THE UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER

Cellular Effects of Silver Diamine Fluoride in Oral Cells

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ABSTRACT

Purpose. This study evaluated SDF's impact on cell viability, inflammation, DNA integrity, and functional properties in oral cells.
Methods. Dental pulp stem cells (DPSCs), periodontal ligament fibroblasts (PDLFs), and gingival fibroblasts (GFs) were exposed to increasing concentrations of silver diamine fluoride (SDF). Cell viability and apoptosis were assessed by MTT and Annexin V assays. Inflammatory and DNA damage–related gene expression was analyzed by qRT-PCR. Wound closure and trans-well migration assays were evaluated for functional effects. To simulate dentin permeability, a trans-well system (0.4 µm pores) was used to test whether SDF diffuses and affects underlying cells.
Results. SDF exposure (0.005–0.01%, 24-48 h) caused a dose-dependent reduction in viability, confirming increased apoptosis across all cell types. qRT-PCR showed upregulation of inflammatory genes (IL-1β and COX2) and downregulation of DNA repair genes (RAD51, PARP1, and XRCC4). Functional assays demonstrated impaired wound closure and reduced cell migration. Notably, indirect exposure through the dentin-mimicking trans-well barrier also significantly reduced cell viability and wound healing.
Conclusions: Low SDF concentrations, even across a dentin-like barrier, can induce inflammation, apoptosis, impaired wound healing, and suppression of DNA-repair pathways in oral cells, indicating cytotoxic risks and the need to reassess therapeutic concentrations for clinical use.

INTRODUCTION

Dental caries remains one of the most prevalent chronic diseases worldwide, affecting individuals across all age groups despite advances in preventive care. This persistent burden highlights the need for effective, minimally invasive, and cost-efficient treatment strategies, particularly in pediatric populations. Silver diamine fluoride (SDF) has emerged as a promising option due to its combined antimicrobial and remineralizing properties. Its components—silver, fluoride, and ammonia—work synergistically to arrest caries, with clinical studies showing high success rates. However, the characteristic black staining of treated lesions continues to limit parental acceptance, and concerns remain regarding its effects on oral tissues.

MATERIALS AND METHODS

DPSCs, PDLFs, and GFs were cultured and exposed to varying concentrations of SDF. Cell viability was assessed by MTT assay, and a trans-well system was used to model dentin permeability for DPSCs. Gene expression (inflammation, apoptosis, DNA damage) was analyzed by RT-PCR, while apoptosis, cytotoxicity, and cell cycle were evaluated using Annexin V/PI staining, live/dead imaging, and flow cytometry. Cell migration was assessed using a scratch wound assay.

RESULTS

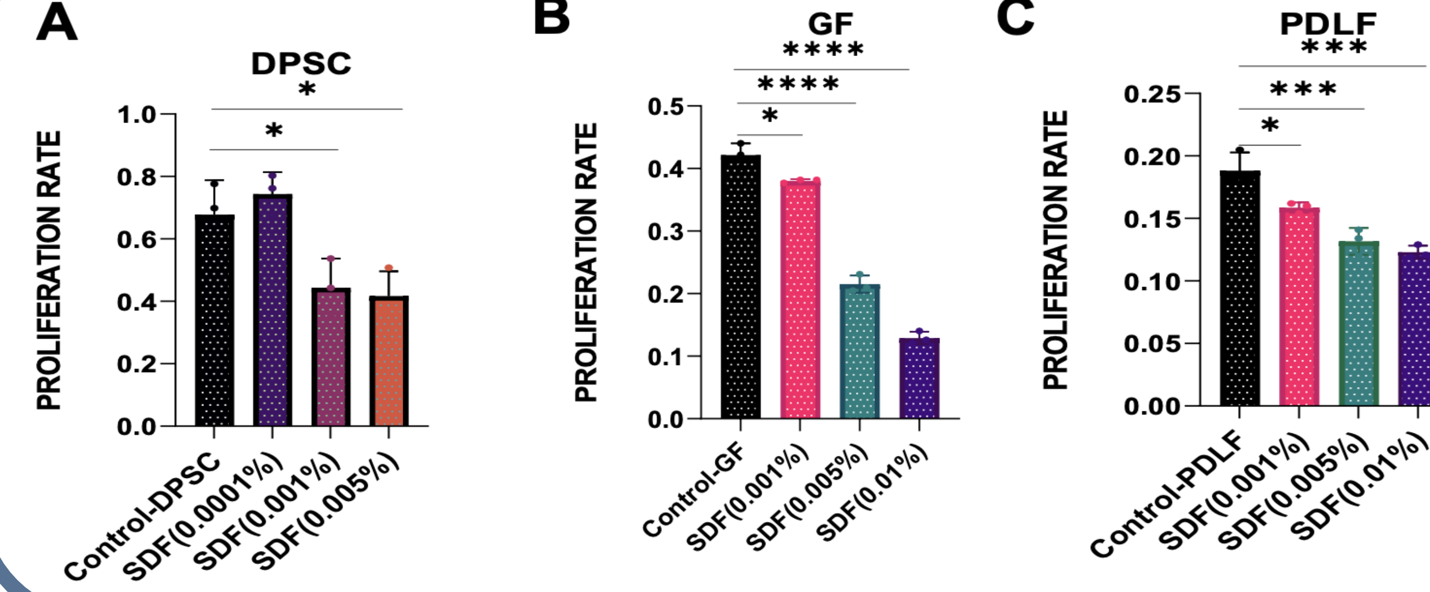

Figure 1. MTT Viability Assay – SDF Treatment 24 Hrs

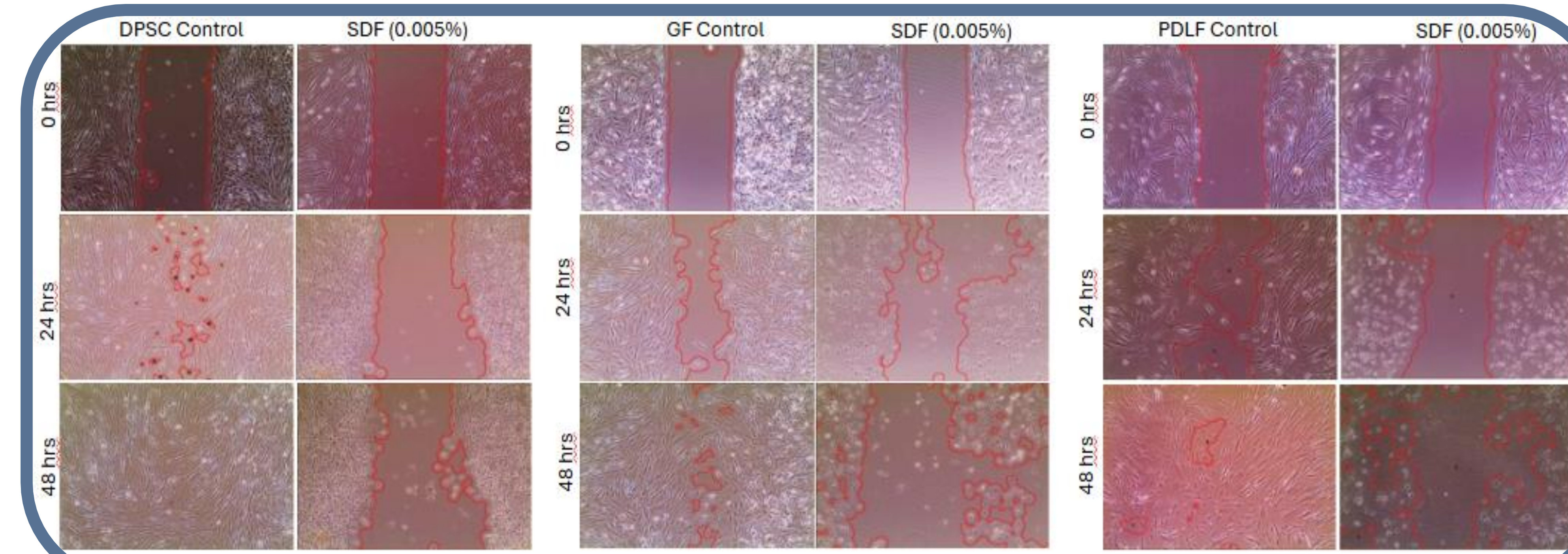

Figure 2. Wound-Healing Assay

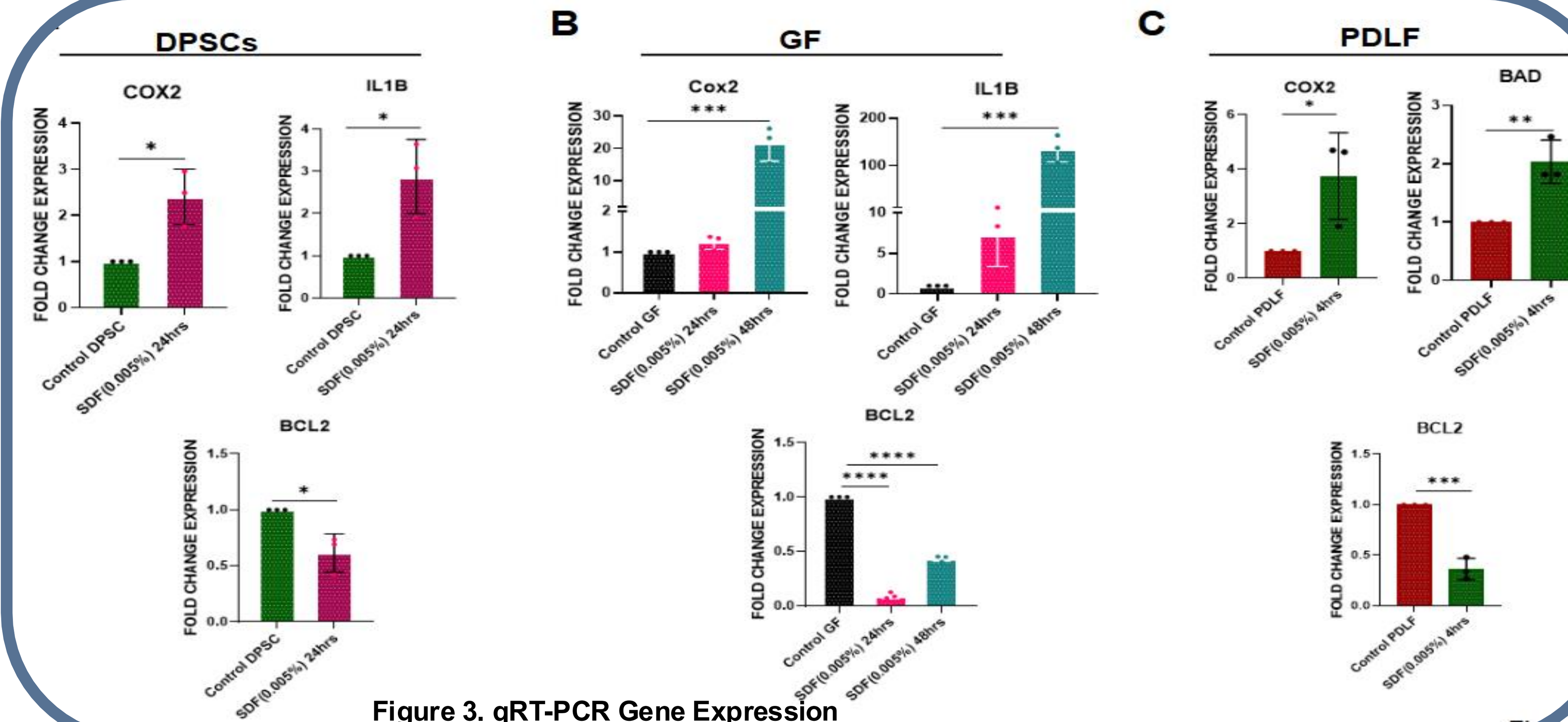

Figure 3. qRT-PCR Gene Expression

- Figure 1: concentration-dependent cytotoxic effects
- Figure 2: inhibited migration, negative wound-closure values (~50%) due to cell retraction and monolayer disruption
- Figure 3: upregulation of inflammatory and pro-apoptotic genes (COX-2, IL-1β); downregulation of anti-apoptotic gene (BCL2)
- Figure 4: DPSC cytotoxicity (~50% viability reduction at 0.005% and ~80% cell death at 0.01%) and impaired migration with dentin-barrier conditions

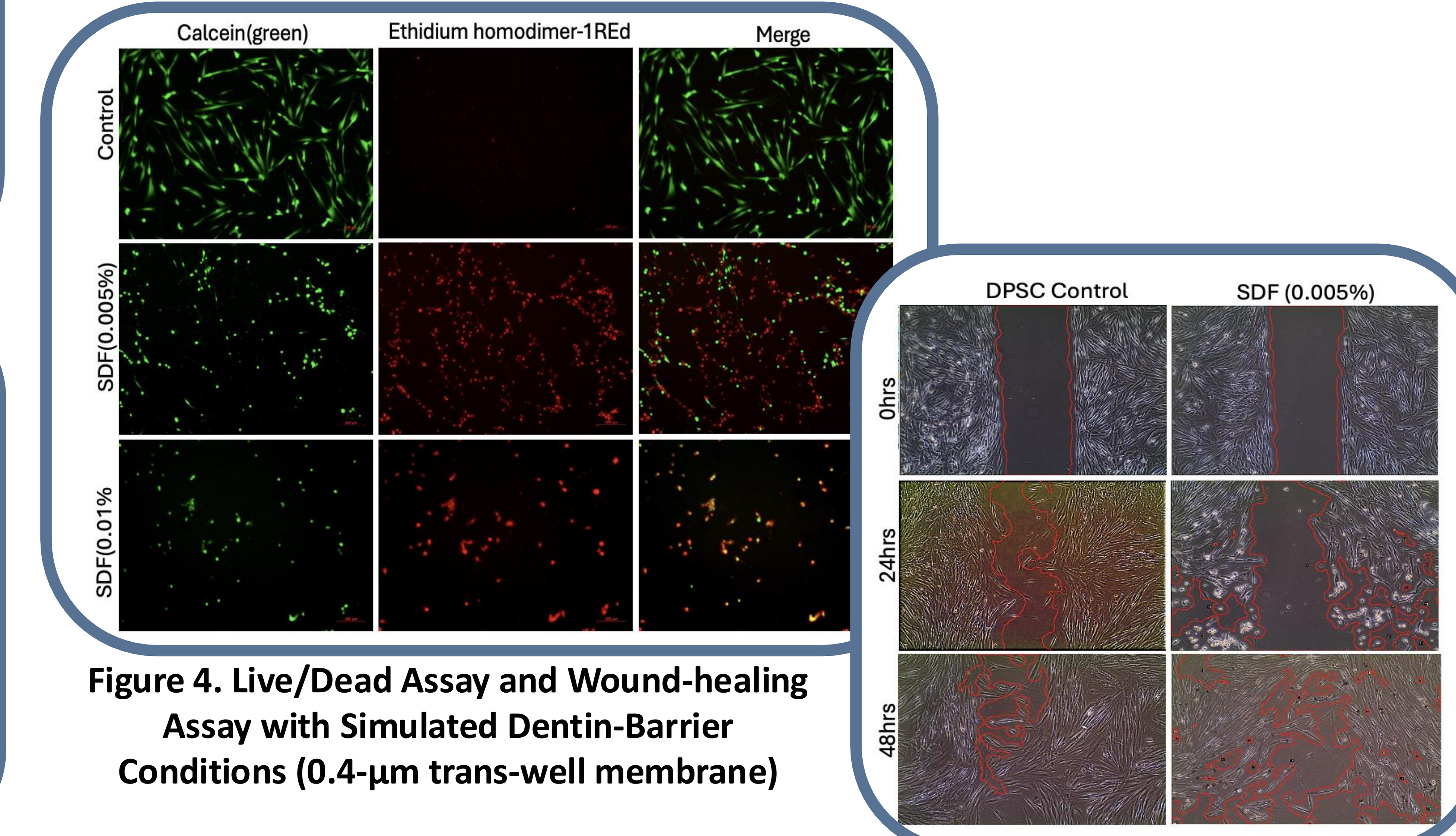

Figure 4. Live/Dead Assay and Wound-healing Assay with Simulated Dentin-Barrier Conditions (0.4-µm trans-well membrane)

CONCLUSION

1. SDF exerts dose-dependent cytotoxicity on DPSCs, PDLFs, and GFs, with apoptosis confirmed by Annexin V analysis and cytotoxic effects persisting even under simulated dentin-barrier conditions.
2. SDF impairs migration across all cell types and disrupts cell-cycle progression, with S-phase accumulation indicating replication stress.
3. SDF upregulates pro-inflammatory and necroptotic genes, downregulates the anti-apoptotic gene BCL2, and suppresses key DNA repair genes, indicating overlapping cell death pathways and genomic instability.

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