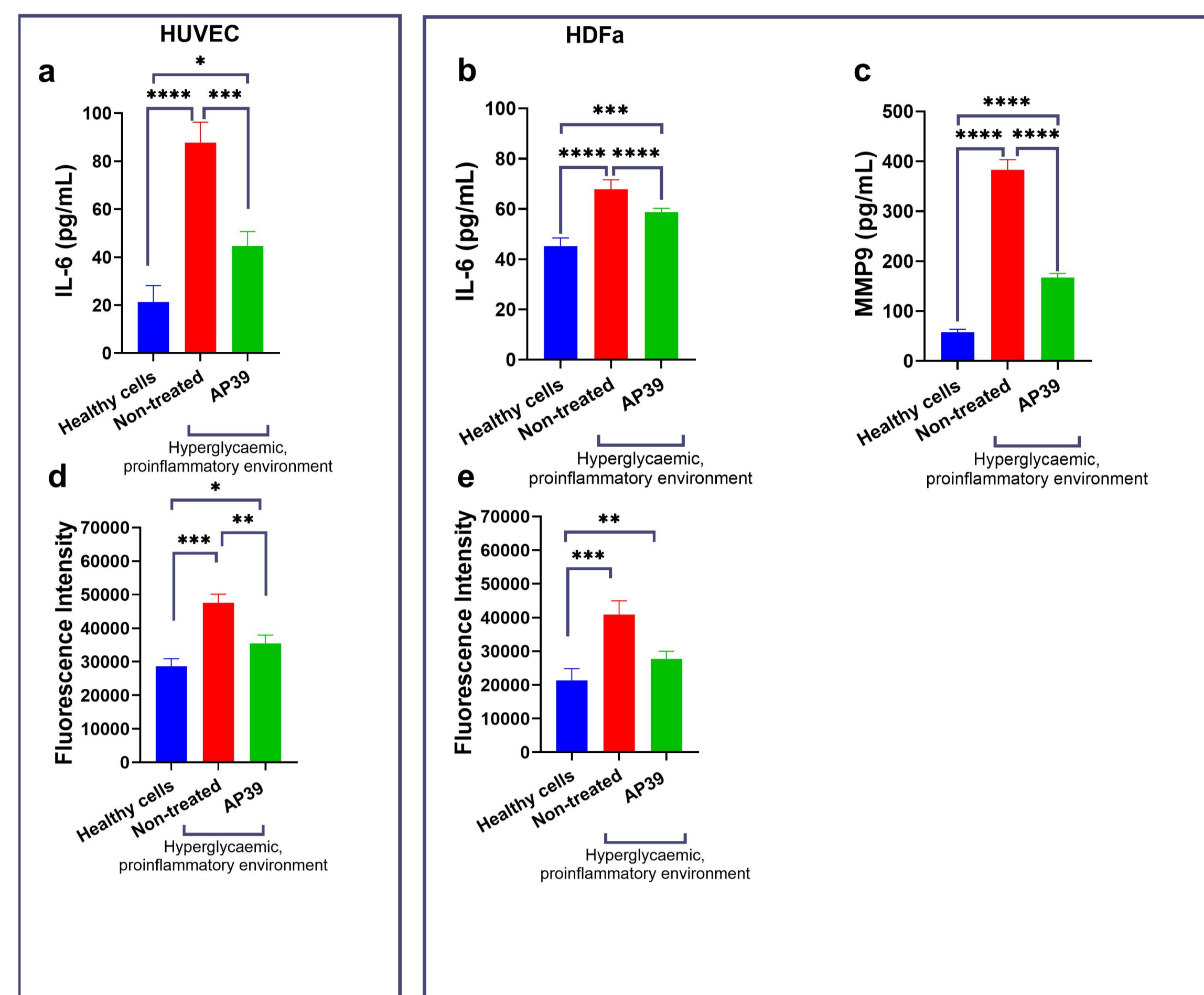


Figure 1: Effect of AP39 on IL-6 production in a) HUVEC and b) HDFa cells following hyperglycaemic and TNF- α insult. Hyperglycaemic and TNF- α increase production of IL-6 and ROS in both HUVEC and HDFa cells however treatment with AP39 was able to reduce IL-6 and ROS produced. Further AP39 treatment reduced MMP-9 levels suggesting its role in improving tissue remodelling. Results are representative or expressed as mean \pm standard deviation and analysed by One-way ANOVA. $n = 3$ independent batches. * $p < 0.05$, *** $p < 0.0001$.

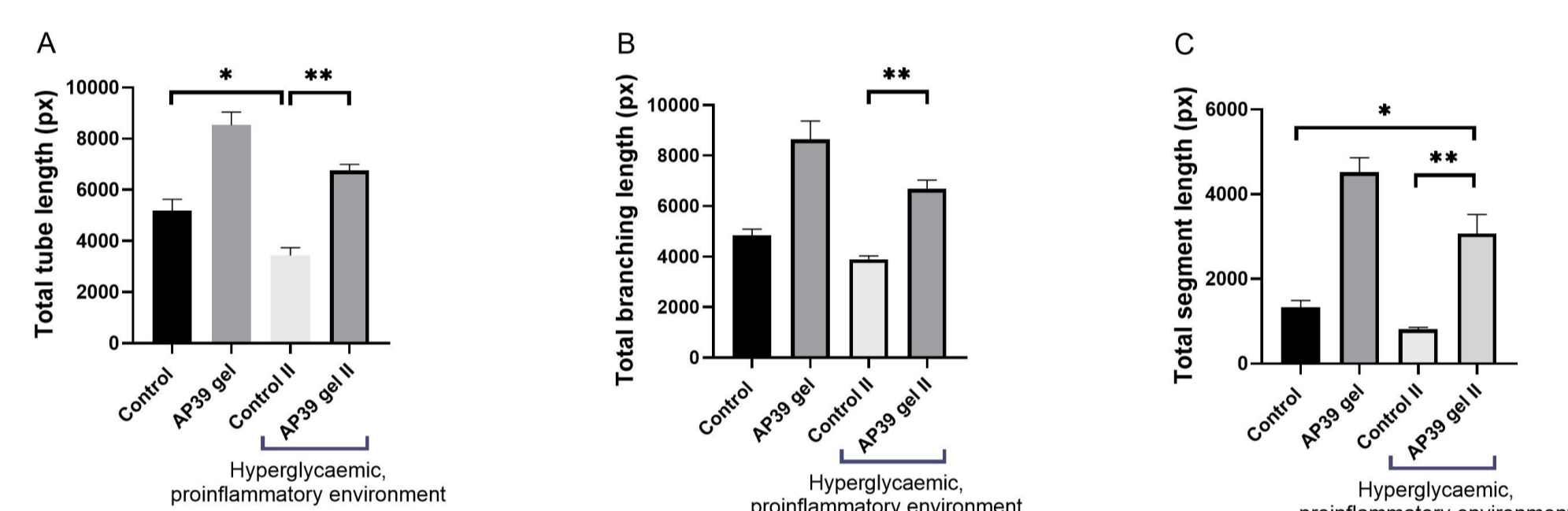


Figure 2: Whilst hyperglycaemia and TNF- α decreased tube formation, AP39 gels increased total tube length in these HUVEC cells. (A) total tube length, (B) total branching length and (C) total segment length comparing control with TNF- α treated and AP39 treated HUVECs. (D) Example fluorescent imaging of tube formation assay. Results are representative or expressed as mean \pm standard deviation and analysed by ANOVA. $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

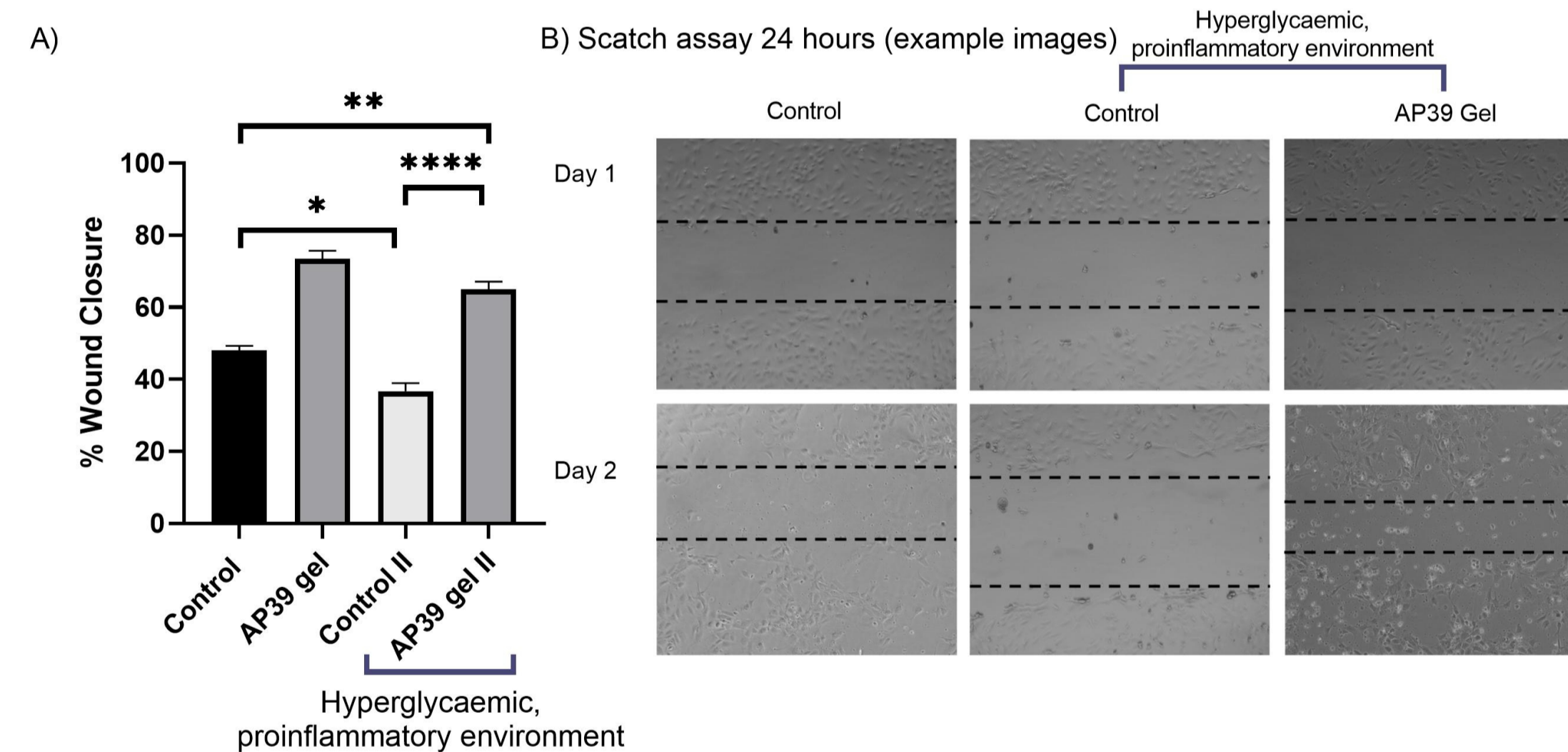
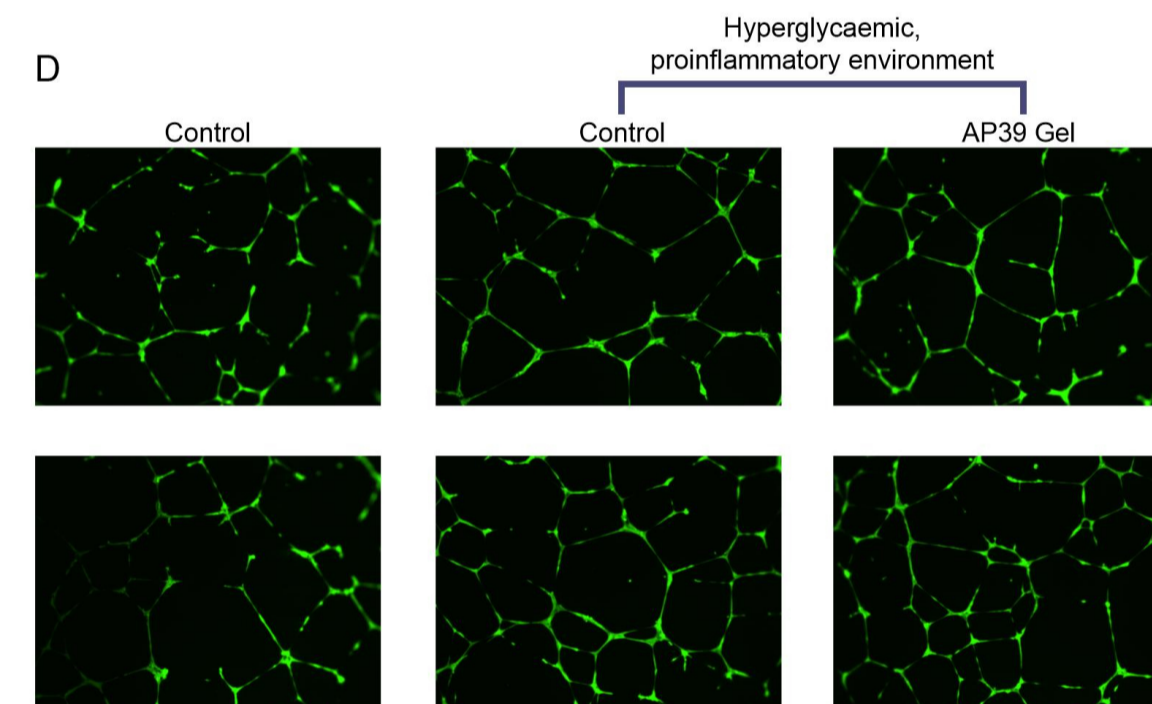


Figure 3: HUVEC wound assay observed whilst hyperglycaemia and TNF- α decreased wound healing, AP39 gels increased wound healing in these HUVEC cells (A) Wound areas were calculated and the results shown are mean (\pm SD) $n = 3$ * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. (B) example images for wound healing assay, black lines indicate width of "wound."

CONCLUSIONS

Hydrogels effectively preserved the stability of AP39, allowed sustained release, and demonstrated non-toxicity. In HUVECs and HDFs, treatment with AP39-loaded hydrogels reduced oxidative stress, a key driver of cellular senescence, as indicated by fluorescence markers, and decreased inflammation, measured through IL-6 levels. The reduction in inflammation is significant given its role in propagating the senescence-associated secretory phenotype, which exacerbates tissue dysfunction and impairs wound healing. In HDFa cells, a reduction in MMP-9 expression further supports the role of AP39 in enhancing tissue remodeling, a critical process in wound healing that is often impaired in senescent cells.

Moreover, in HUVECs cultured under hyperglycaemic conditions with TNF- α -induced inflammation, a significant reduction in wound healing was observed compared to controls. However, treatment with the AP39-loaded hydrogel following the TNF- α inflammatory insult resulted in increased cell proliferation and migration, demonstrating its potential to promote diabetic wound healing. These findings underscore the therapeutic potential of AP39 in addressing inflammation and senescence-associated dysfunction in diabetic wounds.

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