

# Cytotoxicity Analysis of Pulp Stem Cells After Silver Diamine Fluoride Application

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## ABSTRACT

**Purpose:** To evaluate the cytotoxicity of silver diamine fluoride (SDF) to human dental pulp stem cells (hDPSC).

**Methods:** hDPSC were exposed to dilutions of 38% SDF ( $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$ ) and incubated for 24 hours. Cell viability was assessed with colorimetric detection assay (XTT) after 24 hours. Fresh media was used as a negative control and 0.1% Sodium Dodecyl Sulfate (SDS) was used as a positive control. Three independent experiments were performed in triplicates. Cell viability data were analyzed using ANOVA and Tukey's multiple comparison's test.

**Results:** Cells exposed to dilution of SDF  $10^{-3}$  had an average cell viability of  $17 \pm 3.5\%$ . Cells exposed to SDF  $10^{-4}$  and  $10^{-5}$  had an average cell viability of  $101 \pm 2.5\%$  and  $94 \pm 4.4\%$ , respectively. Dilution of  $10^{-3}$  had a significantly lower cell viability than the negative control ( $P < 0.0001$ ). Dilution of  $10^{-4}$  and  $10^{-5}$  SDF had significantly higher cell viability than the positive control ( $P < 0.0001$ ) and cells treated with dilution of  $10^{-3}$  ( $P < 0.0001$ ).

**Conclusions:** SDF was cytotoxic to hDPSC at a dilution of  $10^{-3}$ , but not at  $10^{-4}$  and  $10^{-5}$ . These findings demonstrate a dose-dependent cytotoxicity of SDF to hDPSC.

## BACKGROUND

- Silver Diamine Fluoride (**SDF**) is currently used as a non-invasive treatment option for caries arrest.
- Current clinical recommendation is to refrain from using SDF on exposed pulp.<sup>1</sup>
- There are currently no studies testing cytotoxicity of SDF on human dental pulp stem cells (**hDPSC**).
- Potential cytotoxicity of SDF to hDPSC is relevant to clinical decision making for application of SDF on teeth with exposed pulp tissue.



Active caries before and after SDF application<sup>1</sup>

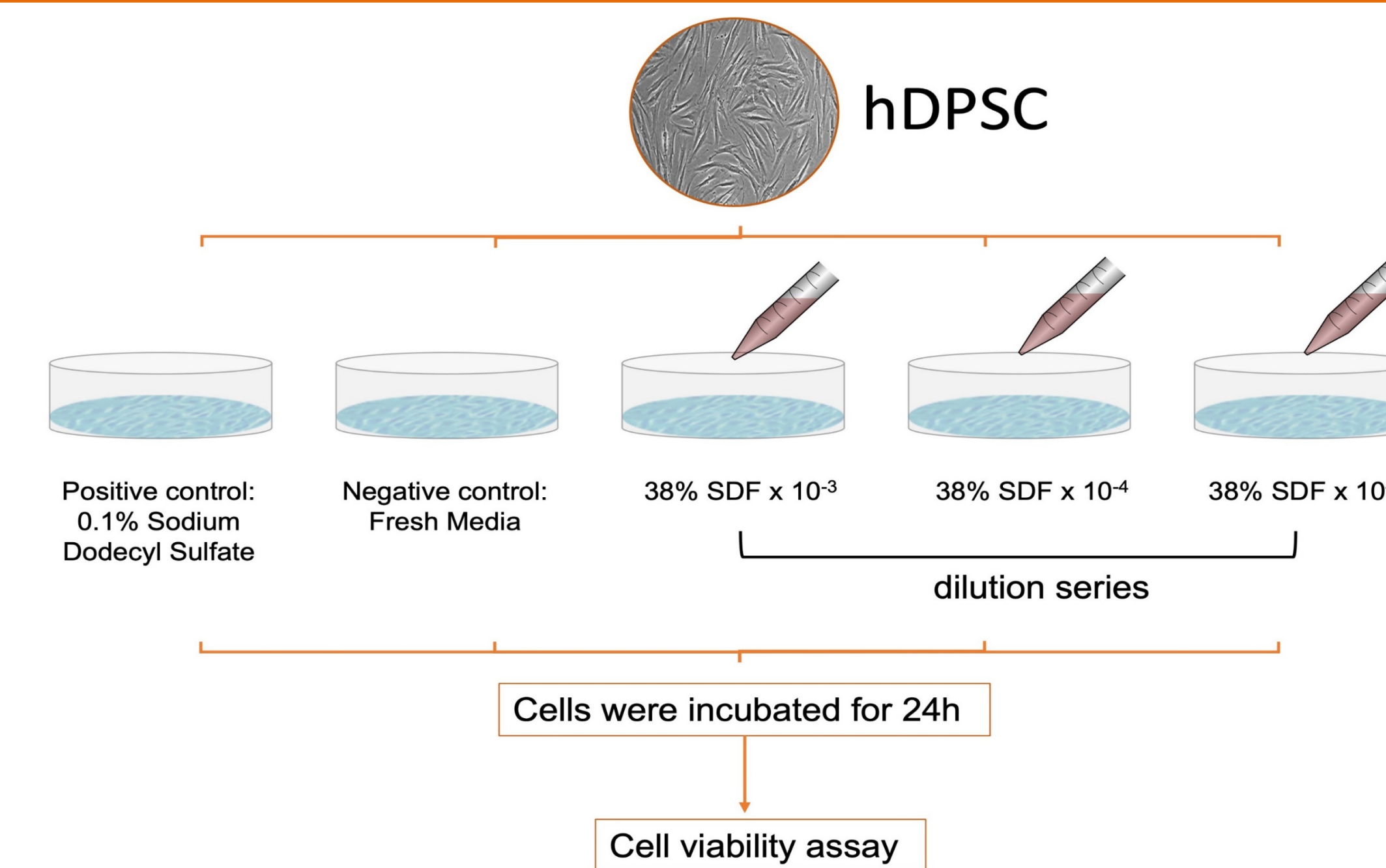
## AIM

To investigate the effect of SDF on hDPSC viability.

**Null hypothesis:** There will be **no decrease in cell viability** of hDPSC after exposure to serial dilutions of 38% SDF.



## MATERIAL & METHODS



- Three independent experiments were performed in triplicates.
- Cell viability was assessed with colorimetric detection assay (XTT) after 24 hours.
- Data was analyzed using ANOVA and Tukey's multiple comparisons test.

## RESULTS

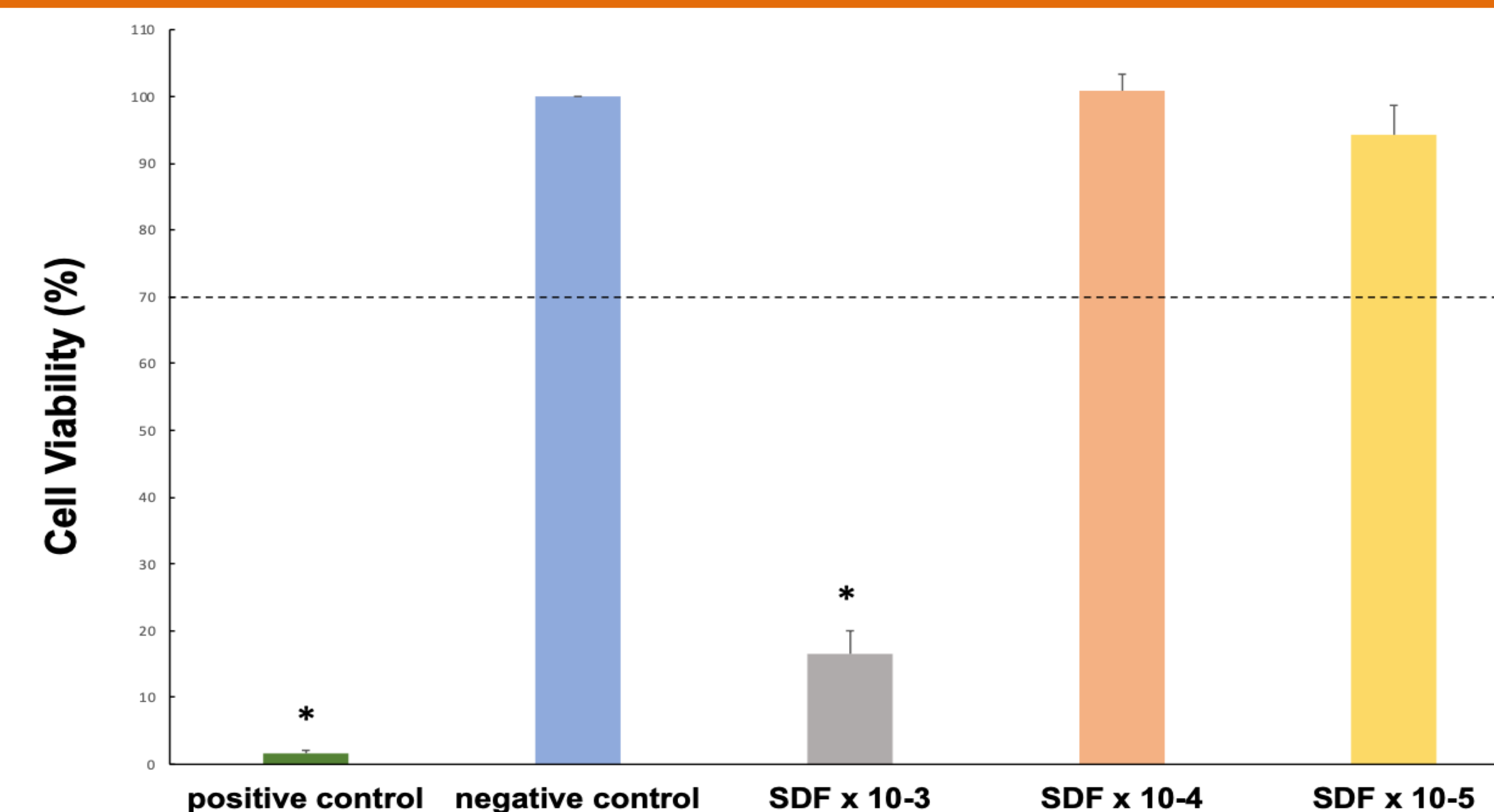


Figure 1- Results of Cell Viability Assay.  
hDPSC cell viability (%) after exposure to different dilutions of 38% SDF and controls. Dashed line represents the cell viability cutoff level established by the ISO (10993-513).<sup>5</sup> (\*statistically significant  $P < 0.01$ )

- Cells exposed to 38% SDF x  $10^{-3}$  had **significantly lower** cell viability than cells exposed to the negative control ( $P < 0.0001$ ) but **showed significantly higher** cell viability than the positive control ( $P = 0.0004$ )
- Cells exposed to 38% SDF x  $10^{-4}$  and  $10^{-5}$  showed **significantly higher** cell viability, than cells exposed to the positive control ( $P < 0.0001$ ) and to  $10^{-3}$  SDF treated cells ( $P < 0.0001$ ).
- No significant differences** were observed between  $10^{-4}$ ,  $10^{-5}$  SDF-treated cells and cells exposed to the negative control ( $P > 0.05$ ).

## DISCUSSION

- Our null hypothesis was rejected since there was significant decrease in cell viability of hDPSC after exposure to various dilutions of 38% SDF**
- Despite the dose-dependent cytotoxicity noted in this study, a concentration of SDF lower than 38% is not recommended for caries arrest because lower concentrations seem to be less effective at arresting caries<sup>2-4</sup>
- A clinical approach should balance the aim of arresting caries with minimizing adverse pulpal effects.
- Based on the results of our study it is reasonable to avoid direct application of SDF on exposed pulp

### Limitations:

- Time-dependent cytotoxicity in hDPSC exposed to dilutions was not observed.
- As an *in vitro* experimental study, we understand the limited clinical generalization of our findings. Despite these limitations, we demonstrated that 38% SDF is cytotoxic to hDPSC if applied directly.

**Future research** should focus the depth of the cytotoxic effect of SDF once it comes in contact with the pulp. Additionally, the depth of SDF penetration into the pulp based on the remaining dentin thickness of carious lesions should be explored.

## CONCLUSION

Based on this study's results, the following conclusions can be made:

- SDF demonstrates dose-dependent cytotoxicity to hDPSC.
- The authors support the recommendations of the American Academy of Pediatric Dentistry (AAPD) of avoiding the application of SDF on exposed pulps.<sup>1</sup>

## ACKNOWLEDGEMENTS

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References